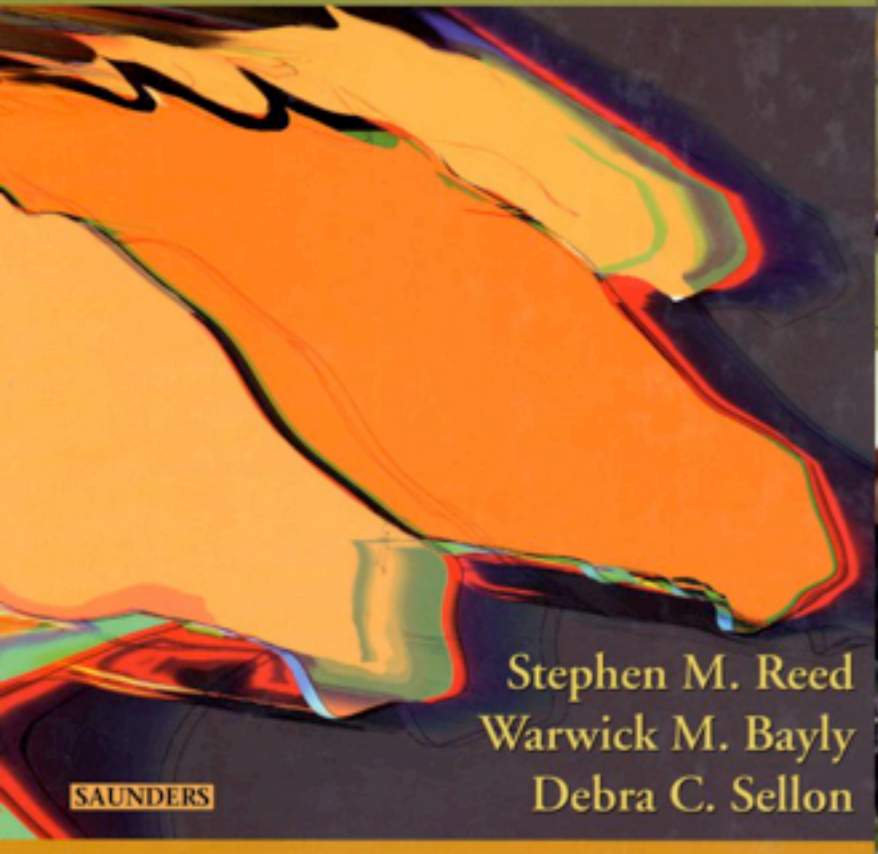
EQUINE INTERNAL MEDICINE

SECOND EDITION



Stephen M. Reed
Warwick M. Bayly
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SAUNDERS

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Front Matter

DEDICATION

This book is dedicated to our parents, who taught us to appreciate the value of education and the utility of knowledge, and to Karen, Abby, Nick, Della, Matt, Dan, Ben, Caitlin, Rance, and Ethan for their endless patience and support.

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PREFACE

The 5 years since the publication of the first edition of *Equine Internal Medicine* have witnessed the continued evolution of equine internal medicine as a specialty veterinary discipline. The progressive globalization of the world's societies and associated growth in the international movement of horses has been linked with increased global expectations in the standard of veterinary care and evaluation of sick horses. The sophistication of specialist training programs and the increased number of equine internists also taking advantage of postgraduate doctoral opportunities have resulted in a wealth of new information and the maturing of an increasingly complex and challenging discipline — equine internal medicine. The delivery of superior health care and increased client expectations that have been associated with the growth of this discipline have led to the development of a specialist, the equine internist, and extremely well-informed and astute equine general practitioners. Partly as a result of more training opportunities, the number of equine internists in practice is growing, and many veterinary teaching hospitals

are now tertiary care facilities rather than secondary referral centers. More than ever before, equine internal medicine now stands as an autonomous specialty in the veterinary profession.

The aim of the first edition of *Equine Internal Medicine* was to promote a clearer comprehension of the principles of disease or problem development by focusing on the basic pathophysiologic mechanisms that underlie the development of various equine diseases. The objectives behind the publication of the second edition of this book are no different. Basic information is presented and then related to the clinical characteristics of each disease and its therapy and management.

All the chapters that appeared in the first edition have been updated and a number of them have been extensively revised. Sections on the mechanisms by which infectious agents establish themselves contain a considerable amount of new material as do those dealing with the diseases of foals, the reproductive system, the endocrine system, and the gastrointestinal tract. In addition, new chapters have been contributed on the epidemiologic approach to outbreaks of disease and critical care of patients, while those on clinical pharmacology have also been rewritten.

Although the bulk of the chapters address specific diseases along systems-based lines, we realize that the practitioner is initially confronted with a specific problem that may have its origin in one or more of the body's systems. The first section of the book is therefore devoted to an in-depth discussion of the basic mechanisms by which the problems may develop and the principles underlying the treatment to many of them. The reader can build on this foundation by reading about specific disorders in the second section of the book, which is divided into chapters dealing with problems of a particular body system or of a specific nature.

Many true experts have contributed to this text. Their depth of knowledge about all aspects of equine internal medicine is encyclopedic. We are grateful for their efforts and diligence in helping us to produce what we hope will come to be regarded as the definitive text on medical diseases of horses. We trust the second edition of *Equine Internal Medicine* will prove to have as much universal appeal and application as the first one.

We would be remiss if we did not thank the many people at Elsevier for their persistence and efforts. Teri Merchant, Liz Fathman, Jody McBride, Cheryl Abbott, John Dedeke, and Linda Duncan particularly deserve our gratitude. They and many others have assisted in manuscript preparation, correspondence, and all the other tasks that must be accomplished to get a book like this into print. Without them and the generosity of our colleagues, this book would not have been published. We trust that everyone's efforts have been worthwhile.

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1 # CHAPTER 1 THE EQUINE IMMUNE SYSTEM

1.1 ## 1.1—Equine Immunology

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David W. Horohov

Although much of modern immunology has focused on human beings and murine models of human diseases, the horse has played a significant role in the understanding of immunological processes. These contributions include the earliest work on serotherapy and passive transfer, immunoglobulin structure and function, immunity to infectious agents, immunodeficiencies, and more recently, reproductive immunology. Work in the horse continues in many of these areas in equine medicine and comparative immunology. The overall organization and function of the equine immune system is similar to other mammalian species, though differences exist. The reader is referred to any one of a number of texts[1–4] for a more in-depth summary of immunology. This chapter focuses on those aspects of the immune system of most interest to equine researchers and clinicians. When possible, pertinent references to equine work are provided.

1.1.1 ### Innate Immunity and the Acute Inflammatory Response

Immune defenses include *innate responses* and *adaptive responses,* each mediated by cellular and soluble components. Although the innate and adaptive responses often are regarded as separate, they in fact are related intimately, sharing many of the same processes and components. The major difference lies in the specificity and recall capability that characterize the adaptive response. The specificity of adaptive responses, mediated by antibodies or by effector cells such as cytotoxic T lymphocytes (CTLs), and the phenomena of immunological memory are responsible for the capacity to protect an animal completely against a particular pathogen. Nevertheless, the role of innate responses in prompting the adaptive response and providing valuable time for specific adaptive responses to develop cannot be overstated.

The horse, like every other species, is under constant assault from a variety of microbes that share its living space. Although most of these organisms are harmless, their disease-causing potential is evident when they cause opportunistic infections in individuals with compromised immune systems.[5] Mammals have evolved a variety of defensive measures to prevent infection. The first line of defense includes the physical barriers of the skin and mucosal surfaces of the digestive, respiratory, and urogenital tracts. In addition to providing a barrier to penetration, the surface of the skin contains various enzymes, fatty acids, and oils that inhibit the growth of bacteria, fungi, and viruses. Mucous membranes and mucosal secretions contain bacteriolytic enzymes, bactericidal basic polypeptides, mucopolysaccharides, and antibodies that prevent colonization and penetration of these surfaces. Mucus also provides a physical barrier that entraps invading organisms and leads to their eventual 2
disposal.[6] Particles trapped in the mucous secretions of the respiratory tract, for example, are transported upward through the action of ciliary cells to the trachea where they are swallowed.[7] Once the particles are swallowed, the acidic secretions and digestive enzymes of the stomach destroy most organisms. Normal epithelial and tissue architecture is essential for successful exclusion of bacteria, and the disruption of this mechanism makes the host susceptible to infection by bacteria that normally colonize the upper airway.[8,9]

1.1.1.1 ACUTE PHASE PROTEINS, PROINFLAMMATORY CYTOKINES, AND COMPLEMENT

Once the integumentary and mucosal barriers are breached, the host presents a variety of internal defenses to contain and eliminate potential pathogens. Invading organisms can initiate an inflammatory response via the activation of plasma protease systems directly, such as by bacterial cell wall components, or by the secretion of toxins or other proteins that can activate the inflammatory response directly. Injured cells also release products that initiate plasma protease cascades or produce proinflammatory cytokines that augment the inflammatory process. Resident macrophages that encounter invaders complement the inflammatory response through the production of proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor α (TNF-α).[10] Cytokines are hormonelike proteins that mediate a variety of cellular responses. A vast number of cytokines are involved in the regulation of innate and adaptive immune responses. IL-1, for example, is a pleiotropic mediator of the host response to infections and injurious insults (Box 1.1-1). Many of the effects of IL-1 are mediated through its capacity to increase the production of other cytokines, such as granulocyte colony-stimulating factor, TNF-α, IL-6, IL-8, platelet-derived growth factor, and IL-11 (see the following discussion of cytokines, chemokines, and interleukins). IL-6 is responsible for the increased production of *acute phase proteins* (Table 1.1-1) by the hepatocytes. Although the function of all of the acute phase proteins remains unclear, many of these proteins and the cytokines that elicit them are responsible for the characteristic physical signs of inflammation, including increased blood flow and vascular permeability, migration of leukocytes from the peripheral blood into the tissues, accumulation of leukocytes at the inflammatory focus, and activation of the leukocytes to destroy any invading organisms.[11] The acute phase proteins include a number of *complement* proteins. The complement system is an interacting series of proteases and their substrates that produce the physiologically active intermediaries that can damage membranes, attract neutrophils and other cells, increase blood flow and vascular permeability, and opsonize bacteria and other particles for phagocytosis.[12] The complement cascade can be activated in two ways (Figure 1.1-1). The *classical pathway* involves the recognition and binding of the first component of complement (C1) to antigen-antibody complexes. Bound C1 is proteolytic and cleaves C4. This cleavage of C4 leads to the binding of C2 to C4b. C2 in turn is cleaved by C1 into C2a. The C4bC2a complex is referred to as the classical pathway C3 convertase because the complex is a protease capable of cleaving C3 into C3a and C3b. Another C3 convertase is generated via the alternate pathway. The activation of complement via the *alternate pathway* does not involve antibodies; instead, certain microbial products (zymosan and lipopolysaccharide) stimulate the association of factor D, a proteolytic enzyme, with the complex of factor B and C3b leading to the formation of the C3bBb complex, which is the alternative pathway C3 convertase. C3a and C4a can bind to mast cells, causing them to degranulate, and are referred to as anaphylatoxins. C3b serves as an opsonin for C3b receptor–bearing phagocytic cells. C3b also is required for the formation of the membrane attack complex by the terminal complement components C5 to C9. In this process C5 is cleaved by the C4b2a3b (classic pathway C5 convertase) or by C3b, Bb, and properidin (alternate pathway C5 convertase). C5 is cleaved into C5a and C5b. C5a is a chemoattractive factor for neutrophils and monocytes.[13] C5b forms a complex with C6, C7, and C8 on cell surfaces, which leads to the insertion and polymerization of C9 that forms a pore in the membrane leading to cell lysis.

1.1.1.1.1 BOX 1.1-1 BIOLOGIC ACTIVITIES OF I NTERLEUKIN-1

Activates T cells.

Activates B cells.

Enhances natural killer cell killing.

Activates fibroblast growth factor.

Stimulates prostaglandin E synthesis.

Stimulates bone resorption.

Is chemotactic for neutrophils.

Activates osteoclasts.

Induces fever.

Is cytotoxic for some tumor cells.

Is cytostatic for other tumor cells.

Stimulates collagen production.

Stimulates keratinocyte growth.

Stimulates mesangial cell growth.

Activates neutrophils.

Induces interleukin-6 production.

2

TABLE 1.1-1 Acute Phase Proteins

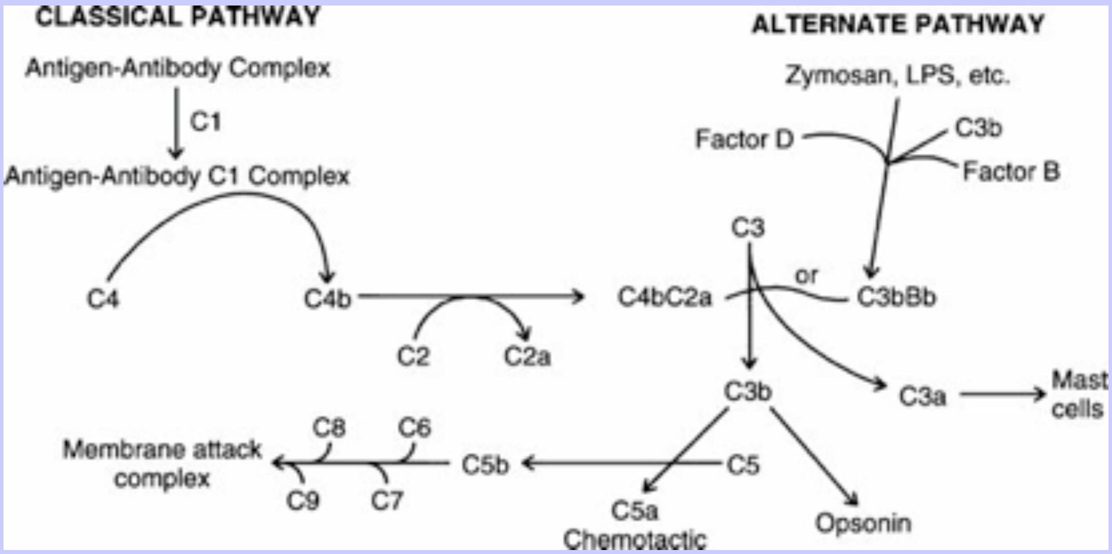
NAME	FUNCTION
C3, C4, and factor B	Opsonins
C-Reactive protein	Opsonin and complement activator
Fibrinogen	Fibrin precursor and clotting factor
Kininogen	Kinin precursor
α1-Acid glycoprotein	Function unknown; possibly immunomodulatory
Ceruloplasmin and ferritin	Iron restriction
Haptoglobin	Binding free hemoglobin
Hemopexin	Binding free heme
Serum amyloid A	Lipid transporter; possible inhibitor of neutrophil function
Serum amyloid P	Lipid transporter; possible inhibitor of neutrophil function
α1-Antichymotrypsin	Protease inhibitor
α2-Macroglobulin	Protease inhibitor
Cysteine protease inhibitor	Protease inhibitor

1.1.1.2

LIPID MEDIATORS

Prostanoids are lipid mediators that regulate the inflammatory response.[14,15] The prostanoid group includes the prostaglandins, leukotrienes, and prostacyclin; these are the products of cyclooxygenase cleavage of arachidonic acid followed by endoperoxidation (Figure 1.1-2). The major sources of prostanoids in acute inflammation are phagocytes, endothelial cells, and platelets. Although prostanoids in general mediate the cardinal effects of pain, fever, and edema characteristic of the acute inflammatory response, their particular roles are confounding and can be pro- or antiinflammatory (Table 1.1-2).[16] Prostanoid production depends on the activity of the two isoforms of the cyclooxygenase enzymes within cells: COX-1, which is present in most cells and the expression of which is generally constitutive, and COX-2, the expression of which is low or undetectable in most cells but increases dramatically on stimulation, particularly in cells of the immune system. Increased COX-2 expression by inflammatory stimuli likely accounts for the high levels of prostanoids found in chronic inflammatory lesions and is the basis for the development of COX-2–specific inhibitors for treating chronic inflammatory diseases. However, studies using mice have indicated that the earliest prostanoid response to deleterious environmental stimuli depends on COX-1, and only as the inflammatory process progresses does COX-2 become the major source of prostanoids.[17] As such, recent emphases on developing specific COX-2 inhibitors as treatments for inflammatory diseases may need to be reconsidered.[18]

Figure 1.1-1 Classical and alternate pathways of complement activation (see text for explanation). *LPS,* Lipopolysaccharide.



Both COX isoforms produce prostaglandin H_2 (PGH_2), which is the common substrate for a series of specific synthase enzymes that produce PGD_2, PGE_2, PGF_2, PGI_2, and thromboxane A_2 (see Figure 1.1-2). The differential expression of these enzymes within cells present at sites of inflammation determines the profile of prostanoid production. For example, mast cells predominantly generate PGD_2, whereas resting macrophages produce thromboxane A_2 in excess of PGE_2, though this ratio changes to favor PGE_2 production after activation. Likewise, the biological effect of a prostanoid depends on binding to G protein–coupled cell-surface receptors. The receptors for PGF_2, PGI_2, and thromboxane A_2 are called FP, IP, and TP, respectively. In contrast, PGD_2 acts through two receptors, the DP receptor and the recently identified CRTH2 receptor, and PGE_2 has four subtypes of receptors, termed EP1 to EP4. The prostanoid receptors themselves are coupled to various G protein–coupled intracellular signaling pathways. The DP, EP2, EP4, IP, and one isoform of the EP3 receptor can couple to G_s and thus increase the intracellular cyclic adenosine monophosphate concentration. In 3
T cells and other inflammatory cells, coupling generally is associated with inhibition of effector cell functions. 4
By contrast, the EP1, FP, IP, and TP receptors, as well as other EP3 iso forms, couple to G_q. Activation of these receptors leads to increased intracellular calcium concentrations and immune cell activation. Finally, TP, CRTH2, and yet another EP3 receptor isoform each can couple to G_i, causing cyclic adenosine monophosphate levels to decline while mobilizing intracellular calcium. Many cells of the immune system express multiple 4
receptors that couple to these apparently opposing pathways. The array of receptors the cells express and the 5
intracellular pathways to which they are coupled determine the impact of prostanoids during an inflammatory response. Activation of these receptors, even when coupled to similar pathways, might evoke different responses because of differences in the levels of expression (constitutive and induced) or in the patterns of desensitization. The role of prostanoids in a given inflammatory response depends not only on the presence of the lipid mediators in the lesion but also on the receptor profile on immune cells and the biochemical signaling pathways of these receptors.[17] Thus PGE_2 is considered proinflammatory because it promotes vasodilation by activating cyclic adenosine monophosphate–coupled EP2 receptors on vascular smooth muscle and increases

vascular permeability indirectly by enhancing the release of histamine and other mediators from tissue leukocytes such as mast cells. Prostaglandin E_2 is also the prostanoid responsible for development of fever. As inflammation progresses, increased expression of COX-2 and prostaglandin-E synthase enhance PGE_2 synthesis by macrophages. Increased PGE_2 inhibits leukocyte activation and mast cell degranulation and relaxes smooth muscle contractions. In the lung, PGE_2 promotes bronchodilation through activation of G_s-coupled EP2 and EP4 receptors. In these situations, PGE_2 may be considered antiinflammatory.

Figure 1.1-2 Lipid mediators of inflammation (see text for explanation). *HPETE,* Hydroperoxyeicosatetraenoic acid; *HETE,* hydroxyeicosatetraenoic acid; *LTA_4,* leukotriene A_4. (From Davies P, Bailey PJ, Goldenberg MM et al: The role of arachidonic acid oxygenation products in pain and inflammation, *Annu Rev Immunol* 21:337, 1984. Reprinted with permission by Annual Reviews, www.annualreviews.org.)

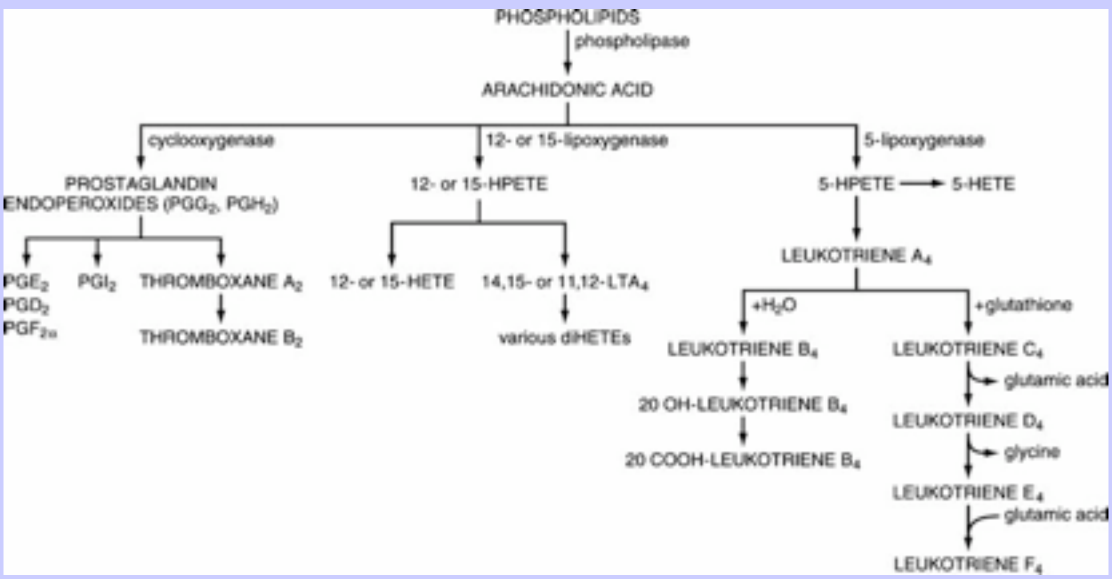


TABLE 1.1-2 Physiologic Effects of Lipid Mediators

	LIPID MEDIATOR*									
EFFECT	PGD_2	PGE_2	$PGF_{2\alpha}$	PGI_2	TXA_2	LTB_4	LTC_4	LTD_4	LTE_4	PAF
Constricts smooth muscle	X		X	X	X	X	X	X	X	X
Dilates systemic vasculature	X									
Increases vascular permeability	X			X		X	X	X	X	X
Inhibits platelet aggregation	X			X						
Aggregates platelets					X					X
Increases vasodilation									X	
Regulates arteriolar constriction and vasodilation							X	X		
Increases mucus production							X			X
Provides chemoattractant for neutrophils						X				
Inhibits leukocyte chemotaxis		X								
Relaxes smooth muscles		X								
Inhibits mediator release		X								
Stimulates mediator release						X				X

* *PGD_2,* Prostaglandin D_2; *PGE_2,* prostaglandin E_2; *$PGF_{2\alpha}$,* prostaglandin $F_{2\alpha}$; *PGI_2,* prostaglandin I_2; *TXA_2,* thromboxane A_2; *LTB_4,* leukotriene B_4; *LTC_4,* leukotriene C_4; *LTD_4,* leukotriene D_4; *LTE_4,* leukotriene E_4; *PAF,* platelet-activating factor.

1.1.1.3

CHEMOTAXIS AND LEUKOCYTE TRAFFICKING

One of the initial and most crucial aspects of the acute inflammatory response is the recruitment of leukocytes (primarily neutrophils) to the site of injury. Neutrophils constitute the first line of cellular defense and are the

initial cells involved in an inflammatory response. These phagocytic cells are derived from multipotent stem cells located chiefly in the bone marrow. Under the influence of a variety of signals provided from within and without the bone marrow, these stem cells become committed to developing into cells of the granulocyte lineage. The critical signal is provided by a family of growth factors known as *colony-stimulating factors* that provide proliferative and differentiative signals leading to the development of granulocytes and other leukocytes. Once released into the circulation, these cells must find their way to the site of the inflammatory response. The production of various chemotactic factors by host cells, bacteria, and other invaders causes various leukocytes to enter the circulation and to be carried to the site of the injury.[19] Chemokines are soluble proteins produced by host cells that induce the directional migration and activation of leukocytes, as well as other somatic cell types, and thus play a major role in the inflammatory response. IL-8 plays a central role in this process. Other chemokines promote humoral and cell-mediated immune reactions; regulate cell adhesion, angiogenesis, leukocyte trafficking, and homing; and contribute to lymphopoiesis and hematopoiesis.[20]

The specific trafficking of leukocytes from the blood to inflammatory sites depends on the production of chemotactic factors and the interaction of specific receptors on the leukocytes with corresponding adhesion molecules on the endothelial surface of the blood vessels. Neutrophil adherence is a two-step process first involving endothelial cell surface molecules known as selectins.[10] Small venular endothelium overlying a site of inflammation and exposed to thrombin, platelet-activating factor, IL-1, histamine, or other mediators released by clotting, platelet activation, or mast cell activation expresses P-selectin.[21] P-selectin mediates the process in which neutrophils initially interact with the endothelial surface. In a process known as rolling, the circulating neutrophil interacts with the endothelial cell before actual adherence.[22] Selectins bind to carbohydrate ligands present on the cell surface. In the case of neutrophils the ligand is sialylated Lewis-X antigen for the endothelial E-selectin. The second part of the adherence process is the tight binding of integrins on the neutrophil surface with intracellular adhesion molecules on the endothelial cell surface. Leukocyte integrins are heterodimeric proteins with distinct α and shared β polypeptide chains. The α and β chains can combine in different heterodimers to form multiple shared and unique specificities (Table 1.1-3). Neutrophil expression of $\alpha_M\beta_2$ and $\alpha_X\beta_2$ is activation-dependent. Neutrophils can be activated by a number of soluble proteins including formylmethionylleucylphenylalanine, N-formylated peptides present in bacterial but not eukaryotic proteins. Host factors present at the site of inflammation—notably the complement proteins (C5a and C3a), cytokines such as IL-8 and TNF, and immune complexes—also can activate neutrophils.[23] Expression of integrins by the activated neutrophil allows tethering to the endothelial surface. The migration of neutrophils through the vascular wall is less well understood than these initial events leading to firm adhesion. The β_2 integrins—as well as $\alpha_V\beta_3$, platelet/endothelial cellular adhesion molecule-1, and integrin-associated protein—appear to play a role in this process. Endothelial cell–produced IL-8 also is believed to have a critical role in this process. Once through the endothelium, phagocytes may adhere to other cells during migration to the site of inflammation. These interactions also depend on $\alpha_M\beta_2$ and $\alpha_X\beta_2$ integrins. Migration through the extracellular matrix is mediated by $\beta_1\beta_3$ and β_5 integrins recognizing specific protein ligands.

Neutrophils recruited and activated in this manner actively phagocytose microscopic invaders and attempt to destroy them using reactive oxygen products generated via an NADPH-oxidase-dependent “respiratory 5
burst.”[22,24] In this process, neutrophils release additional proinflammatory mediators, amplifying this response. 6
Among those cells attracted to the area are *natural killer* cells capable of lysing virus-infected and other abnormal cells. The production of interferon-α and interferon-β by macrophages and other cells enhances the cytolytic activity of the natural killer cells, which can be the source of interferon-γ, another proinflammatory cytokine. Depending on the magnitude of the initial insult and the susceptibility of the invader to neutrophil-mediated destruction, the inflammatory response may be acute or chronic.

TABLE 1.1-3 Integrins and Their Ligands

INTEGRIN PROTEINS	EXTRACELLULAR MATRIX PROTEINS	CELL SURFACE*
β_1 SUBFAMILY		
$\alpha_1\beta_1$	Collagen, laminin	
$\alpha_2\beta_1$	Collagen, laminin	
$\alpha_3\beta_1$	Collagen, laminin, fibronectin	
$\alpha_4\beta_1$	Fibronectin	VCAM-1, MAdCAM-1
$\alpha_5\beta_1$	Fibronectin	
$\alpha_6\beta_1$	Laminin	
$\alpha_7\beta_1$	Fibronectin, vitronectin	
β_2 SUBFAMILY		
$\alpha_L\beta_2$		ICAM-1, ICAM-2, ICAM-3
$\alpha_M\beta_2$	C3bi, factor X, fibrinogen	ICAM-1
$\alpha_X\beta_2$	C3bi, fibrinogen	ICAM-1 (?)
$\alpha_D\beta_2$		ICAM-3
β_3 SUBFAMILY		
$\alpha_V\beta_3$	Vitronectin, fibronectin, von Willebrand factor, finbrinogen, laminin, thrombospondin	PECAM-1
β_4 SUBFAMILY		
$\alpha_6\beta_4$	Laminin	
β_7 SUBFAMILY		
$\alpha_4\beta_7$	Fibronectin	MAdCAM-1, VCAM-1
$\alpha_E\beta_7$	E-cadherin	

* *VCAM*, Vascular cell adhesion molecule; *MAdCAM*, mucosal addressin cell adhesion molecule; *ICAM,* intracellular adhesion molecule; *PECAM*, platelet/endothelial cellular adhesion molecule.

Acute inflammation is a rapid response to an injury, characterized by accumulation of fluid, plasma proteins, and neutrophils that rapidly resolves once the initial inflammatory stimulus is removed. Deactivation signals include PGE_2, cortisol, IL-10, and transforming growth factor β (TGF-β). Some of those chemotactic agents responsible for initiating the response (IL-8, N-formyl-methionyl-leucyl-phenylalanine, C5a, leukotriene B4, and platelet-activating factor) also serve to downregulate its intensity by inducing the shedding of IL-1 receptors from neutrophils.[25] The shedding of this decoy receptor may have antiinflammatory effects as it

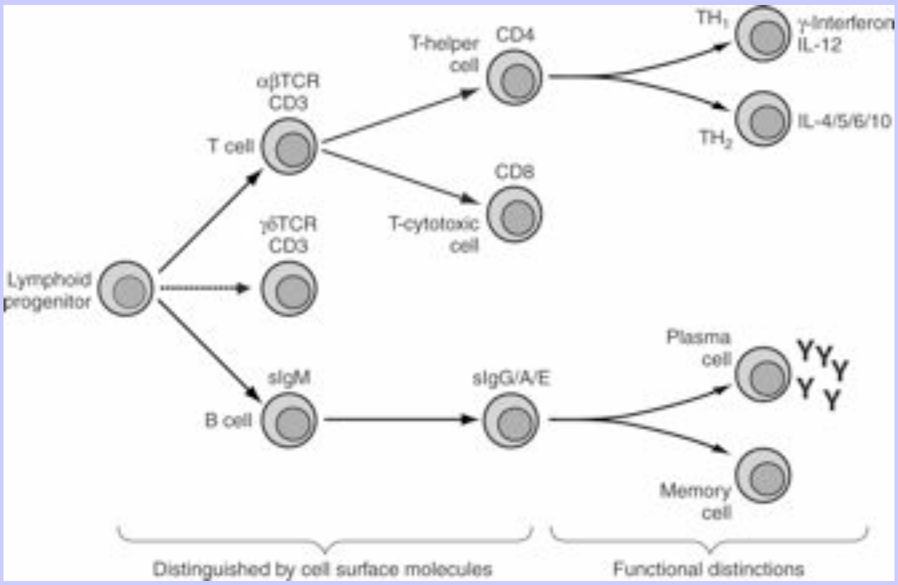
effectively binds and neutralizes this cytokine. Likewise, many acute phase proteins may have immunomodulatory activity, downregulating neutrophil function.[26] Acute inflammatory responses often may be subclinical and resolve without complications. However, if the invader is resistant to neutrophil-mediated destruction or the degree of injury is great, the response may become more chronic with the added recruitment of macrophages and lymphocytes and growth of fibroblasts.

The essential characteristic of the innate immune response is that it does not exhibit specificity for the invading organism. The induction of an innate immune response does not require prior exposure to the invading organism and is not augmented by repeated exposure to the same organism. Although resistance may be controlled genetically, the genes encoding resistance are not found within the gene complex that controls adaptive immune responses. In most instances, these mechanisms are adequate for eliminating casual invaders. However, pathogenic organisms have evolved various methods for avoiding elimination. In response to these organisms, the specialized cells and products of the adaptive immune response are mobilized.

1.1.2 Adaptive Immunity

The adaptive immune response follows an encounter with a foreign agent and depends on antigen-specific 6
immune responses mediated by different divisions of the lymphocyte family (Figure 1.1-3). In contrast to the 7
nonspecific nature of the innate immune response, an important characteristic of the adaptive immune response is its specificity. Exposure of the host to a particular microbe or parasite results in the induction of immune responses that are directed against specific components of the invading organism that do not affect unrelated organisms. The specificity of the adaptive immune response results from the interaction of specific molecular structures or *antigens* of the invader with antigen-specific receptors on lymphocytes. All types of chemical structures can serve as antigens; however, not all antigens can induce an immune response. *Immunogens,* those antigens that can stimulate an immune response, are usually chemically complex molecules of high molecular weight. Proteins, nucleic acids, lipids, and polysaccharides all can serve as immunogens. Large immunogens such as proteins contain multiple *antigenic determinants* or *epitopes* that interact with lymphocytes via their antigen-specific receptors. *Haptens* consist of single antigenic determinants and can combine effectively with the binding site of antibody molecules. However, because they consist only of a single antigenic determinant, they cannot cross-link B cell receptors (antibody molecules) and they are also unable to stimulate T cell responses. Haptens therefore cannot stimulate an immune response unless multiple haptens are attached physically to a larger molecule known as a *carrier*. Though these distinctions between antigens, haptens, and immunogens appear minor, they provide the underlying basis for understanding many allergic and autoimmune responses.

Figure 1.1-3 Major divisions of the lymphocyte family. To the left of the diagram different populations of lymphocytes are distinguished by expression of different cell surface molecules. To the right of the diagram the distinctions are functional.

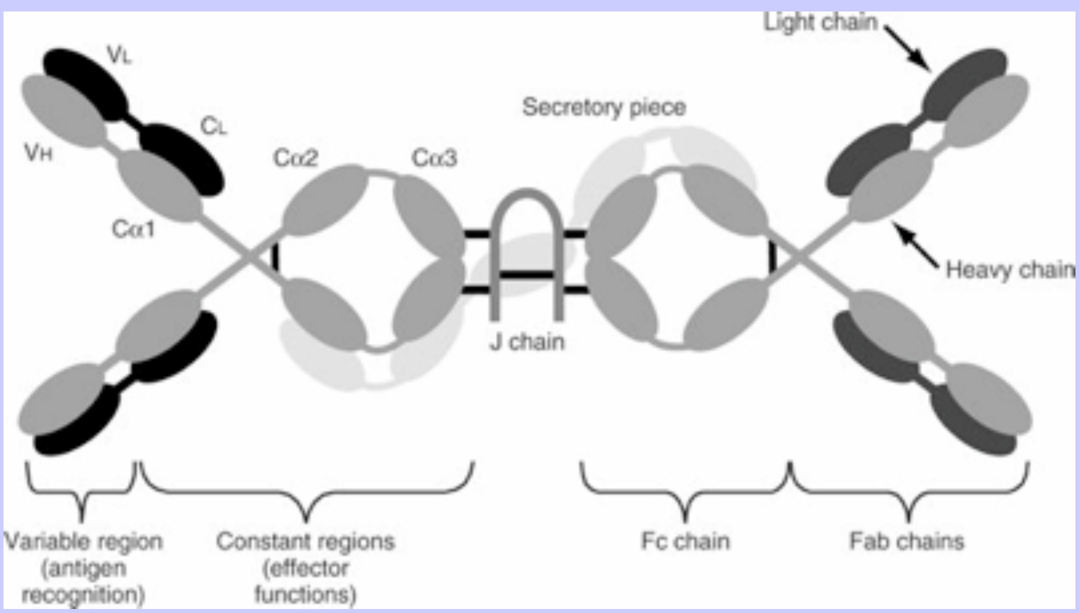


Like the innate response, the adaptive immune response to a specific antigen consists of humoral and cellular effector mechanisms. The humoral component is mediated by *immunoglobulins* or *antibodies* found in plasma and tissue fluids. Antibodies are produced by B lymphocytes, small lymphoid cells characterized by the cell surface expression of immunoglobulin molecules. B cells represent less than 15% of circulating peripheral blood mononuclear cells but are present in higher proportions in lymph nodes and spleen. B cells are derived from the fetal liver and bone marrow of mammals and the bursa of Fabricius of birds. In the bone marrow, B cells are the products of a putative lymphoid stem cell derived from the pluripotent stem cell. Under the influence of various cytokines produced by bone marrow *stromal* cells, the B cell precursor undergoes its 3-day development into a mature B cell. On stimulation with specific antigen, B cells differentiate into *plasma cells* that produce enormous quantities of specific antibody. The activation, proliferation, and differentiation of B lymphocytes into plasma cells depends on other cells, including T lymphocytes, which represent the cellular component of the adaptive
immune response. The T lymphocyte also is derived from the multipotent stem cell and lymphoid precursor in the 7
bone marrow, though its subsequent development into the mature T cell occurs in the *thymus*. Within the thymic 8
environment, prothymocytes undergo a developmental and selective process while emigrating through the cortex into the medullary region of the thymus. Less than 3% of all immature thymocytes in the cortex survive to become peripheral T cells.

Although the induction of an antibody response requires the interaction of B and T lymphocytes, these cells recognize different epitopes on the same antigen. Indeed, antigen recognition by B cells and T cells is fundamentally different. B cells and antibodies recognize antigens in solution or on cell surfaces in their native conformation, whereas T cells only recognize antigen in association with self-molecules known as *major histocompatibility complex* antigens found on most cell surfaces. The adaptive immune response thus differs from

innate immunity in that it is antigen driven. Those cells that mediate the adaptive immune responses, T and B lymphocytes, express specific receptors for the antigen. Because the immune system responds to antigens of live and killed pathogens, stimulating immunity without causing infection is possible and is the basis of vaccination. Although this principle appears to be straightforward, vaccination does not always yield the expected result. Why some vaccines work and others fail is a complex issue, a major component of which is the nature of the antigen-specific receptors of lymphocytes.

Figure 1.1-4 Molecular structure of secretory immunoglobulin A. This schematic illustrates the major features of immunoglobulin molecules. Although the illustrated IgA molecule is dimeric, with the two immunoglobulin units joined by a J chain and a series of disulphide bonds, IgG molecules are monomeric. Each immunoglobulin unit consists of two heavy chains and two light chains. The heavy chains have four subunits, and the light chains have two. One end of the immunoglobulin unit has a highly variable protein structure and is involved in antigen recognition, whereas the remainder of the immunoglobulin unit has a constant structure in each immunoglobulin class and subclass. This structure determines the functional characteristics of the molecule, such as binding complement or recognition by macrophages or neutrophil Fc receptors. This specialized dimeric IgA molecule also has a secretory piece that increases its stability in the harsh mucosal environment.



1.1.2.1 IMMUNOGLOBULIN: ANTIGEN-SPECIFIC RECEPTOR OF B LYMPHOCYTES

The antigen-specific receptor of the B cell is cell surface–bound antibody. An antibody molecule is composed of two identical light chains and two identical heavy chains that form a disulfide-linked Y-shaped molecule (Figure 1.1-4). The light chain can be divided into two domains, a conserved carboxy-terminal domain and a highly variable amino-terminal domain. Analysis of heavy chains reveals a similar domain structure with the amino-terminal domain being highly variable and the presence of three constant domains. The antigen-binding region of an antibody molecule is formed by the association of the amino ends of a light and a heavy chain, whereas the carboxyl end of the heavy chain determines the isotype of the molecule. Five different *isotypes* of antibody molecules have been identified in most species: immunoglobulin D (IgD), IgM, IgG, IgA, and IgE (Table 1.1-4), although evidence for the existence of IgD in horses currently is lacking.[27] Additionally, the IgG 8
isotype can be subdi vided into subclasses based on physiochemical properties. Restriction analysis of equine 9
genomic DNA has indicated the existence of one IgE, one IgA, and up to six IgG genes.[28] Four IgG subclasses have been previously identified serologically as IgG(a), IgG(b), IgG(c), and IgG(T).[29] The IgG(a) is tentatively identified as the first IgG gene, IgG(T) as the third IgG gene, and IgG(b) as the fourth IgG gene.[28,29a] The serological identification of the remaining genes has not yet been accomplished.

TABLE 1.1-4 Immunoglobulin Isotypes

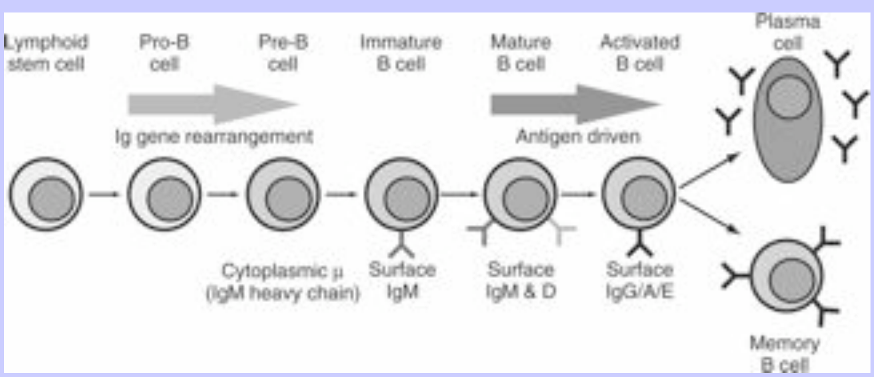
ISOTYPE	IMMUNOLOGICAL FUNCTION
IgD	Antigen receptor of naïve B lymphocytes. Currently no IgD heavy chain gene has been identified in the horse.
IgM	Surface IgM is found on naïve, activated, and memory B cells. Secreted IgM is a pentamer and represents the major antibody produced during a primary response. IgM efficiently mediates agglutination, neutralization, opsonization, and complement activation.
IgG	IgG is the principle immunoglobulin found in plasma, representing up to 80% of the total immunoglobulin concentration. Various subclasses of IgG have been identified (see text). Four IgG subclasses occur in the horse (IgGa, IgGb, IgGc, and IgG[T]) as defined by current monoclonal antibodies, although six IgG heavy chain genes have been identified.[29a] The major functions of IgG include opsonization and neutralization reactions. IgGa and IgGb are effective in fixing complement and participate in antibody-dependent cellular cytotoxicity, whereas IgGc and IgG(T) are not effective, although they appear to play an important role in exotoxin neutralization and immunity to parasites.
IgA	IgA, the most abundant antibody in secretions (tears, mucus, saliva, colostrum, etc.), is a dimer composed of two IgA molecules joined by a J chain. IgA in the plasma is predominantly monomeric. IgA antibodies can be neutralizing but only activate complement via the alternative pathway.
IgE	Most IgE is found associated with the surface of mast cells and basophils and only small amounts are present in the plasma. The cross-linking of two IgE molecules with specific antigen results in the degranulation of the mast cells and basophils. Thus IgE is the primary antibody responsible for type I hypersensitivity reactions and appears to play a central role in immunity to parasites.

Membrane-bound IgM and IgD serve as the antigen-specific receptors for B lymphocytes. Each contains a membrane spanning region near its carboxy end that is inserted into the messenger RNA during differential splicing of the heavy chain exons. Although rarely detectable in the circulation, IgD is present in large quantities on the surface of naïve B lymphocytes. Following activation, the surface expression of IgD is lost, though the cell may continue to express the membrane form of IgM. Early in an immune response the B cell secretes large amounts of the pentameric form of IgM. As the immune response proceeds, the B cell switches the isotype of its heavy chain. Isotype switching involves the substitution of one heavy chain constant region for another. The genes encoding the five different constant regions of the heavy chain are sequentially arranged on the chromosome (Cδ, Cμ, Cγ, Cε, and Cα). Initially, the first two constant region genes encoding the δ and μ constant regions are used to form the heavy chain. The 5′ region of each constant region gene segment contains repetitive regions of DNA known as *switch sequences*.[30] The switch sequences appear to play a role in this rearrangement and may serve as the target for specific recombinases. When switching occurs, a new constant region segment is selected and the intervening genes are removed by splicing or looping out. Isotype switching only affects the heavy chain constant domains and has no effect on the antigen specificity of the immunoglobulin molecule. The signals for B cells to undergo isotype switching are provided by T lymphocytes in the form of various cytokines.[31] For example, IL-4 induces isotype switching to the IgE isotype, whereas interferon-γ blocks this induction and augments IgG production.[32,33] IgA is produced in response to the combination of the cytokines IL-4, IL-5, and TGF-β.[34]

The combination of the variable domains of the light and heavy chains determines the antigen specificity of a particular antibody molecule (and the B cell that produces it). The association of these two domains results in the formation of an antigen-binding groove or pocket that contains regions of hypervariability that define the specificity of a particular antibody molecule. More than 1×10^8 different antibody specificities have been estimated to be possible. The generation of this tremendous diversity in antibody specificity occurs during B cell ontogeny in the bone marrow.[35] Within a given B cell the genes encoding the heavy and light chains of an anti body molecule are organized into specific gene segments. Thus the light chain is formed from variable (V_l), joining (J_l), and constant (C_l) gene segments that together form the variable and constant domains of the light chain. In the germ line of an undifferentiated cell are several hundred different V_l and several dozen J_l gene segments. Likewise, the heavy chain of a B lymphocyte is composed of V_H, diversity (D), and J_H segments that form the variable domain. These join to the constant region genes to form the complete heavy 9
chain molecule. Similarly, in the germ line are a large number of V_H gene segments and a smaller number of D 10
and J_H segments. During the differentiation of a B cell (Figure 1.1-5), sequential selection and rearrangement of a V_L segment with a J_L segment occurs with the accompanying deletion of intervening V_L and J_L segments (Figure 1.1-6). The rearranged VJC sequence is transcribed into mRNA and translated into the light chain. A similar sequence follows for heavy chains except that two rearrangements are necessary, a D to J_H rearrangement followed by a V_H to DJ_H rearrangement. Once rearrangement is completed, the VDJ segment is brought into proximity of the appropriate C_H segment and transcribed. Not all of the gene segment rearrangements produce functional genes. Because a B cell has two sets of heavy chain genes, one on each chromosome, and most species, including the horse, have two different sets of light chain genes,[36,37] several chances exist to form appropriate heavy and light chains. Once the heavy and light chain gene segments are recombined successfully, neither do the genes on the sister chromosome recombine nor are they expressed. This process of *allelic exclusion* ensures that the B cell produces antibodies of a single specificity. Although this random assortment of gene segments accounts for much of the diversity in antibody specificity, additional mechanisms also are involved, including *junctional diversity,* which results from the imprecise joining of gene

segments, and *somatic mutations,* which are point mutations in the hypervariable region of the heavy or light chain that occur during the proliferation of antigen-activated B lymphocytes. Such mutations appear to play a role in increasing antibody affinity for its antigen. Thus fewer than 1000 genes can give rise to more than 1 × 10^{8} molecules of the various specificities needed to recognize the vast number of antigens the host may encounter.

Figure 1.1-5 B cell differentiation. Different stages of B lymphocyte development are recognizable by expression of immunoglobulin molecules. This maturation requires a series of gene rearrangements to select the genes that will encode the antigen-binding part of the immunoglobulin molecule (variable region) and subsequently to select the genes that determine the class or subclass of the antibody molecule. Initially, immature B cells express immunoglobulin M (the majority of peripheral blood B cells), but after antigen exposure the B cell becomes activated and may express any of the immunoglobulin classes or subclasses. This determination depends largely on cytokine signals from T helper cells. Finally, activated B cells mature into short-lived antibody secreting plasma cells or become long-lived memory B cells.

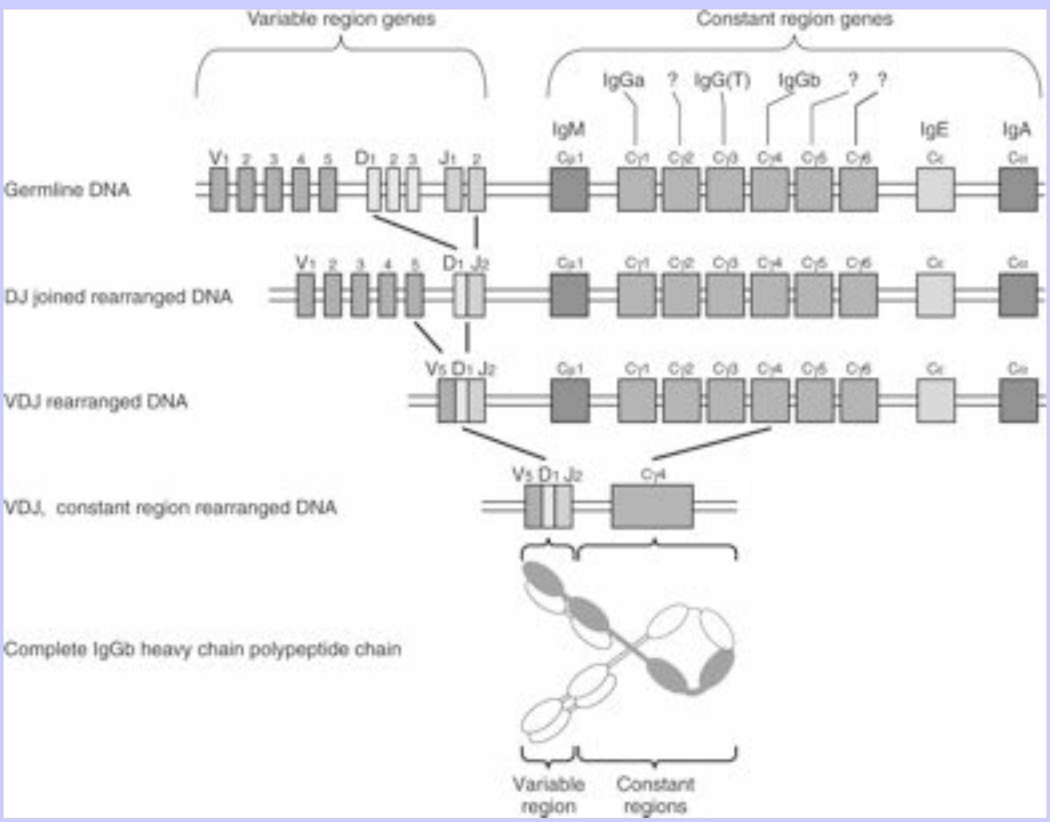


1.1.2.2 T CELL RECEPTOR AND CD3 COMPLEX: ANTIGEN-SPECIFIC RECEPTOR OF T CELLS

T lymphocytes can be differentiated from B lymphocytes in that they do not express surface immunoglobulins but instead express the T cell receptor (TCR). T cells also express another antigen called CD3. (The designation *CD* stands for *cluster designation* and resulted from an international workshop to standardize the terminology used to describe leukocyte surface antigens recognized by monoclonal antibodies.) The TCR and CD3 form a multimeric complex on the T cell surface, and this complex is involved in antigen-specific recognition.[38] The

TCR structure was first identified using antibodies that recognized a surface antigen expressed on a cloned T
lymphoma cell line. This antibody recognized a disulfide-linked heterodimer composed of an acidic (α) and a
basic (β) protein of 40,000 to 45,000 molecular weight. Similar heterodimers were found on a variety of
antigen-specific T cell lines, but not on B cells. Peptide mapping studies of the α and β chains from many differ
ent T cell lines demonstrated that they contained variable and constant domains reminiscent of immunoglobulin
structure. Further analysis indicated that like immunoglobulin genes, the TCR genes underwent gene
rearrangements during T cell development. Subsequently, two additional TCR genes were identified, the γ 10
chain and δ genes corresponding to a second heterodimer. Thus two TCRs exist, an α/β heterodimer that 11
constitutes the TCR on almost 90% of all T cells and a γ/δ-heterodimer present on approximately 10% of the
peripheral T cells. The significance of these two different TCR heterodimers has not yet been determined. One
should note that γ/δ T cells have not yet been identified in the horse. Clearly, γ/δ T cells represent a
functionally distinct populations of T cells typically associated with mucosal surfaces.[39] As such, they are
thought to play an important role in immunological surveillance.

Figure 1.1-6 Immunoglobulin gene rearrangement–somatic recombination process for production of an immunoglobulin heavy chain. The figure shows a hypothetical series of V, D, and J variable heavy chain genes, positioned 5′ to the known equine heavy chain constant region gene loci. In the first step in somatic recombination a D and a J gene segment are joined, and in the second step a V gene segment is joined to complete the VDJ recombination and form a gene capable of encoding the variable region. Subsequently, one of the six equine γ heavy chain constant regions, labeled with their corresponding immunoglublin G subclass when known, was selected to complete the gene rearrangement. Because the Cγ4 heavy chain constant region gene was selected, this leads to production of an IgGb heavy chain.



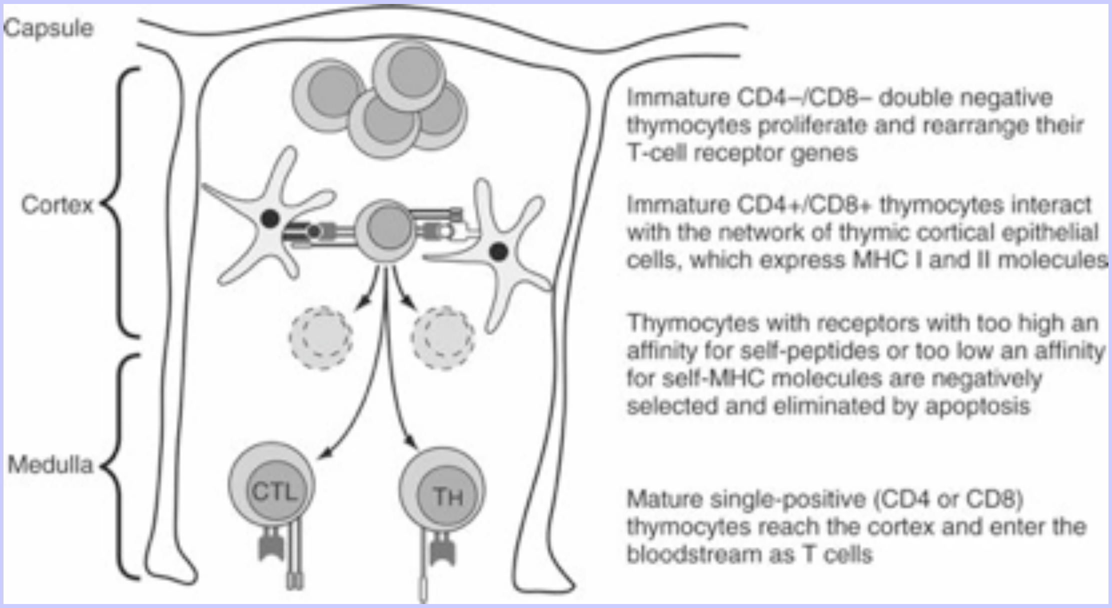
Analysis of the predicted amino acid sequences for the TCR proteins confirms a structural similarity with antibody molecules. One peculiarity in the structure of the TCR occurs in the amino acid sequence analysis. Although the α and β chains of the TCR contain a transmembrane region, both proteins have short cytoplasmic tails. That TCR itself could transmit any cytoplasmic signal in response to antigen binding therefore seems unlikely. This led to the search for other proteins associated with the TCR. Solubilization of the T cell membranes revealed that five other proteins could be immunoprecipitated with the TCR. Similar results were obtained when anti-CD3 antibodies were used. Thus the TCR heterodimer is associated noncovalently with the CD3 complex of proteins. The five proteins of the CD3 complex (γ, δ, ε, ζ, and ξ) are involved in signal transduction following TCR binding to antigen.[40] Unlike the TCR α and β proteins, the CD3 proteins have 11
large intracellular domains, some of which are phosphorylated in response to stimulation of the TCR. In 12
addition to providing a signaling mechanism for the TCR, the CD3 complex also is required for expression of the TCR heterodimer on the cell surface.[38]

The generation of diversity in the TCR during T cell ontogeny involves a mechanism that is similar to that used to generate immunoglobulin diversity. The TCR α and γ chains resemble immunoglobulin light chains in that they are composed of V, J, and C gene segments. The particular V, J, and C segments used are selected from a germ line configuration containing a few (C region) to several hundred (V region) gene segments. The selection and rearrangement of the gene segments is similar to that used by the immunoglobulin light chain and appears to involve the same recombinase. Likewise the β and δ chains resemble heavy chains, each being composed of V, D, J, and C gene segments, and their selection and rearrangement from germ line genes also parallels immunoglobulin heavy chain rearrangement. Thus the generation of diversity results from the combination of multiple gene segments and junctional diversity. However, unlike immunoglobulins, the TCR genes do not undergo somatic mutations.

1.1.2.3 T LYMPHOCYTE SUBSETS

Mature thymocytes and T lymphocytes can be divided further into two distinct populations based on their expression of the CD4 or CD8 antigen.[41] The expression of these antigens is correlated directly with the specificity of the T cell. The expression of CD4 or CD8 also correlates with the function of the T cell. Those cells that express the CD8 antigen are typically cytotoxic effector cells (cytotoxic T lymphocytes, or CTLs), whereas those that express the CD4 antigen are typically helper cells that produce cytokines to enhance antibody and cell-mediated immune responses. Although T lymphocytes in the periphery express CD4 or CD8 antigen, cortical thymocytes express both antigens. During the process of thymic selection, these cells convert to $CD4^+$ or $CD8^+$ cells or they are eliminated (Figure 1.1-7). At this stage of their development T cells are said to “learn” to recognize antigen. Also at this stage autoreactive T cells are eliminated. Although experimental studies have shown that positive and negative selection of T cells occurs, the exact mechanism of these selective processes remains unknown. Interestingly, although T cells expressing the α/β heterodimer of the TCR can be $CD4^+$ or $CD8^+$, γ/δ cells are $CD8^+$ or $CD4^-CD8^-$. These results suggest that the γ/δ cells undergo a different developmental process than α/β cells. Like the CD3 complex, CD4 and CD8 antigens are involved in the intracellular signaling event following TCR engagement with its specific antigen. Unlike B cells and antibodies that recognize antigens in solution or on cell surfaces in their native conformation, T cells only recognize *processed* antigen in association with self-molecules known as major histocompatibility complex (MHC) antigens.

Figure 1.1-7 Thymic development.



1.1.2.4 MAJOR HISTOCOMPATIBILITY ANTIGENS AND ANTIGEN PRESENTATION

The MHC originally was defined in terms of its role in allograft rejection. Following rejection of a primary allograft, antibodies that react with the allograft can be found in the recipient's sera. These antibodies can be used to identify or type tissues to determine the suitability of a donor for transplantation. Multiparous females 12
have similar antibodies in their sera because of exposure to paternal MHC antigens on the fetus.[42] Using these 13
sera, one can identify a large number of serologically defined transplantation antigens. Genetic analysis of the MHC region demonstrates that a number of closely linked genes encode several different, though related, antigens that are involved in allograft rejection. These closely related genes are referred to collectively as MHC I genes and their products as MHC I antigens. In addition to the serologically defined MHC I antigens, another group of antigens was identified within the MHC that are involved in the stimulation of mixed lymphocyte responses and the control of immune responsiveness. These MHC II antigens are structurally and functionally distinct from the MHC I antigens, except that both are involved in T cell recognition of antigen.

MHC I antigens are cell surface glycoproteins consisting of two noncovalently associated proteins, an MHC-encoded transmembrane protein of approximately 44-kd (α chain) and a β_2-microglobulin, a 12-kd protein encoded outside of the MHC.[43] MHC I antigens are expressed on the surface of most nucleated cells. The highest level of expression is on lymphoid cells, with lower expression on fibroblasts, muscle cells, and neural cells. MHC I antigens are not detectable on early embryonal cells, placental cells, and some carcinomata. The level of expression of MHC I antigen can be modified by treatment with cytokines or infection with viruses. Interferons and TNF-α augment MHC I antigen expression. This augmented expression results from increased production of MHC I mRNA. The regulatory region of the MHC I antigen genes contains interferon and TNF-α responsive elements that control the transcriptional activity of these genes.

The MHC I region of most animal species, including the horse, contains a number of MHC I α chain genes, some of which are pseudogenes and are not expressed.[44] In the horse these genes are located on chromosome 20, and those genes that are expressed exhibit a great deal of polymorphism.[45,46] Much of this polymorphism is localized in the α_1 and α_2 domains, the α_3 domain being more conserved. The polymorphism of these two domains is related to their role in presenting antigen to T cells.

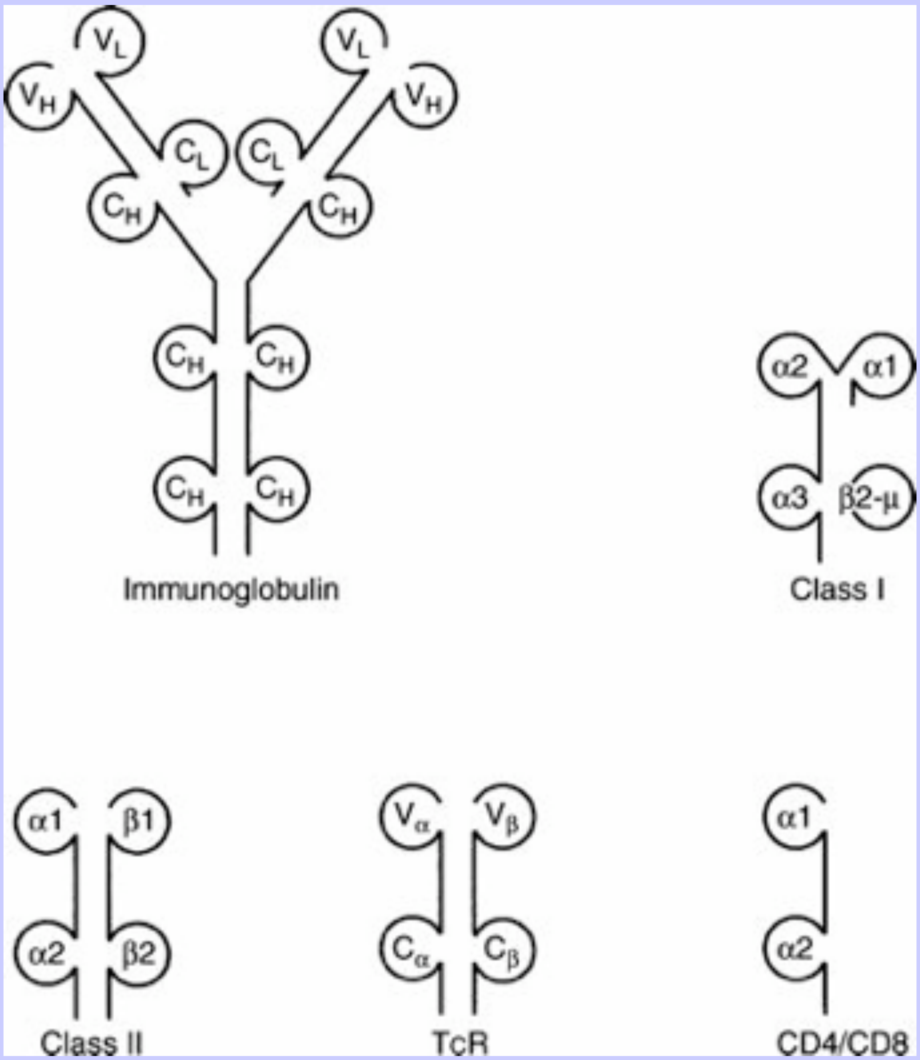
The physiologic role of MHC I antigens was defined when cytotoxic T cell (CTL) lysis of virus infected cells was discovered to be restricted to target cells expressing the same MHC I antigen as the CTL.[47] This observation led to the realization that T cells recognize the combination of self-MHC and foreign antigen. Furthermore, those T cells that recognize MHC I antigens invariably express the CD8 co-receptor. The nature of the association between MHC I and the foreign antigen remained unclear until x-ray crystallographic studies of human MHC I antigen were performed. In addition to revealing the structural organization of the domains of the MHC I antigen, the image also revealed a cleft that lay between the α_1 and α_2 domains. Researchers proposed that this cleft binds the processed peptide epitopes for presentation to the T cell receptor. Indeed, the cleft of the crystallized protein used for x-ray diffraction studies contained a contaminating peptide.[48] Other experiments showed that the incubation of cells with purified viral peptides resulted in the lysis of the cells by virus-specific, MHC I–restricted CTLs. Together these results support the notion that *endogenous processing* of viral antigens leads to the association of the viral peptides with MHC I antigens on the surface of the infected cell, and this is recognized by the TCR-CD3 complex in association with CD8.[49] These viral antigens get to the cell surface by a peptide transport system the function of which is to transport processed peptides from the cytosol to the endoplasmic reticulum.[50] Once in this compartment, peptides are handed off to newly formed MHC class I molecules and they stabilize a trimolecular complex with β_2-microglobulin. This complex is transported to the cell surface, where antigen presentation occurs. Because this is a normal cellular process for eliminating degraded proteins from the cell, MHC I antigens are normally loaded with these self-peptides. Indeed, this encounter with MHC I loaded with self-peptides in the thymus is responsible for the deletion of autoreactive clones during T cell ontogeny.

MHC II antigens are heterodimeric, transmembrane glycoproteins composed of an acidic α chain (25 to 35 kd) and a basic β chain (25 to 30 kd).[51] A third chain, the invariant chain, is associated with the MHC II antigen during assembly in the endoplasmic reticulum but is not expressed on the cell surface. The α and β polypeptides are encoded within the MHC region. Both polypeptides possess two extracellular domains. The α chain has a single disulfide bond located in its membrane proximal (α_2) domain, whereas the β chain has a disulfide bond in both of its extracellular domains. Structurally, MHC II antigens resemble MHC I antigens and are also members of the immunoglobulin superfamily, a group of proteins with structural similarities to immunoglobulin molecules (Figure 1.1-8).[52]

The MHC II genes are functionally and structurally distinct from the MHC I genes. Unlike MHC I antigens, the MHC II antigens are restricted in their expression to certain cells of the immune system: B lymphocytes, dendritic cells, macrophages, and activated T lymphocytes of some species. Other cells may express MHC II antigens after treatment with various cytokines.[53–55] Interferon-γ, TNF-α, 1,25-dihydroxyvitamin D_3, and granulocyte-macrophage colony-stimulating factor can induce MHC II antigen expression on monocytes, macrophages, and other cells. IL-4 enhances MHC II antigen on B cells. A number of agents have been shown 13
to downregulate MHC II antigen expression including glucocorticoids, prostaglandins, and α-fetoprotein. 14
Although MHC II antigen expression also is regulated at the transcriptional level, no interferon or TNF-α response elements have been identified in the regulatory regions of MHC II genes. In fact, the regulatory region

of MHC I and MHC II genes are different, and this fact is probably responsible for the differences in tissue distribution for these antigens.[43]

Figure 1.1-8 The immunoglobulin superfamily. Immunoglobulin serves as the prototype model for the superfamily. The heavy and light chains of an immunoglobulin molecule can be divided into variable (V_H and V_L) and constant domains (C_H and C_L). Analogous regions have been identified on a variety of other molecules involved in immune recognition including class I and II antigens, the T cell antigen receptor *(TCR),* and the CD4 and CD8 antigens found on T cells (see text). Disulfide bonds forming the domains are not shown.



Like the MHC I genes, the MHC II region contains genes for multiple MHC II antigens, some of which appear to be pseudogenes and are not expressed. Those α and β chains that are expressed exhibit a high degree of allelic variability, though typically the β chain exhibits the most polymorphism. Unlike the MHC I genes the variability in the MHC II genes results from point mutations. Correspondingly, less polymorphism occurs in the MHC II genes compared with the MHC I genes. The MHC II region of other species, including the horse, have been studied using human DNA probes and extensive polymorphism involving several genes has been identified.

Although antigens processed via the endogenous pathway are associated with MHC I antigens, antigen processed via the *exogenous pathway* is associated with MHC II antigens (Figure 1.1-9).[49] Endocytosed antigen, such as that phagocytosed by a macrophage, is partially degraded in a prelysosomal compartment of low pH and limited proteolytic activity. The processed protein associates with a peptide binding site at the junction of the α_1 and β_1 domains of the MHC II molecule. This association of the epitope with the MHC II molecule protects it from further degradation. The MHC II molecule then is reexpressed on the cell surface for subsequent presentation to the T cell. The immune system contains a distinct group of antigen-presenting cells called dendritic cells that are specialized to capture antigens and initiate T cell immunity. They move freely from epithelial surfaces to adjoining lymph nodes.[56] Dendritic cells can be found in a variety of locations in the body and often are named based on their microscopic appearance. Hence interdigitating cells found in lymph nodes, veiled cells in lymphatics, and Langerhan's cells in skin are dendritic cells. Immature dendritic cells can take up antigens by micropinocytosis using their extensive cellular processes or receptor-mediated phagocytosis. This results in activation and migration to a regional lymph node where antigen presentation to T lymphocytes occurs. Mature dendritic cells have high levels of MHC II expression on their surface and are no longer phagocytic but are efficient stimulators of MHC I- and MHC II-restricted T cell responses in the draining lymph node (Figure 1.1-10).

In a complex immunogen, certain antigenic determinants are particularly effective at stimulating an antibody response. These *immunodominant* epitopes often are located at exposed areas of the antigen such as in polypeptide loops. These types of structures often are mobile and may allow for easier access to the antibody binding site. T cell epitopes possess a particular structural characteristic resulting in the formation of amiphipathic helices. However, structure alone does not determine the immunogenicity of a particular antigen. T cell recognition of foreign antigen requires more than just the expression of the processed antigen on the surface of the antigen presenting cell. Additional signals provided by the antigen presenting cell are required for the activation of the T lymphocytes. Among these are signals provided by other accessory molecules found on the antigen presenting cell and various cytokines present in the extracellular environment.

1.1.2.5 SIGNALING THROUGH THE ANTIGEN-SPECIFIC RECEPTORS

The encounter of specific antigen by a T cell or a B cell antigen-specific receptor results in an intracellular 14
signaling cascade that eventually leads to the production of various proteins and the proliferation of the 15
stimulated cell. T cell recognition of antigen involves the engagement of a TCR-CD3-CD4 or TCR-CD3-CD8 complex with processed peptide in cleft of an MHC II or MHC I molecule (Figure 1.1-11).[57] The engagement of the TCR-CD3 complex with the appropriate MHC antigen–containing peptide results in the binding of CD4 or CD8, depending on the MHC antigen, with the TCR-CD3 complex. In doing so, the *lck* protein—tyrosine kinase, which is associated with the cytoplasmic tail of CD4/CD8—phosphorylates the cytoplasm of the CD3 proteins in regions known as *immunoreceptor tyrosine-based activation motifs* (Figure 1.1-12). These motifs serve as docking sites for other kinases including ZAP70 and *fyn*. Recruitment of ZAP70 to CD3 results in its

subsequent phosphorylation and activation by *lck*. Once activated, the ZAP70 subsequently can phosphorylate other signal proteins including phospholipase C. Activation of phospholipase C leads to the cleavage of phosphatidylinositol bisphosphate into inositol 3-phosphate (IP_3) and diacylglycerol. IP_3 and diacylglycerol are second messengers. IP_3 causes release of stored Ca^{2+} from the endoplasmic reticulum; diacylglycerol activates protein kinase C. The increase in intracellular Ca^{2+} levels and activation of protein kinase C leads to phosphorylation of various transcriptional factors. These transcriptional factors regulate the expression of the genes for various cytokines and their receptors (Figure 1.1-13). The process subsequently is downregulated by various phosphatases that are recruited to and subsequently dephosphorylate the CD3 immunoreceptor 15
tyrosine-based activation motifs. A similar process occurs in B cells when the surface immunoglobulin receptor 16
is cross-linked on binding to specific antigen.

Figure 1.1-9 Antigen processing pathways. This figure depicts major histocompatibility complex I (MHC I) antigen presentation to the left of the diagram, and MHC II antigen presentation to the right. In MHC I antigen presentation, *(a)* peptides generated by degradation of proteins in the cytoplasm are transported into the endoplasmic reticulum *(b)*. In this location MHC I molecules bound by the membrane protein calnexin bind the peptides, which allows release of the MHC I molecules by the calnexin and transport through the Golgi complex to the cell surface (*c*). In MHC II antigen presentation, antigen is taken up by phagocytosis *(1)* into the endosome compartment and routed to lysosomses for degradation. Vesicles containing MHC II molecules produced in the endoplasmic reticulum fuse with the endosomes *(2),* and the MHC II molecules bind with the degraded peptides for transport back to the cell surface *(3)*. The MHC II molecules are prevented from binding the endogenous peptides in the endoplasmic reticulum by the presence of invariant chain that is only lost in the acidic endosomal environment.

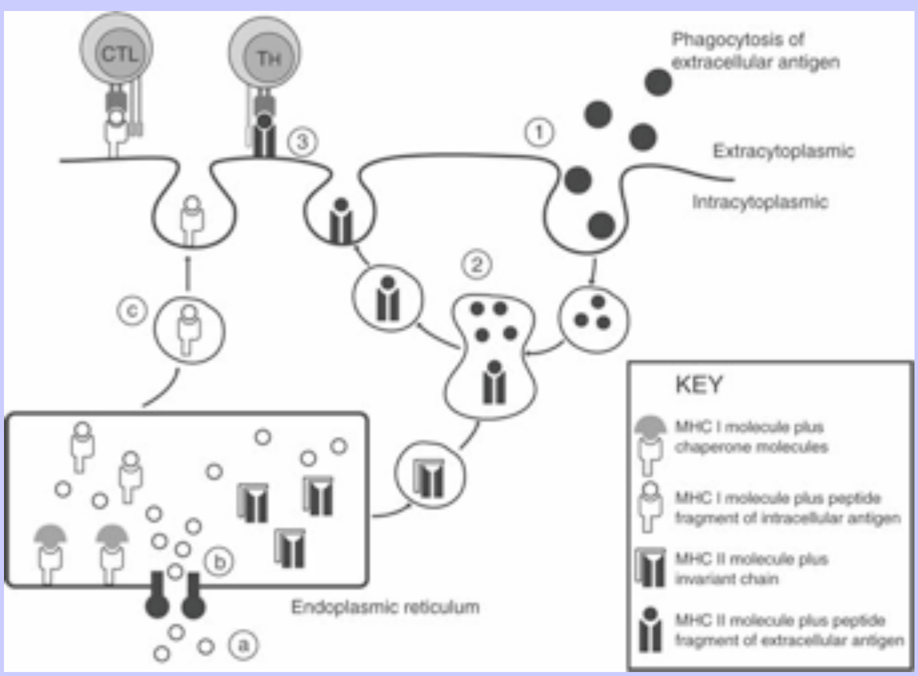


Figure 1.1-10 The role of professional antigen-presenting cells. In this figure, pathogen invasion is followed by antigen uptake by a dendritic cell, the most potent of the antigen-presenting cell family. The dendritic cells become activated and migrate to a local lymph node where they are effective at stimluating naïve T cells, including T helper cells and cytotoxic T lymphocytes.

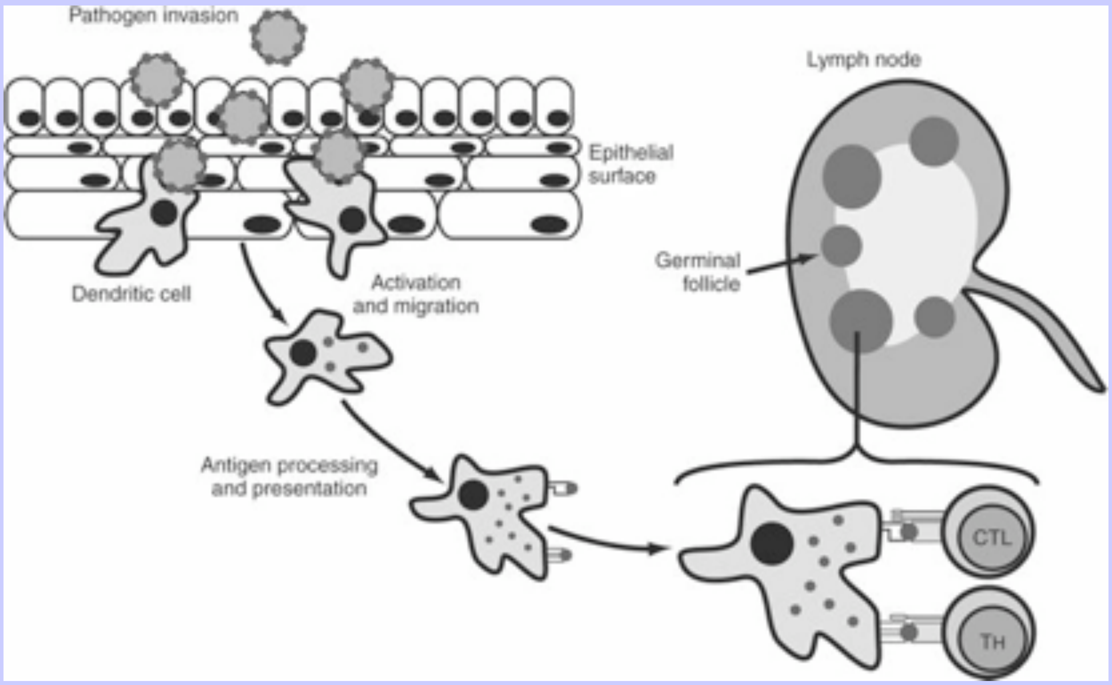
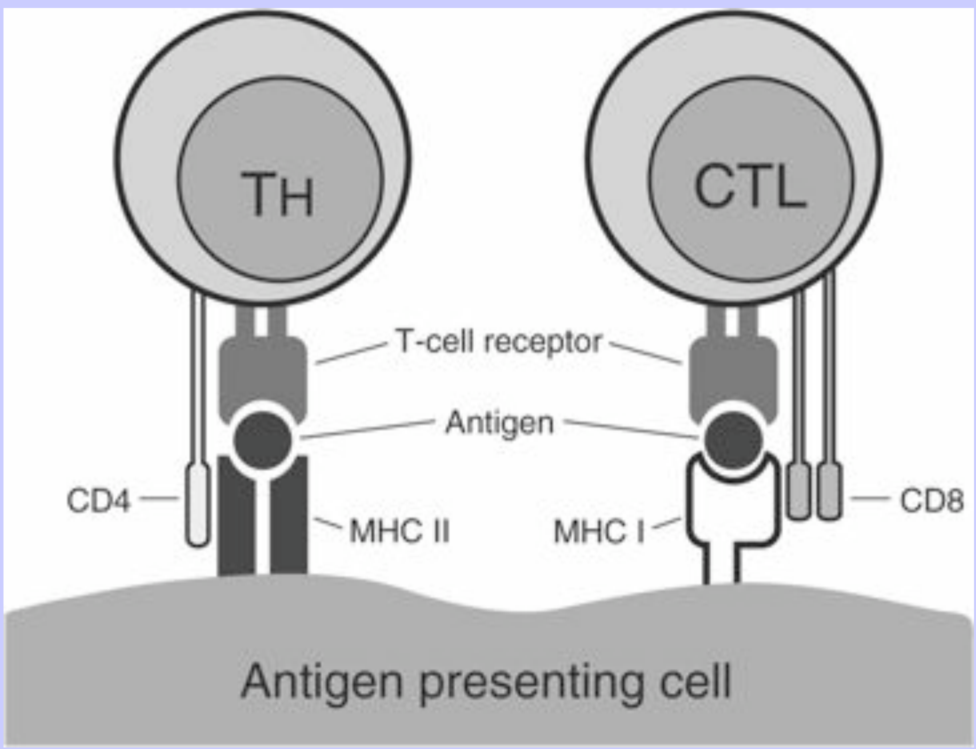


Figure 1.1-11 Class I and II restricted T cell recognition: the role of T cell CD4 and CD8 molecules. T cells use their T cell receptors to recognize processed antigen presented in combination with major histocompatibility complex molecules I or II. T cells exclusively express CD4 (T helper cells) or CD8 (cytotoxic T lymphocytes). The CD4 molecule is required for interaction with MHC II molecules, whereas CD8 is required for interaction with MHC I. As a result, T helper cells recognize antigen presented by MHC II molecules, and cytotoxic T lymphocytes only recognize antigen presented by MHC I molecules.



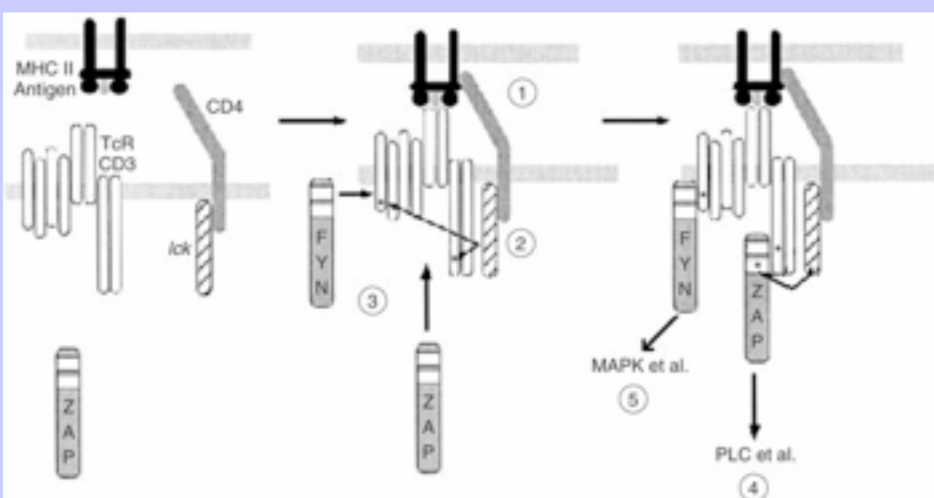
1.1.2.6

CO-STIMULATORY SIGNALS

In addition to the interaction of TCR-CD3 and CD4/CD8, other cell surface antigens are involved in the signaling pathways.[58] Of greatest importance is the interaction of CD28 on the T cell with B7 on the antigen presenting cell. In the absence of CD28/B7 co-stimulation, T cells are rendered functionally inactive or anergic. On restimulation, these anergic T cells fail to proliferate or produce cytokines such as IL-2. The induction of anergy can be prevented by the addition of exogenous IL-2 or, more importantly, by interaction of the CD28 cell surface antigen with its ligands, B7-1(CD80) and B7-2(CD86). Stimulation of CD28 appears to be

necessary for subsequent intracellular signaling events following TCR stimulation. CD28 cross-linking enhances various biochemical events triggered by TCR-mediated signaling, including the activation of phospholipase C, *lck,* and Raf-1 kinase, as well as inducing the influx of Ca^{2+} and generation of phosphoinositides. Other molecules including the TNF-receptor family member CD40 also have been shown to 16
regulate T cell growth or cell death. The engagement of CD40 on the T cell with its ligand, CD40L, on the 17
antigen presenting cell leads to NF-κB activation and promotes cell survival and cell cycle progression. The binding of other members of this family, notably TNF-α, to their receptor on activated T cells typically results in the activation of a biochemical cascade of caspases that lead to apoptosis. The cytocidal activity of these receptors results from the death effector domains within the intracytoplasmic portion of the receptor. By contrast, CD40 lacks intracellular death domains and instead has amino acid motifs that bind TNF-R–associated factors and promote NF-κB activation. In addition to their role in promoting T cell activation and growth, the CD28/B7 and TNF-receptor pathways also may play a dominant role in the induction of specific T helper cell subsets.

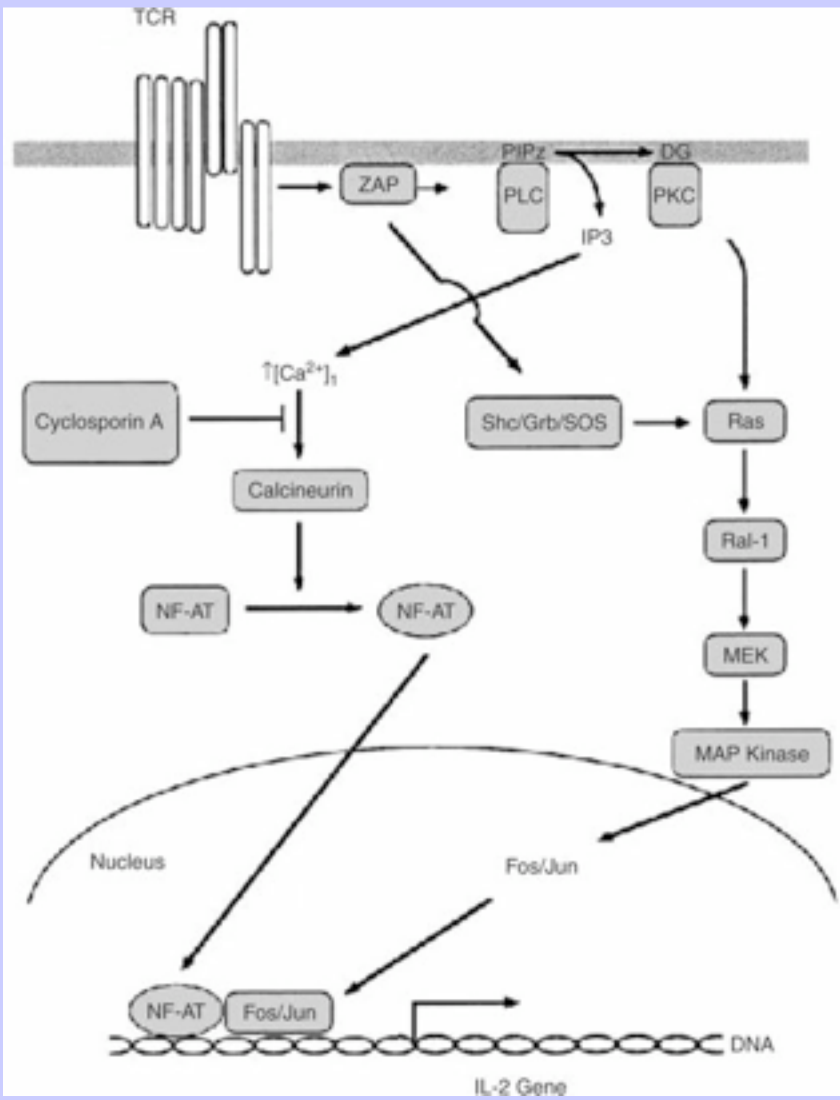
Figure 1.1-12 Intracellular signaling by the T cell–CD3 receptor. T cell receptor recognition of its specific peptide in the peptide-binding groove of a major histocompatibility complex molecule on an antigen-presenting cell results in the attraction of CD4/CD8 to the complex *(1)* and the phosphorylation of CD3 proteins by *lck* associated with CD4/CD8 *(2)*. The phosphorylation of these sites (*) on CD3 leads to the attraction and binding of other kinases (*fyn* and ZAP70) to CD3, where they in turn are phosphorylated and activated *(3)*. Activation of ZAP70 leads to the subsequent activation of phospholipase C *(4)*. Activation of *fyn* ultimately leads to the mitogen-activated protein (MAP) kinases pathway and cell division *(5)* (see also Figure 1.1-13).



1.1.3 Cytokines, Cytokine Receptors, and T Helper Cell Subsets

Frequent mention has been made in this chapter of the role of cytokines in regulating immune responses. Indeed, this particular area of immunology has grown tremendously over the past several years. This rapid acquisition of knowledge results from the application of modern molecular biology techniques to the identification and characterization of specific cytokines. Initial studies of the role of soluble factors in the regulation of immune responses often were confounded by the heterogeneous nature of the culture supernatants used as the source of the cytokine activity. Furthermore, using biologic assays to identify specific cytokines resulted in the practice of assigning descriptive names to newly discovered cytokines (lymphocyte activating factor, T cell growth factor, etc.).[59] Such naming quickly led to confusion because individual cytokines often exhibited multiple biologic activities and biologic assays were not specific for a particular cytokine. Once the genes for the cytokines had been cloned and the resulting proteins identified, eliminating much of the confusion was possible. The adoption of the interleukin terminology for naming cloned immunoregulatory cytokines has clarified further the biologic function and role of particular cytokines.[60] After the gene for a new cytokine is identified and the biologic activity of the purified protein is characterized, the gene is assigned an interleukin designation. To date, more than 50 different cytokines and chemokines have been cloned, sequenced, and synthesized in bacterial and eukaryotic expression systems. This work has led to a better understanding of cytokine function and to their use in a variety of clinical settings. Table 1.1-5 contains a list of interleukins and their known biological activity. Not all 17
cytokines have been given interleukin designation. Interferons, certain growth fac tors (platelet derived growth 18
factor, TGF-β), and TNF-α have retained their original names. That other cells besides T cells produce cytokines and interleukins also should be emphasized. For example, monocytes and macrophages are the major source of IL-1, IL-6, and TNF-α. Thus the term *lymphokine* that was used originally to describe immunoregulatory products of lymphocytes has been replaced with *cytokine,* which denotes the more varied sources of immunoregulatory molecules.

Figure 1.1-13 Intracellular signaling pathway. After activation of ZAP70 and other receptor-associated kinases, a propagation of the signal occurs as subsequent kinases and target proteins are phosphorylated. Increases in intracellular Ca^{2+} leads to the activation of calcineurin, which is necessary for NF-AT activation. This step is the target for cyclosporin A, a potent and specific immunosuppressive agent. Activation of the transcriptional factors NF-AT and *fos/jun* leads to their translocation into the nucleus and the binding to regulatory DNA sequences upstream of the promoter for the interleukin-2 gene.



Many cytokines have similar structures and can be grouped into like families. Helical cytokines have α helices as the predominant structure. IL-2 serves as the prototypical cytokine for this family (Figure 1.1-14). This family can be divided further into two subclasses based on the length of the helices: long helical (many growth factors including IL-3 and IL-7) and short helical (IL-2, IL-4, and IL-13). IL-1 is a β-trefoil cytokine with 12 antiparallel 18
β strands forming a bowl-like structure. Most chemokines and other smaller cytokines contain α helices and β 19
sheets, typically a single α helix and more than two β sheets. TNF-α is the prototype for the β-sandwich family the structure of which characteristically consists of five antiparallel strands with an overall jelly roll structure.

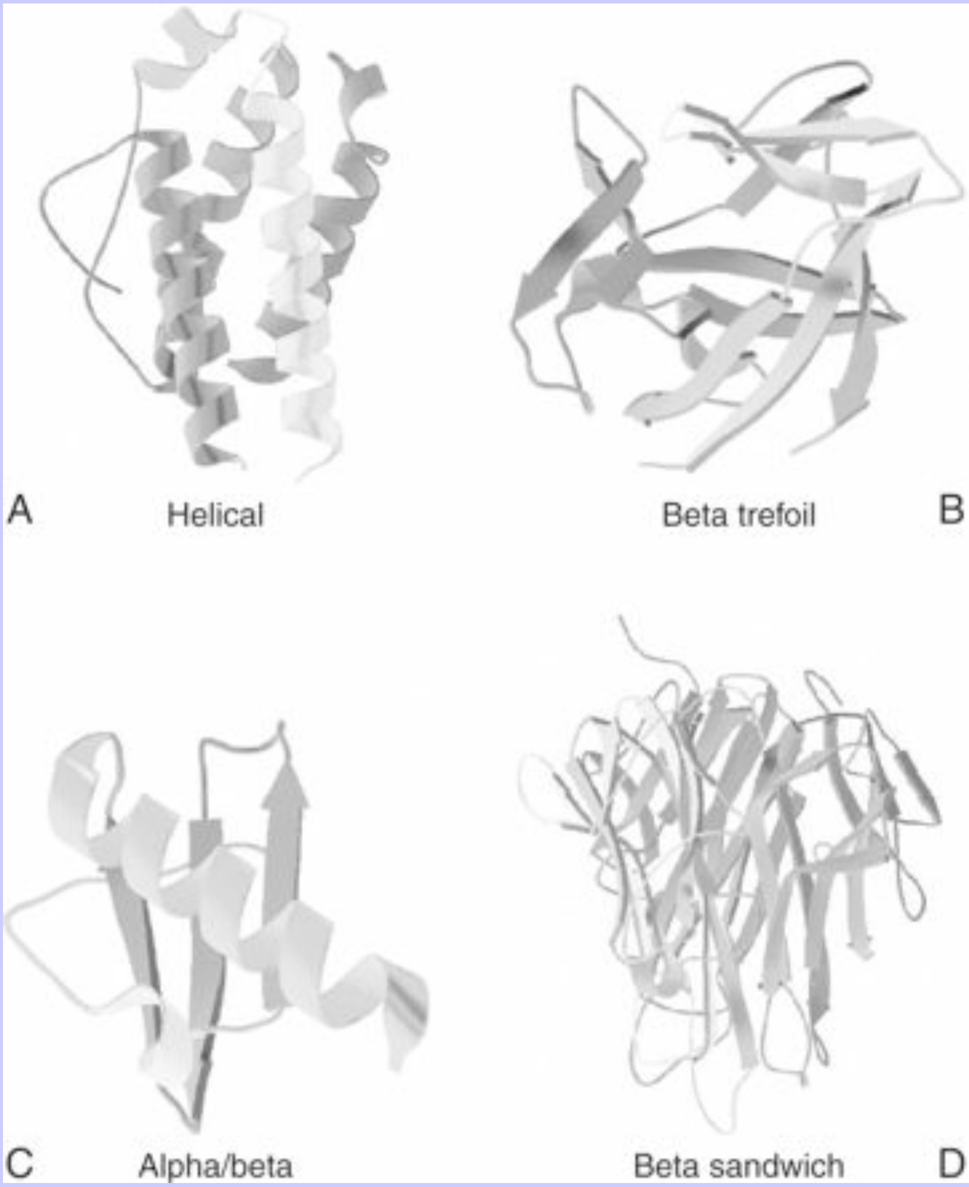
TABLE 1.1-5 Interleukins

INTERLEUKIN	BIOLOGIC ACTIVITIES AND SOURCE
1	Lymphocyte-activating factor. Enhances multiple biological activities affecting a variety of lymphoid and nonlymphoid cells.
2	T cell growth factor. Provides proliferative signal for T cells. Also affects B cells, macrophages, and natural killer cells. High concentrations of IL-2 stimulate cytolytic activity in natural killer cells and T cells. Produced by activated type 1 T helper cells and some $CD8^+$ cells.
3	Multi–colony-stimulating factor. Promotes the growth of various hematopoietic cell precursors. Produced by T cells and myelomonocytic cell lines.
4	B cell stimulatory factor 1. Stimulates growth, maturation, and differentiation of B cells. Also provides proliferative and differentiation signal for some T cells. Produced by type 2 T helper cells.
5	T cell replacing factor. Stimulates B cell proliferation and immunoglobulin synthesis. Also stimulates T cell proliferation and differentiation, as well as eosinophil formation in the bone marrow. Produced by type 2 T helper cells.
6	B cell differentiation factor. Promotes maturation and immunoglobulin production by B cells. Stimulates T cell growth and IL-2 synthesis. Induces the production of acute phase proteins by hepatocytes. Produced by macrophages, T cells, stromal cells, fibroblasts, and a variety of other cell lines.
7	Pre-B cell growth factor. Stimulates proliferation and maturation of early B and T cells as well as mature T cells. Produced by stromal cells derived from bone marrow.
8	Neutrophil chemokine. Produced by monocytes and hepatocytes.
9	Also known as P40. Supports the growth of certain T cell clones. Produced by $CD4^+$ T cells.
10	Cytokine synthesis inhibitory factor. Inhibits the production of IL-2 and interferon-γ by type 1 T helper cells. Produced by type 2 T helper cells.
11	An IL-6–like factor produced by bone marrow stromal cells.
12	Natural killer cell differentiation factor. Augments natural killer cell function and stimulates generation of type 1 T helper cells. Produced by macrophages.
13	Produced by type 2 T helper cells. Downregulates cytokine production by macrophages/monocytes while activating B cells.
14	A high-molecular-weight B cell growth factor produced by T cells and some B cell lines.
15	A T cell growth factor similar in function to IL-2.
16	Chemokine for $CD4^+$ T cell subset. Produced by T cells, mast cells, and eosinophils.
17	A family of related cytokines. Enhances expression of the intracellular adhesion molecule 1 on fibroblasts. Also stimulates epithelial, endothelial, or fibroblastic cells to secrete IL-6, IL-8, granulocyte colony-stimulating factor, and prostaglanding E_2.
18	An inducer of interferon-γ production by T-cells.

19	A homolog of IL-10.
20	An autocrine factor for keratinocytes that regulates their participation in inflammation.
21	Stimulates proliferation of B cell by cross-linking of the CD40 antigen, bone marrow progenitor cells, and naïve T cells.
22	A proinflammatory cytokine. Increases the production of acute phase proteins.
23	Produced by dendritic cells. Stimulates the production of interferon-γ by T cells.
24	Selectively suppresses the growth of tumor cells by promoting cell death by apoptosis.
25	Produced by stromal cells. Supports proliferation of cells in the lymphoid lineage.
26	Induces the expression of IL-6, IL-8, and intracellular adhesion molecule 1 in primary bronchial epithelial cells.
27	Similar to IL-17. Expressed in the brain.

The availability of cloned cytokines has permitted the identification and characterization of cytokine-specific receptors. Cytokine receptors also can be grouped into major families: class I or class II receptor families, immunoglobulin superfamily receptors, the TNF receptor family and Toll-like receptors (IL-1 and IL-18).[61] The best characterized of these is the class I receptor for IL-2, which is composed of three subunits; α, β, and γ. Although the α and β subunits are involved in the specific binding to IL-2, the γ chain is involved in signal transduction after IL-2 is attached to the receptor. Five different immunologically important cytokines share this common cytokine receptor γ chain, though each has its own unique α or αβ binding subunits (Figure 1.1-15). Other common signaling chains of this type of receptor include βc (IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor) and gp130 (IL-6 and IL-11). Class II receptors are illustrated by the interferons with receptors that consist of at least two chains. In contrast to the chains being denoted α and β analogous to the nomenclature for type I cytokine receptors, the chains are called IFNAR-1 and IFNAR-2 for α/β interferons and IFNGR-1 and IFNGR-2 for interferon-γ. A third receptor, CRF2-4 is a component of the IL-10 receptor. The TNF receptor family is composed of two separate receptors, TNF-RI and TNF-RII. Although both can bind TNF, no 19
structural homology is found in their intracellular domains, indicating that they signal by distinct mechanisms. 20
TNF-RI is thought to be the main signaling receptor because many biological actions of TNF, including cytotoxicity, fibroblast proliferation, and the activation of NF-κB, can be elicited in the absence of TNF-RII. The IL-1 receptor is a member of the Toll-like receptor family. This receptor superfamily represents an ancient signaling system that was identified initially in *Drosophila melanogaster*. The introduction of a pathogen into *Drosophila* leads to the activation of proteases that cleave a precursor and generate an extracellular ligand of a receptor called Toll, the intracellular part of which is homologous to the IL-1 receptor cytoplasmic tail. Other Toll-like receptors are involved in other signaling pathways in innate immune and inflammatory responses, indicating this receptor superfamily represents an ancient signaling system.[62]

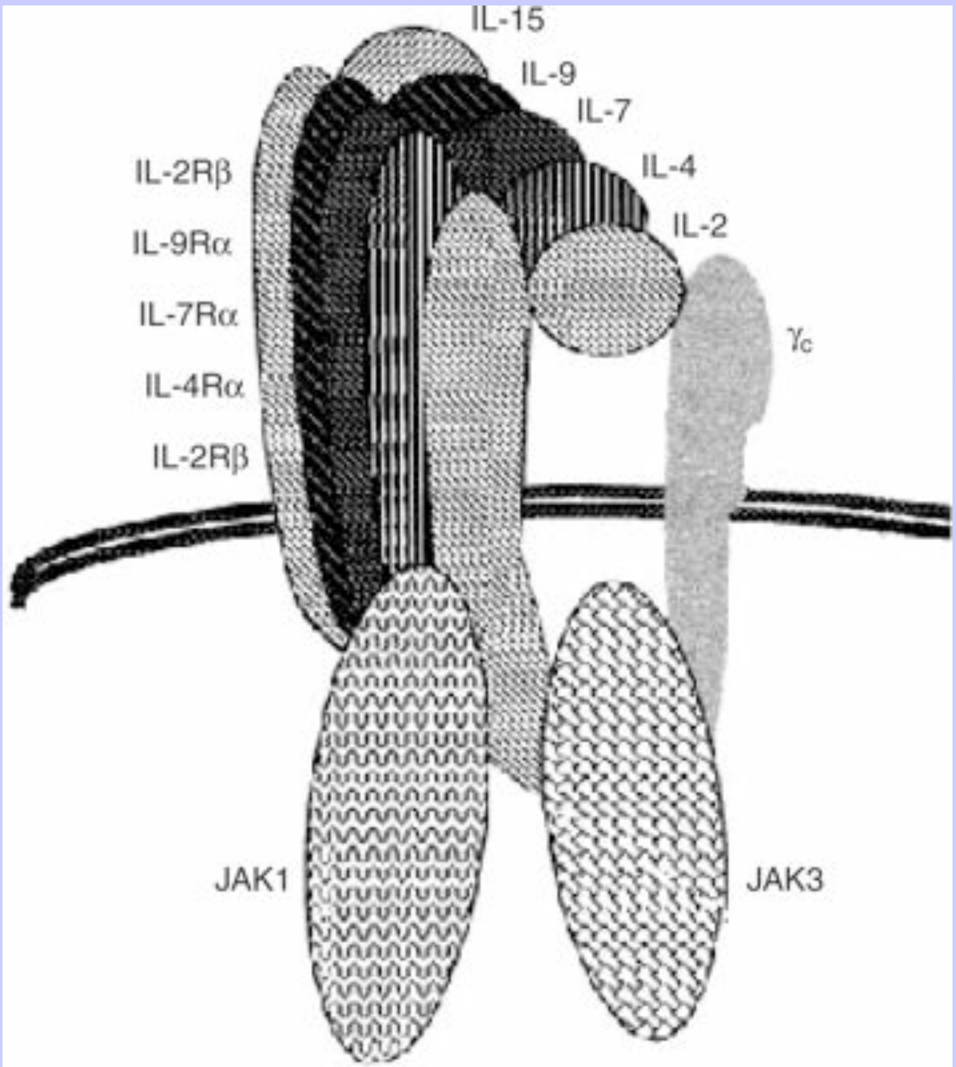
Figure 1.1-14 Cytokine structural families. **A,** Helical cytokine. **B,** β-Trefoil. **C,** α/β. **D,** β sandwich.



A common feature of all receptor families is that signaling is initiated through the recruitment of protein tyrosine kinases and other cytosolic proteins to the receptor.[63,64] Although most cytokine receptors lack intrinsic kinase activity, they do have a family of Janus protein tyrosine kinases (JAKs) associated with their cytoplasmic tails. Following binding of a ligand to its cognate receptor, receptor-associated JAKs are activated. A family of transcriptional factors known as STATs (signal transducers and activators of transcription) in turn are activated by tyrosine phosphorylation by the activated JAKs, allowing the STAT to dimerize (Table 1.1-6). After dimerization, the STAT translocates into the nucleus and binds to the DNA sequence it recognizes via a DNA-binding domain

on the protein. The binding of the STAT protein to the DNA subsequently modulates gene expression. The sharing of receptor subunits combined with a similar sharing of JAKs and STATs accounts for similar biological functions of many cytokines (see Table 1.1-5).

Figure 1.1-15 Type I cytokine receptors. These receptors are characterized by cytokine-specific α and β chains involved in ligand binding and a shared or common γ chain used for intracellular signaling. The Janus kinases *(JAK)* are associated with the cytoplasmic tails of these receptors and are responsible for signal transmission (see Table 1.1-6).



In addition to the JAKs and STATs, other transcriptional factors can activate multiple genes involved in inflammatory responses and apoptosis. One of these transcriptional factors, NF-κB, regulates many

proinflammatory cytokines including TNF-α, IL-1, and IL-8. NF-κB itself is activated by a number of cytokine receptor signaling cascades including TNF receptors.[65] In the cytoplasm, NF-κB is associated with an inhibitory protein, IκB, which prevents its translocation to the nucleus. Phosphorylation of IκB leads to its degradation and the translocation of NF-κB to the nucleus, where it binds to its corresponding DNA motif, altering gene transcription. NF-κB activation also is associated with resistance to apoptosis, probably as the result of its effect 20
on IL-8 transcription because this chemokine is antiapoptopic.[66] Increased levels of IL-8 in inflammatory lung 21
lesions and the increase in NF-κB activation likely accounts for the neutrophil accumulation observed in some forms of human asthma[67] and equine recurrent airway obstruction.[68]

TABLE 1.1-6 Cytokines and the JAKs and STATs They Activate[*]

CYTOKINE	JAKs	STATs
Interferon-α/β	JAK1, Tyk2	STAT1, STAT2
Interferon-γ	JAK1, JAK2	STAT1
Interleukin-10	JAK1, Tyk2	STAT3
Interleukin-12	JAK2, Tyk2	STAT3, STAT4
Granulocyte colony-stimulating factor	JAK1, JAK2	STAT3
γC	JAK1, JAK3	STAT5A, STAT5B, STAT3
Interleukin-4		STAT6
Interleukin-13		STAT6
bc	JAK2, JAK1	STAT5A, STAT5B
gp130	JAK1, JAK2, Tyk2	STAT3

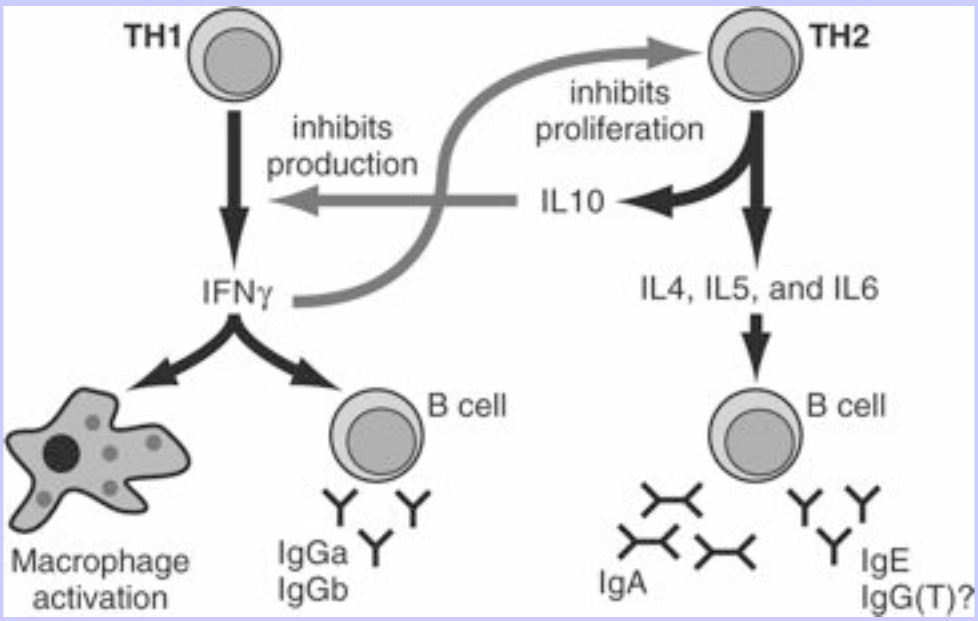
* *JAK,* Janus protein tyrosine kinase; *STAT*, signal transducer and activator of transcription.

1.1.3.1 IMMUNOREGULATION

The generation of an immune response requires the interaction of multiple leukocyte subsets including macrophages, dendritic cells, B cells, and $CD4^+$ and $CD8^+$ T cells. Although the initial interactions of the B and T cells involve recognition of specific epitopes, which in the case of the T cell are presented in the context of MHC antigens, cytokines produced by the various cells mediate subsequent interactions. Although macrophages, B cells, and even nonhematopoietic cells produce a variety of cytokines with immunoregulatory activity, the T helper cell plays a central role in regulating immune responses. Much effort over the past decade has focused on the characterization of helper T cells and the soluble factors they produce. What is apparent now is that $CD4^+$ helper cells may be divided further into distinct helper cell subsets based on the cytokines they produce (Figure 1.1-16). T helper type 1 (TH1) cells produce interferon-γ and IL-2, cytokines involved in the induction of cell-mediated immune responses. TH2 cells produce IL-4, IL-13, and IL-5, cytokines involved in the induction of antibody responses. The best evidence for separate T helper cell populations comes from the study of intracellular parasite infections in mice. Those strains of mice resistant to *Listeria donovani* infection develop a cell-mediated immune response characterized by activated macrophages and TH1 cells. By contrast, the susceptible BALB/c strain of mice generates a vigorous antibody response and TH2 helper cells. TH1 cells also have been implicated in various autoimmune diseases characterized by the induction of self-reactive

cytotoxic cells. TH2 cells play a central role in resistance to extracellular parasites such as intestinal helminths and in induction of allergic diseases. A similar contribution of TH1 and TH2 responses in protective and pathologic responses in the horse have been described.[69,70]

Figure 1.1-16 T helper cell types 1 and 2 regulation. The TH1 lymphocyte subsets provide help for macrophage activation, cytolytic activity, and production of a subset of immunoglobulin G subclasses. The TH2 promotes antibody responses, including IgA, IgE, and the remainder of the IgG subclasses. The response is mediated by production of cytokines that have a regulatory effect on one another.



Although what determines whether a helper cell will be a TH1 or TH2 cell is unclear, one proposal is that the initial encounter with the antigen during the innate immune response may determine the fate of the helper cell. Multiple factors probably are involved in this process; however, the single most important factor is the type and amounts of cytokines present at the time of the initial encounter with the antigen. Among the cytokines that may play a role, IL-12 and interferon-γ are the main inducers of TH1 responses, and IL-4 and IL-10 play a similar role for TH2 responses. IL-12, produced by macrophages and dendritic cells, is a potent inducer of interferon-γ that inhibits TH2 cell induction. The evidence from a variety of models to date suggests that IL-12 is the single most important factor in regulating the differentiation and magnitude of the TH1 response; however, this should not preclude the possibility that additional factors, such as IL-18, may have an equally important role in regulating TH1 responses in some situations. What is also apparent is that IL-4 plays a similar, crucial role in the induction of TH2 immune responses. IL-4 production by mast cells and IL-10 production by macrophages favors TH2 development in part by inhibiting TH1 cells. Other signals (e.g., PGE_2)

can induce differentiation of potent antigen-presenting cells with dendritic morphology that produce low levels of IL-12 and high levels of IL-10 preferentially inducing TH2 differentiation. 21

22

In addition to cytokines, another influence on T helper cell differentiation is the interaction of the B7/CD28 and CD40/CD40L co-stimulatory pathways. In particular, these co-stimulatory pathways may regulate the differentiation of TH1 and TH2 cells by affecting the intensity and the strength of signals through the CD3/TCR complex and those provided through the co-stimulators. High-intensity stimulation favors TH2 development, whereas lower-intensity signals favor TH1 cells. The association of stronger co-stimulation with TH2 responses contrasts with the ability of higher concentrations of antigen mostly to induce TH1 responses, whereas lower doses induce TH2 responses, suggesting that T helper cell differentiation may be influenced differently by the strength of signals through the CD3/TCR complex versus signals delivered through co-stimulators and their associated enzymes.[71]

1.1.3.2 T HELPER CELL PARADIGMS

The role of cytokines in regulating immune responses can be illustrated best in two scenarios, the first involving the induction of a TH1 immune response to viral infection and the second involving an allergic response to inhaled mold antigens. In the first scenario, viral antigen present at the site of an ongoing infection in the respiratory tract is processed by resident macrophages via the exogenous pathway. The processed epitope is presented on the surface of the macrophage or a dendritic cell in the context of MHC II antigen to a $CD4^+$ T cell in a regional lymph node. These cells produce IL-12 that induces natural killer cells, attracted to the site of the infection, to produce interferon-γ that, along with antigen presentation, activates the T cell and drives it towards a TH1 phenotype. Meanwhile, $CD8^+$ T cells encounter viral antigen on the surface of virus-infected cells that has been processed via the endogenous pathway and is now associated with the MHC I antigens on the infected cell surface. Once antigen-activated, these T cells express the high-affinity form of the IL-2 receptor. The $CD4^+$ TH1 cell produces IL-2 and interferon-γ. The interaction of IL-2 with its receptor drives the clonal proliferation of the activated $CD8^+$ T cells. The interferon-γ also stimulates $CD8^+$ cells to differentiate into CTLs that produce additional interferon-γ. These CTLs can lyse the target cells through production of TNF-α or via activation of the *fas* receptor on the target cell via the *fas* ligand expressed on the activated CTL. Both pathways lead to target cell apoptosis via the activation of cytoplasmic caspases in the target cell. Meanwhile, virus-specific B cells have encountered antigen and, in the presence of interferon-γ, differentiate into IgG-secreting plasma cells. This combination of IgG antibodies and CTL cells serve to eliminate the virus. Prostaglandin E_2 and IL-10 production by macrophages exert antiinflammatory effects on the response and that, coupled with the production of soluble cytokine receptors, dampens the immune response as the invader is eliminated.

In the second scenario, the introduction of mold antigens into the respiratory tract leads to the processing of antigen by macrophages and dendritic cells as described previously. However, in the absence of IL-12 and interferon-γ and perhaps the presence of IL-3, IL-4, IL-9, IL-10, or PGE_2, induction of TH2 cells produces additional IL-4 and IL-13. These cytokines cause those B cells recognizing the allergens to switch isotype to IgE antibodies that bind to mast cells. Subsequent degranulation of these mast cells, resulting from antigen binding to the IgE, leads to the production of other mediators, including IL-4 and PGE_2 that exacerbate this response. In the continued presence of the allergen, a secondary inflammatory response develops characteristic of recurrent airway obstruction.

1.1.3.3 ROLE OF CYTOKINES IN THE HORSE

The field of equine immunology continues to expand with the development of better reagents. Recent advances in gene cloning technology have led to the cloning and expression of a number of equine cytokines. A number of equine cytokines have been cloned and sequenced, and specific protocols are now available to measure their expression (Table 1.1-7). Using these procedures, identifying the role of TH1 and TH2 cells in protective and pathologic responses in the horse has been possible (Table 1.1-8).[69,72,73] The results from these and other studies confirming the role of inflammatory cytokines in equine sepsis and joint and airway diseases emphasize the similarities between equine and human immune systems. As such, the potential for manipulating these responses using recombinant cytokines or anticytokine reagents is as applicable to equine medicine as to human medicine.

1.1.3.4 LYMPHOCYTE TRAFFICKING PATHWAYS

Leukocyte trafficking has been reviewed already, with a particular emphasis on the innate immune response. Lymphocytes involved in adaptive immune responses differ in their migration in that they recirculate instead of making one-way trips. Memory and naïve T lymphocytes, with their different capacities for response to antigen, differ also in their migration pathways through the body. Two general pathways of lymphocyte recirculation have been demonstrated. Naïve T lymphocytes take the most common route, which involves entry into the lymph node by extravasation from the high endothelial venule (HEV) and return to the peripheral circulation via the efferent lymphatic. The endothelial cells of HEVs have a distinctive appearance and specialized receptors and can support a large lymphocyte migration. Such characteristics allow rapid repeated 22
circulation of naïve lymphocytes through lymph nodes to where the greatest chance of exposure to their 23
specific antigens occurs. Memory lymphocytes leave the bloodstream in peripheral vascular beds, particularly in inflamed tissues, and return to lymph nodes via afferent lymphatics. This circulation leads to the exposure of primed memory lymphocytes to the most likely early sites of antigenic encounter and allows for an early response to recall antigens. Thus memory lymphocytes are most common in inflammatory lesions and in the epithelial surfaces of the lung and gut wall. Differing expression of the adhesion and homing molecules may play an important role in mediating these different migration pathways.

TABLE 1.1-7 Cloned Equine Cytokines

CYTOKINE*	GENEBANK ACCESSION NUMBERS†
IL-1	D42146, E13117, E13117, U92480
IL-1	D42147, D42165, E13118, U92481
IL-2	L06009, X69393
IL-4	L06010, AF305617, AF035404
IL-5	U91947
IL-6	U64794, AF005227, AF041975
IL-8	AF062377
IL-10	U38200
IL-12 p35	Y11130
IL-12 p40	Y11129
IL-18	Y11131
Interferon-α	M14540, M14541, M14542, M14543
Interferon-β	M14546
Interferon-γ	M14544, M14545, AH001204, D28520, U04050
Interferon-ψ	AH001204
TNF-α	M64087, AB035735, AF503366
GM-CSF	AF448481
G-CSF	AF503365
Eotaxin	AJ251188
Rantes	AF506970
MCP-1	AJ251189
MCP-2	AF506972
TGF-β	AF175709

* *IL,* interleukin; *TNF,* tumor necrosis factor; *GM-CSF,* granulocyte-macrophage colony-stimulating factor; *G-CSF,* granulocyte colony-stimulating factor; *MCP*, macrophage chemotactic protein; *TGF,* transforming growth factor.

† Multiple accession numbers indicate multiple reports of sequences for the same genes with the exception of the interferons, for which multiple gene families exist.

TABLE 1.1-8 Helper Cell Paradigm in the Horse

CELL TYPE	PROTECTION	IMMUNOPATHOLOGY
TH1	Equine influenza virus	Equine recurrent uveitis
TH2	*Strongylus vulgaris*	Recurrent airway obstruction (chronic obstructive pulmonary disease and summer pasture-associated obstructive pulmonary disease

For lymphocytes to follow the maturation and migration pathway previously described, the first step is for the naïve lymphocyte to get into a lymph node so that it can meet its antigen on a professional antigen-presenting cell. To achieve this, the T-lymphocyte must exit in the HEV. The naïve lymphocyte expresses L-selectin, which can bind to the vascular addressins GlyCAM-1, CD34, and MAdCAM-1, which are expressed on HEVs and promote rolling similar to that mediated by P- and E-selectin when they bind to phagocytes. These molecules are expressed on a variety of tissues, but in HEVs they have specific patterns of glycosylation that permit binding to L-selectin. These differences represent the key to the specificity of the migration of lymphocytes to HEVs. This weak interaction initiates the process of extravasation, which is promoted by locally bound chemokines (e.g., IL-8) that increase the affinity of the lymphocyte integrins for their ligands.

Approximately 25% of lymphocytes passing through an HEV leave, and this could mean 1.4×10^{4} cells in a single lymph node every second. In the human body, 5×10^{6} lymphocytes may extravasate through HEVs every second. The "sticking" process (rolling, activation, and arrest) takes a few seconds, with transendothelial migration and passage through the HEV basement membrane occurring in about 10 minutes. After leaving the blood, most T cells travel through the lymph node uneventfully and leave via efferent lymphatics; however, in rare events a naïve T cell recognizes its specific peptide-MHC complex and becomes activated, eventually leading to formation of effector and memory T-cells. That process takes 4 to 5 days, and once activated, the migration pathway of memory T-cells differs considerably from naïve cells. All activated T-cells lose the L-selectin molecules that mediated homing to lymph nodes and increase the expression of other adhesion molecules. The homing of individual lymphocytes to specific sites is regulated by expression of specific adhesion molecules. Memory cells are attracted specifically to areas of inflammation because of the increased concentrations of adhesion receptor ligands expressed on vascular endothelium in these regions. The inflammation typically results from TNF-α production by regional macrophages encountering infections. Memory cells also migrate randomly throughout the body. When memory cells encounter their antigen, they can produce cytokines such as TNF-α, which in turn causes local endothelial cells to increase expression of E-selectin, vascular cell adhesion molecule 1, and intracellular adhesion molecule 1. Such expression 23
subsequently causes recruiting of more effector and memory cells to the region. 24

1.1.3.5 ONTOGENY OF THE EQUINE IMMUNE SYSTEM

Few studies of the prenatal development of the equine immune system have been done. As in other species, the thymus is the first lymphoid organ to develop, and mitogen responsive cells can be identified in the organ from day 80 of the 340-day gestational period of the horse.[74] Subsequently these cells appear in peripheral blood at 120 days, in lymph nodes at 160 days, and in the spleen at 200 days. Cells responsive in mixed lymphocyte reactions are detectable in the thymus at 100 days and in the spleen at 200 days. Immunoglobulin production is detectable before 200 days, and newborn foals typically have IgM concentrations in their serum of

approximately 165 μg/ml. Overall, functional T lymphocytes apparently are present by day 100 and B lymphocytes by day 200 of gestation. Immunologic competence of the equine fetus has been assessed in terms of specific antibody responses. In utero immunization of foals in late gestation with keyhole-limpet hemocyanin in an alum adjuvant results in detectable specific antibody production and T cell responsiveness at the time of birth.[75] In addition, the equine fetus can respond to coliphage T2 at 200 days and to Venezuelan equine encephalitis virus at 230 days.[76,77]

Detailed studies of the appearance of lymphocyte subpopulations defined by monoclonal antibodies have not been performed in the equine fetus. However, some information regarding the maturation of thymocytes in young horses is available. During thymic maturation of T cells, stem cells migrate into the thymus and mature into T cells under the influence of the epithelial microenvironment.[78,79] In this process, different patterns of cell surface differentiation antigen expression distinguish successive stages of thymocyte maturation. In human beings the earliest thymic precursor cells express low levels of CD4.[80] This CD4 expression is lost as early thymocytes become double-negative $CD4^{-}CD8^{-}$ cells and then demonstrate their T cell commitment by TCR-β gene rearrangement, which is an essential trigger for subsequent events and leads to low levels of expression of a cell surface TCR-β–CD3 complex.[81] Intermediate thymocytes are $CD4^{lo}CD8^{lo}$, but after TCRα gene rearrangement and expression of cell surface TCR-αβ they rapidly become $CD4^{hi}CD8^{hi}TCR\text{-}CD3^{hi}$.[80] Subsequently thymocytes selected on the basis of productive TCR gene rearrangement and lack of self-reactivity become mature T cells expressing CD4 or CD8 (single positive) in combination with high levels of TCR-CD3. Using two-color fluorescence-activated cell sorter analysis, similar patterns of EqCD3, EqCD4, and EqCD8 antigen expression in the equine thymus can be demonstrated.[82,83]

1.1.3.6 IMMUNOCOMPETENCE IN FOALS

Infectious disease in neonatal foals is associated with high morbidity and mortality. Although failure of passive transfer is a major cause of this problem, as discussed in Chapter 1.3, immaturity of the immune system also has been considered a potential contributing factor. As a result, a number of studies of neonatal immunocompetence have been completed.

1.1.3.7 INNATE IMMUNE RESPONSES IN FOALs

A number of studies have reported neutrophils to be fully functional from birth[84–86]; however, neutrophil function is impaired significantly before absorption of colostral antibodies, which are required for opsonization.[86,87] A recent study of foal neutrophil development over the first 8 months of life demonstrated killing (measured by chemiluminescence) to be reduced in the first 2 weeks of life, as was phagocytic ability when assays were performed using autologous serum.[88] When serum from adult horses was used, neutrophil phagocytic ability in foals was normal. This latter difference may have been caused by absence of adequate immunoglobulin or complement in foal serum. A similar study of foals less than 7 days of age confirms that phagocytosis and oxidative burst activity of neutrophils is reduced in foals of this age, although the use of adult serum did not improve phagocytosis.[89] Sim ilarly, alveolar macrophages recovered from bronchoalveolar lavage fluid may be low in number up to 2 weeks of age and may have impaired chemotactic function.[90]

The importance of complement in foals is illustrated by the finding that the opsonic capacity of foal serum for bacteria is halved by heat inactivation.[91] Interestingly, complement activity in the first week of life is increased

considerably in colostrum-deprived foals, possibly as an alternative defense mechanism.[92] In foals fed bovine colostrum, serum complement concentrations reach adult concentrations by 1 to 3 weeks of age.[93]

1.1.3.8 ADAPTIVE CELL-MEDIATED IMMUNITY IN FOALS

Recent studies have measured lymphocyte numbers and subpopulations in foals.[94–96] Foals are born with B and T lymphocytes and with $CD4^+$ and $CD8^+$ T lymphocyte subsets. Lymphocyte counts increase in the first 4 months of life, and the proportion of B lymphocytes increases. A comprehensive study of lymphoproliferative responses in foals from the day of birth through 4 months of age found no difference between foals and adults.[96] Another study reported foal lymphoproliferation as low on the day of birth, possibly because of high serum cortisol levels.[97] Currently, markers for the development of memory lymphocytes are unavailable in horses, although increased expression of MHC II antigen on T lymphocytes throughout the first year of life may 24
identify a developing population of memory cells.[98] Although evidence exists for the capacity of foals to mount 25
immune responses in utero,[75–77] few studies of antigen-specific immune responses in the first days of life have been done except in the context of the immunosuppressive effect of passive transfer of immunity.[99] When foals are immunized with antigens against which they have no maternally derived specific antibodies, clearly they can mount immune responses from at least 3 months of age and possibly sooner.[100]

1.1.3.9 ANTIBODY-MEDIATED IMMUNITY IN FOALS

1.1.3.9.1 Passively Transferred Maternal Antibody

During the first 1 to 2 months of life, foals depend on passively transferred immunity for protection from infectious disease. The diffuse epitheliochorial nature of the equine placenta does not allow for in utero immunoglobulin transfer to foals. Although minor concentrations of some immunoglobulins can be detected at birth, the foal is born essentially agammaglobulinemic and acquires passive immunity by the ingestion and absorption of colostrum from the dam.[101,102] Colostrum is a specialized form of milk containing immunoglobulins, which are produced during the last 2 weeks of gestation under hormonal influences.[103] Colostrum contains primarily IgGa, IgGb (IgGa plus IgGb is the equivalent of IgG), and IgG(T), with smaller quantities of IgA and IgM, all of which have been concentrated into mammary secretions from the blood of the mare.[104,105] Colostrum is produced only one time each pregnancy and is replaced by milk that contains negligible immunoglobulins within 24 hours of the initiation of lactation.[104,106] This rapid decline in immunoglobulin concentrations in mammary secretions is consistent with equine colostrum production ending at or even before parturition.[105] The absorptive capacity of the gastrointestinal tract of the foal for immunoglobulins is greatest during the first 6 hours after birth and then steadily declines until immunoglob ulins can no longer be absorbed when the foal is 24 hours old. This closure of the gut to absorption of large intact molecules is caused by replacement of specialized enterocytes by more mature cells.[107]

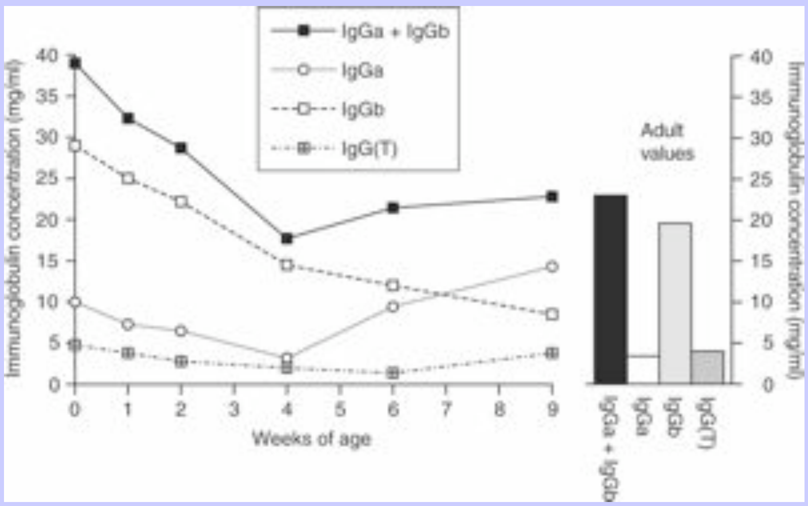
1.1.3.9.2 De Novo Antibody Production in Foals

Few studies of de novo antibody production have been conducted in foals without the effect of passively transferred maternal antibody. In a study of 10 pony foals fed only bovine colostrum, endogenous equine antibody production measured by radial immunodiffusion resulted in serum concentrations of IgG of 200

mg/dl by 2 weeks of age, 400 mg/dl by 1 month, and 1000 mg/dl by 3 months of age.[108] In a smaller study of two colostrum-deprived pony foals, comparing them with 18 colostrum-fed foals and measuring serum γ-globulin levels by immunoelectrophoresis, similar results were obtained, although the colostrum-deprived foals apparently achieved higher serum γ-globulin levels between 6 weeks and 3 months of age than colostrum-fed foals.[101] In a third study, antibody concentrations in six colostrum-deprived foals were substantively higher than in five control foals between 3 and 5 months of age.[92] These three studies provide evidence for substantial endogenous production of IgG in the first month of life in foals deprived of equine colostrum and suggest that the onset of production is earlier and the rate is higher in foals deprived of colostrum. This observation is consistent with nonspecific immunosuppression in colostrum-fed foals or to stimulation of immunoglobulin production in colostrum-deprived foals. In another study of foals from mixed-breed horses fed only bovine colostrum, endogenous IgG production started later and was detected first at 1 month of age in the majority of foals, reaching similar levels in foals fed equine colostrum by 2 months of age.[109]

In colostrum-fed foals, serum IgG concentrations fall to their lowest level at 1 to 2 months of age because of catabolism of maternally transferred immunoglobulin, subsequently rising toward adult levels because of endogenous immunoglobulin production.[88,95,96,102] A study by Sheoran, Timoney, Holmes et al.[105] extended these observations and extensively documented changes in serum IgG subclass concentrations in five Quarter Horse foals in the first 9 weeks of life (Figure 1.1-17). This study showed that IgG (the equivalent of IgGa plus IgGb) concentrations were lowest at 1 month of age. However, the subsequent increase in IgG concentration was due to de novo IgGa production, not IgGb. At the end of this study, at 9 weeks of age still no clear evidence of IgGb production was observed, although IgGa and IgG(T) concentrations had reached or exceeded adult levels. In adult horse serum, IgGb comprises greater than 60% of total serum IgG and is by far the dominant subclass in foal serum after passive transfer of immunity. IgGb also plays a critical role in immunity to a variety of pathogens,[110,111] and possibly the naturally late onset of endogenous production may be a factor in the increased susceptibility of foals at this age to infections such as bacterial respiratory disease.[112,113] This possibility was investigated in a study by Grondahl, Sternberg, Jensen-Waern et al.,[91] in which the opsonic capacity of foal serum was measured during the first 42 days of life using the foal pathogens *Escherichia coli* and *Actinobacillus equuli*. No differences were detected over time, and foal serum was as effective as adult horse serum. Although this study did not provide evidence of decreased opsonization in serum of older foals, the studies were extended only to samples from 42-day-old 25
foals, and in this in vitro system immunoglobulin concentrations may not have been rate limiting. Further 26
investigation of the role of naturally low immunoglobulin subclass concentrations in the pathogenesis of respiratory infections of foals is warranted.

Figure 1.1-17 Immunoglobulin G subclass concentrations in foals and adults. Line graph shows mean serum concentrations of serum IgG subclass data from five Quarter Horse foals during the first 9 weeks of life as measured using an enzyme-linked immunosorbent assay system. Bar graph shows mean adult concentrations measured in 27 mixed-breed horses. (Data is from Sheoran, Timoney, Holmes, et al. 105)



A factor that significantly affects de novo immune responses in foals is the suppressive effect of passively transferred antigen-specific maternal antibodies. The rate of decline of these antibodies varies for individuals and different infectious agents. The half-life for maternal IgG in foals is estimated at 20 to 30 days.[106] Recent studies of antigen-specific antibodies demonstrated similar half-lives for antiinfluenza virus and antitetanus antibodies of 27 to 29 days for IgGa, 35 to 39 days for IgGb, and 35 days for IgG(T).[114] For many important pathogens, the concentration of maternal antibodies in foals falls to nonprotective levels by 2 to 3 months of age.[115,116] However, the remaining antibody still may render the foal unresponsive to vaccination for weeks or even months. In the case of equine influenza virus[117,118] and tetanus toxin, maternal antibodies can persist until 6 months of age and prevent immune responses in foals vaccinated before that age.[114] When foals are vaccinated against antigens against which they have no passively transferred antibody, normal antibody responses have been documented from at least 3 months of age.[100]

1.1.3.10 IMPLICATIONS FOR IMMUNOCOMPETENCE IN FOALs

The evidence presented suggests that the immune system of a foal is competent in many regards, with the innate immune system completely functional at least by the second week of life and with the full complement of lymphocytes present from birth. Antibody is provided entirely by passive transfer at first, although endogenously produced immunoglobulin is detectable within a few weeks of birth and predominates from 1 to 2 months of age. Nevertheless, some key features of the foal immune system can limit its ability to defend

against infection. A critical factor is antigen-specific and nonspecific immuno suppression resulting from transferred maternal antibody. As the foal ages, the continuing immunomodulatory effect of maternal antibody may limit foal immunoresponsiveness while no longer providing comprehensive protection itself. Of similar importance is the fact that although the lymphocytic immune system is complete from the time of birth, it is naïve. Neonates can mount normal immune responses but require appropriate presentation of antigen and co-stimulatory signals.[119] Antigen presentation in the absence of co-stimulatory second signals from (for example, T helper cells) can induce immune deviation or a failure to mount the appropriate immune response, and particularly so in neonates.[120–122] The absence of memory responses and a well-developed repertoire of immune responses is a serious handicap that only appropriate antigenic encounters can overcome.

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1.2 1.2—Hypersensitivity and Autoimmunity

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Hypersensitivity refers to an altered state of immunoreactivity resulting in self-injury. Four different types of hypersensitivity are defined by the type of immunologic process underlying the tissue injury, as originally proposed by Coombs and Gell.[1] Table 1.2-1 presents the general features of this classification system. The most common and important type of hypersensitivity disease is type I hypersensitivity, mediated by immunoglobulin E (IgE). In these diseases, individuals produce IgE antibodies against a normally innocuous antigen, termed an allergen. Exposure to the allergen triggers mast cell degranulation as described later, and a series of responses result that are characteristic of allergy. Allergic diseases are so important that more is known about the function of IgE in this hypersensitivity disease than about its normal role in host defense. Throughout this chapter, the term *allergy* refers only to type I hypersensitivity diseases mediated by IgE.[2] Other authors may use the term *allergy* to refer to the entire spectrum of hypersensitivity diseases.[3] Other forms of hypersensitivity disease depend on IgG antibodies (type II and III hypersensitivities) or T cells (type IV hypersensitivity). Each of these disease processes can play a role in the immunopathogenesis of autoimmune disease, in which the body mounts an adaptive immune response to self-tissue antigens.

Clinical hypersensitivity diseases such as recurrent airway obstruction (RAO) or purpura hemorrhagica may result
from more than one type of hypersensitivity reaction, limiting the use of this classification for clinical diagnosis. 29
Alternative strategies for classifying these diseases may have greater clinical use. For example, antibody-mediated 30
hypersensitivity diseases (types I, II, and III) are immediate in onset if preformed antibody exists in circulation or tissues, with some variation in time course depending on the antibody isotype involved. Cell-mediated hypersensitivity (type IV) reactions are delayed, even in sensitized individuals, for 1 to 3 days, whereas effector cells are recruited to the site of antigen exposure.[3] The goals of this section are as follows:

- Review the classical hypersensitivity types to explain the immunopathogenesis of hypersensitivity diseases.
- Describe immediate and delayed hypersensitivities of horses and their immunologic basis.
- Identify autoimmune conditions of horses with a known immunologic basis.

Detailed descriptions of clinical aspects of hypersensitivity and autoimmune diseases, their diagnosis and management, are presented elsewhere in this book. Detailed explanations of many immunologic mechanisms involved in these disease processes are provided in Chapter 1.1.

TABLE 1.2-1 The Four Types of Hypersensitivity[*]

	HYPERSENSITIVITY TYPE					
				IV		
Traits	I	II	III			
Immune mediator[†]	IgE	IgG	IgG	TH1	TH2	CTL
Antigen	Soluble antigen	Cell or matrix associated antigen	Soluble antigen in excess (immune complex formation)	Soluble antigen	Soluble antigen	Cell-associated antigen
Effector mechanism	Mast cell degranuation	Fc-receptor–positive cells (phagocytes of reticuloendothelial system)	Fc-receptor–positve cells; complement	Macrophage activation	Eosinophil activation	Cytotoxicity
Examples of hypersensitivity reaction	Systemic anaphylaxis; *Culicoides* hypersensitivity	Penicillin-associated hemolytic anemia	Serum sickness in human being	Equine recurrent uveitis	Chronic *Culicoides* hypersensitivity	Contact dermatitis

* The four types of hypersensitivity can be differentiated by the immune mediator involved, the form of antigen recognized, and the effector mechanism elicited in producing the pathologic response. Equine examples of each condition are given when available.

† *Ig,* Immunoglobulin; *TH,* T helper cell (type 1 or 2); *CTL,* cytotoxic T lymphocyte.

1.2.1 Classical Types of Hypersensitivity Reaction

1.2.1.1 TYPE I HYPERSENSITIVITY

As described previously, type I hypersensitivity, or allergy, is mediated by IgE antibody specific for allergens, which are extrinsic antigens normally not recognized by the healthy immune system.[2] IgE is found predominantly in tissues, where it is bound to mast cells through the high-affinity IgE receptor FcεRI.[4] When antigen binds to IgE on the surface of mast cells, cross-linking two or more IgE molecules and their FcεRI receptors, it triggers the release of chemical mediators from mast cells, which cause type I hypersensitivity reactions. Basophils and eosinophils (when activated) also possess FcεRI receptors and therefore can participate in the same process. In addition to FcεRI receptors, an unrelated low-affinity IgE receptor called CD23 is present on many lymphocytes, monocytes, eosinophils, platelets, and follicular dendritic cells. The role of CD23 appears to be to enhance IgE responses to specific antigens when those antigens are complexed with IgE. Thus CD23 on antigen presenting cells can capture IgE-bound antigens. In the horse CD23 has been identified, and its expression is upregulated by equine interleukin-4 (IL-4).[5]

The selective stimulation of IgE responses depends on characteristics of the antigen (allergen), the individual affected (genetic factors such as major histocompatibility complex [MHC] antigens), and the mechanism of antigen presentation. The antigen must be capable of eliciting a type 2 T helper cell (TH2) immune response to

stimulate IgE production. Small, soluble proteins (frequently enzymes) containing peptides suitable for MHC II antigen presentation and presented to mucosal surfaces at low doses are particularly efficient at generating IgE responses. Low doses of antigen specifically favor TH2 over TH1 responses, and exploiting this relationship is the basis of some therapeutic hyposensitization strategies (see Chapter 1.4). When CD4 T-helper cells are exposed to IL-4, as opposed to IL-12, during antigen presentation by dendritic cells, they are driven toward becoming TH2 cells. This process is critical to promoting IgE responses and may be favored at enteric and respiratory mucosal surfaces or on skin, where parasite invasion typically occurs. This makes teleologic sense, 30
because IgE responses are important for antiparasitic immunity.[6] The dendritic cells at such locations 31
frequently are programmed to stimulate TH2 responses.[2] Cross-linking of FcεRI receptors on granulocytes also results in CD40L expression and IL-4 secretion, which further promotes IgE production by B lymphocytes and sustains allergic reactions.

Some individuals maintain IgE responses to a variety of allergens, and this condition is called atopy. Affected individuals have high levels of IgE in the blood and increased eosinophil populations. In human beings this condition depends partly on genetic factors, including genetic variations in the IL-4 promoter sequence or association with particular MHC II genes. Nevertheless, environmental factors are also important, for atopy is increasingly common in human beings in economically developed parts of the world. Four possible explanations for this are decreased exposure to infectious disease during childhood, environmental pollution, allergen levels, and dietary change. The first explanation currently is favored, and its basis is the proposal that many infectious diseases bias the immune system toward TH1 responses,[2] and that their decreased prevalence results in an increased tendency to mount TH2 responses, which may be the natural bias of the neonatal immune system.[7]

1.2.1.1.1 Effector Mechanisms in Type I Hypersensitivity Allergic Reactions

When triggered by antigen cross-linking of IgE bound to FcεRI cell-surface receptors, activated mast cells release chemical mediators stored in preformed granules and synthesize leukotrienes and cytokines. In type I hypersensitivity reactions the outcome of this reaction can vary from anaphylactic shock to minor localized inflammation. Mast cell degranulation causes an immediate allergic reaction within seconds, but also a sustained late-phase response develops over up to 8 to 12 hours because of recruitment of TH2 lymphocytes, eosinophils, and basophils.

Mast cells are highly specialized cells of the myeloid lineage that are common in mucosal and epithelial tissues near small blood vessels. The range of inflammatory mediators released by degranulating mast cells is extensive and includes enzymes that can remodel connective tissues; toxic mediators such as histamine and heparin; cytokines including IL-4, IL-5, IL-13, and tumor necrosis factor α; chamomiles; and lipid mediators including leukotrienes and platelet-activating factor.[2] Histamine causes an increase in local blood flow and permeability. Enzymes activate matrix metalloproteinases that cause tissue destruction. Tumor necrosis factor α increases expression of adhesion molecules and attracts inflammatory leukocytes. These reactions are all appropriate when the mast cell is reacting to an invading pathogen, but in allergy this response is the basis of the immediate inflammatory response and also the initiating step in the late-phase response.

The role of eosinophils in inflammation is tightly controlled at several levels. Synthesis in the bone depends on IL-5 produced by TH2 cells in the face of infection or other immune stimulation. Transit of eosinophils to tissues depends on two chemokines, eotaxin 1 and eotaxin 2. Activation of eosinophils by cytokines and chemokines induces the eosinophils to express FcεRI and complement receptors and primes the eosinophil

to degranulate if it encounters antigen that can cross-link IgE on its surface. Mast cell degranulation and TH2 activation recruit and activate large numbers of eosinophils at the site of antigen encounter. Basophils are similarly recruited, and together their presence is characteristic of chronic allergic inflammation. Eosinophils can trigger mast cells and basophil degranulation by release of major basic protein. This late-phase response is an important cause of long-term illnesses such as chronic asthma in human beings.

1.2.1.1.2 Clinical Manifestations of Type I Hypersensitivity Reactions Depend on Their Site

The clinical outcome of type I hypersensitivity reactions depends on the amount of IgE present, dose of allergen, and the site of allergen introduction. Direct introduction of allergen into the bloodstream or rapid enteric absorption can lead to widespread activation of connective tissue mast cells associated with blood vessels. This potentially disastrous event is called systemic anaphylaxis and can cause catastrophic loss of blood pressure and airway obstruction because of bronchoconstriction and laryngeal swelling. Anaphylactic shock, for example, can follow administration of drugs against which an individual has an established IgE response. Treatment with epinephrine may control these potentially fatal events.

Penicillin is one example of a drug that can cause type I hypersensitivity reactions in human beings, although the ability of penicillin to induce this type of hypersensitivity reaction in the horse is less certain. Penicillin can act as a hapten (see Chapter 1.1). Penicillin alone can elicit antibody formation by B cells but cannot elicit T helper cell responses because it is not a protein. However, the β-lactam ring of penicillin can react with amino groups on host proteins to form covalent conjugates, and the modified self-peptides can generate TH2 responses in some individuals. The TH2 cells in turn can release cytokines that activate penicillin-binding B cells to produce IgE. In this scenario, penicillin is a B cell antigen and becomes a T cell antigen by modifying self-peptides. Intravenous penicillin results in protein modification and recognition and cross-linking of mast cell IgE leading to anaphylaxis.[2]

Allergen inhalation, in contrast, induces local inflammation of the respiratory tract, for example, in the upper 31
airways as in allergic rhinitis or in the lower airways as in human asthma. Similarly, allergen introduction 32
into the skin causes local histamine release and a wheal-and-flare reaction initially, followed by a late-phase response several hours later. When allergens are ingested and reach the skin from the bloodstream, a disseminated form of the wheal-and-flare reaction occurs that is called urticaria or hives. Prolonged inflammation of the skin results in eczema or atopic dermatitis in some individuals. Ingestion of allergens causes activation of gastrointestinal mast cells resulting in fluid loss across the bowel and smooth muscle contraction. The clinical presentation is diarrhea and vomiting. Sometimes ingestion of allergens can lead to systemic anaphylaxis if allergens are absorbed rapidly or to urticaria, as is sometimes seen after administration of oral penicillin.

1.2.1.2 TYPE II HYPERSENSITIVITY

This form of hypersensitivity disease occurs when the causal antigen is associated with cells or tissue components of the body and an IgG antibody response to this antigen occurs. Phagocytes, or other cells expressing Fcγ receptors, mediate destruction of the affected tissue or removal from the circulation by the reticuloendothelial system in the case of antibody-positive erythrocytes or platelets. Antibody-mediated hemolytic anemia or thrombocytopenia are examples of drug-associated type II hypersensitivities, and in the case of the horse, penicillin is an established cause of hemolytic anemia.[8] Diagnosis can be accomplished using a Coombs' test (Figure 1.2-1). Penicillin binds to the erythrocyte surface and is targeted by antipenicillin

antibodies of the IgG isotype. Interestingly, large numbers of horses have antipenicillin antibodies of the IgM isotype, but this does not lead to disease.

Figure 1.2-1 Direct Coombs' test. *Ab,* Antibody; *RBCs,* red blood cells.

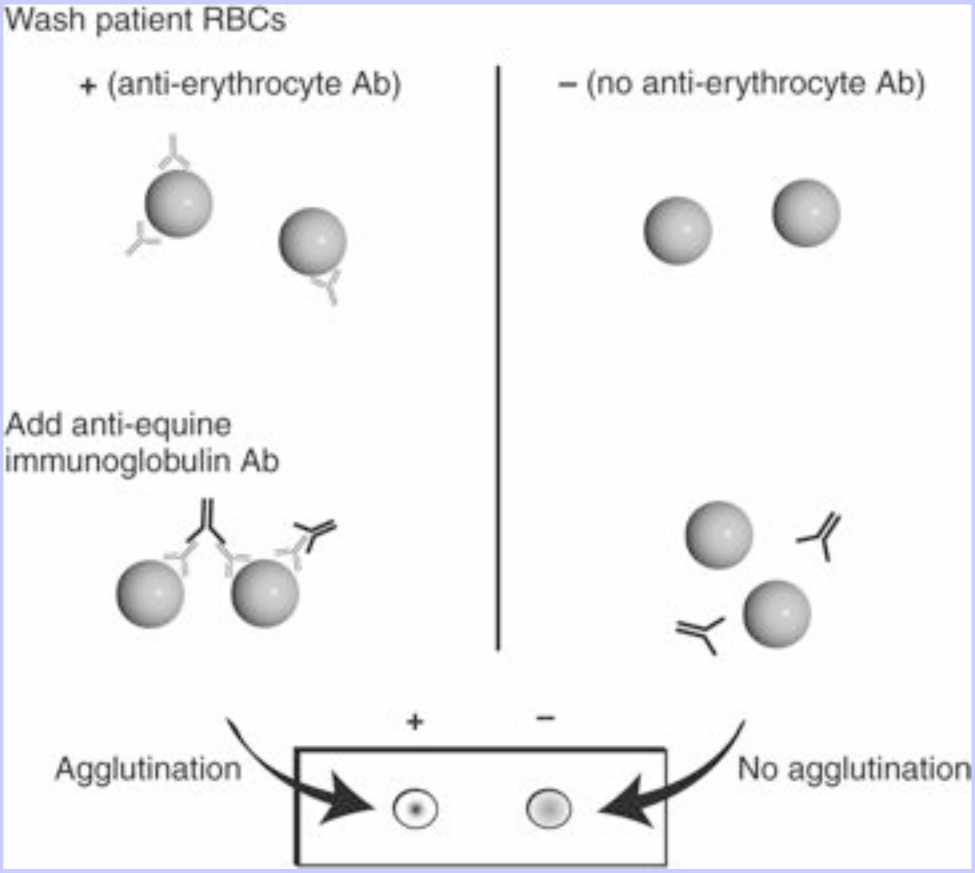
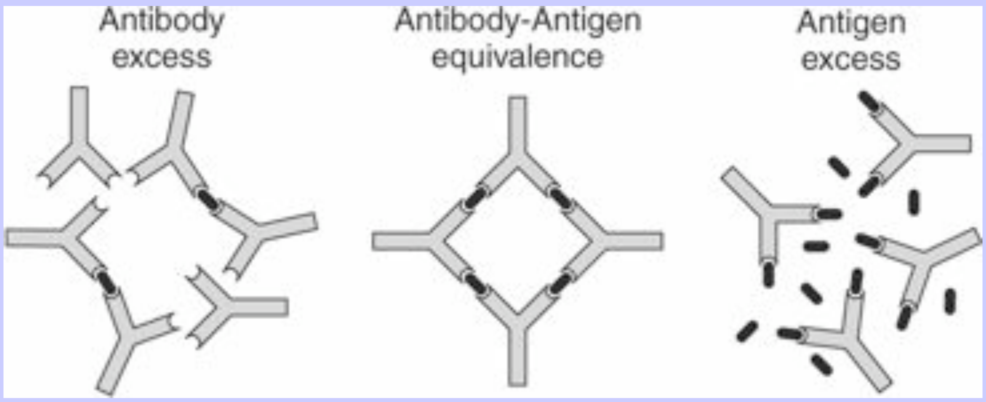


Figure 1.2-2 Antibody-antigen precipitation. Antibody can precipitate soluble antigen in the form of immune complexes. This is most efficient when concentrations of antibody and antigen reach equivalence and large immune complexes are formed. However, when antigen is in excess, some immune complexes are too small to precipitate and can produce pathological changes such as are seen in type III hypersensitivities.



1.2.1.3 TYPE III HYPERSENSITIVITY

In type III hypersensitivity, the antigen is soluble and present in the circulation. Disease results from formation of antibody-antigen aggregates or immune complexes under specific conditions.[2] Although immune complexes are generated in all antibody responses, they are generally harmless. Large complexes fix complement and are removed from circulation by the reticuloendothelial system. However, small complexes can form at antigen excess (Figure 1.2-2), and these can deposit in blood vessel walls and tissues where they ligate Fc receptors on leukocytes, causing an inflammatory response, increased vascular permeability, and tissue injury. Complement activation also contributes to this process. Local injection of antigen sometimes can lead to a necrotizing skin lesion caused by type III hypersensitivity, termed an Arthus reaction.

The classical example of a type III hypersensitivity reaction is serum sickness, which is seen after administration of horse antiserum in human beings, for example, in treating snake bites. After an IgG response to the horse serum is generated (7 to 10 days), signs of fever, urticaria, arthritis, and sometimes glomerulonephritis result. The foreign antigen is cleared as part of this process, which makes this condition ultimately self-limiting. Alternative scenarios for induction of type III hypersensitivity reactions include persistent infectious diseases in which pathogens are not cleared completely from tissues or autoimmune diseases. Inhaled antigens that induce IgG responses can lead to immune complex formation in the alveolar wall, as occurs in farmer's lung, compromising lung function.[2] Any such circumstance in which immune complexes are deposited in tissues can lead to this type of pathologic response. 32

1.2.1.4 TYPE IV HYPERSENSITIVITY

Cell-mediated type IV hypersensitivities cause delayed hypersensitivity reactions. A variety of cutaneous hypersensitivity reactions are seen, such as the contact hypersensitivity seen after absorption of haptens such as pentadecacatechol in poison ivy or the local TH1 response seen in the diagnostic tuberculin reaction. When type IV hypersensitivity results in a TH2 response, the principle outcome is eosinophil activation and recruitment such as in chronic asthma.

1.2.2 Immediate Hypersensitivity Diseases

A major limitation in the ability to study hypersensitivity disease in the horse has been the limited availability of reagents capable of detecting equine IgE. Although equine IgE has long been known to exist,[9,10] and the genetic sequence has been known since 1995,[11–13] the only reagents for studying it have been conventional polyclonal antisera produced by vaccination with physicochemically purified IgE[14,15] or made in chickens after vaccination with recombinant fragments of the IgE heavy chain.[16] Although many valuable studies have been performed using these reagents,[17–20] the development of well-characterized monoclonal antibodies recognizing equine IgE remains a critically important goal for equine immunologists. At the time of writing, this goal seems likely to be realized soon by a number of groups,[21] and future clinical studies and investigations should benefit from this advance.

The following section describes a series of equine diseases with characteristics of immediate hypersensitivity disease. The list of equine hypersensitivity diseases is not exhaustive, and additional examples are found throughout this book.

1.2.2.1 SYSTEMIC ANAPHYLAXIS

The incidence of true systemic anaphylaxis in horses is unknown, although the condition has been reported in association with administration of a wide range of compounds including serum, vaccines, vitamin E–selenium preparations, thiamine, iron dextrans, and antibiotics including penicillin.[22,23] Target organs in experimental equine anaphylaxis are the lung and the intestine.[22] Sudden dyspnea; hypotension, as evidenced by poor peripheral pulse character; rapid onset of urticaria; and collapse are cardinal signs of the onset of systemic anaphylaxis.

The therapeutic goals in treating systemic anaphylaxis are to prevent or reverse the complications caused by mediator release, maintain respiratory integrity, and maintain cardiovascular stability. Not all anaphylactic reactions require therapy. However, rapid recognition of those that do is critical to patient survival. Intravenous access via an indwelling catheter and airway patency should be assured immediately because cardiovascular collapse and upper airway obstruction caused by angioedema can occur rapidly. The conscious horse does not tolerate tracheal intubation, so emergency tracheotomy may be required. Oxygen should be administered if available because bronchoconstriction and cardiovascular collapse result in hypoxemia. The fluid requirement of horses in anaphylactic shock is not known, but large volumes of balanced polyionic fluid should be administered rapidly.

The principal therapeutic agent is epinephrine, which is a potent sympathetic stimulant. Epinephrine administration may cause excitement in the horse. Epinephrine should be administered intramuscularly (10 to

20 μg/kg, equivalent to 5 to 10 ml of 1:1000 dilution of epinephrine for a 450-kg horse) if dyspnea or hypotension are mild. Epinephrine should not be administered subcutaneously because its potent vasoconstriction can lead to poor absorption and tissue necrosis. If dyspnea or hypotension is severe, epinephrine should be administered intravenously or endotracheally if no venous access is available (3 to 5 μg/kg or 1.5 to 2.25 ml of 1:1000 dilution of epinephrine for a 450-kg horse). Epinephrine doses can be repeated every 15 to 20 minutes until hypotension improves. The side effects of epinephrine therapy are tachyarrhythmias and myocardial ischemia, which in themselves can be life threatening. Alternatively, an epinephrine or norepinephrine drip can be used in cases of refractory hypotension. Other therapeutic agents such as antihistamines, β-agonists, or other pressors may be indicated, although their value is less certain. Though the effects may be delayed, glucocorticoid therapy is indicated to help reverse persistent bronchospasm and angioedema and to break the cycle of mediator-induced inflammation triggered during hypersensitivity reactions. Ideally, a rapid-acting glucocorticoid such as prednisolone sodium succinate (0.25 to 10.0 mg/kg intravenously) should be used. Glucocorticoid therapy during the acute phase aids in preventing the late-phase reaction.

1.2.2.2 INSECT HYPERSENSITIVITY

Horses commonly suffer from hypersensitivity to salivary antigens of *Culicoides* and *Simulium* species, leading to an intensely pruritic skin disease with characteristics of immediate and delayed-type hypersensitivity.[24] The clinical sign of urticaria, combined with increased numbers of IgE positive cells in the skin and high levels of *Culicoides*-specific IgE in serum, are evidence of immediate (type I) hypersensitivity in the immunopathogenesis of this disease.[17,18] In some breeds a genetic predisposition based on an MHC-linkage has been demonstrated.[25,26]

1.2.2.3 RECURRENT AIRWAY OBSTRUCTION

RAO is a severe inflammatory disease of middle-aged and older horses induced by exposure of susceptible 33
horses to inhaled organic dust, generally from hay, although a summer pasture-associated form also is observed 34
in the southern United States.[27] Hay dust contains a mixture of mold spores, forage mites, particulates, and endotoxins, which can induce and exacerbate airway inflammation. Removal of the hay dust by returning the horse to pasture leads to decreased inflammation within a few days. In RAO-susceptible horses, exposure to hay dust leads to invasion of the lungs and airways by neutrophils within 4 to 6 hours and concurrent airway obstruction caused by bronchospasm, inflammation, and increased mucus viscosity, which principally affect the bronchioles. RAO-affected horses develop nonspecific airway hyperrespon siveness, bronchospasm, in response to a variety of stimuli including inflammatory mediators and neurotransmitters. Horses affected by RAO demonstrate increased histologic lesions and worsening airway function with increasing age. In addition, significant histopathologic changes are present before abnormal airway function can be detected.

The immunologic basis of RAO remains poorly elucidated. Two pieces of evidence suggest a type I hypersensitivity basis to this disease. First, IgE levels are increased in bronchoalveolar fluid of RAO-affected horses,[14] and second, allergen-specific IgE is increased in affected horses.[20,28] However, the immediate onset of airway obstruction typical of a type 1 reaction to exposure to allergens is never observed because clinical signs are only apparent several hours after antigenic exposure.[27] A number of immunologic processes, including IgE-mediated pathologic processes, may be involved in this disease. A study of immunoregulatory cytokines in RAO using in situ hybridization demonstrated evidence for a TH2 bias in RAO, with increased levels of IL-4 and IL-5 and decreased interferon-γ messenger RNA in bronchoalveolar cells.

Evidence indicates that the neutrophilic inflammation characteristic of RAO in horses is induced by IL-8 production, and IL-8 levels in bronchoalveolar fluid are increased in RAO-affected horses.[29,30] When RAO-susceptible horses are antigen challenged, peripheral blood and airway neutrophils are primed, as demonstrated by increased superoxide production and respiratory burst activity. In airways, neutrophil degranulation products are increased, including neutrophil elastase and matrix metalloproteinase 9 (MMP-9). Much of the neutrophil elastase is inactive, and much of the MMP-9 is active. However, in contrast to human chronic obstructive airway disease, airway remodelling in RAO-affected horses is minimal. During the resolution of RAO, apoptosis of airway neutrophils is increased, and consequently neutrophil function and the resulting tissue damage are reduced. In contrast, during exacerbations of RAO, neutrophil apoptosis is reduced, possibly through cytokine-mediated upregulation of NF-κB.[31]

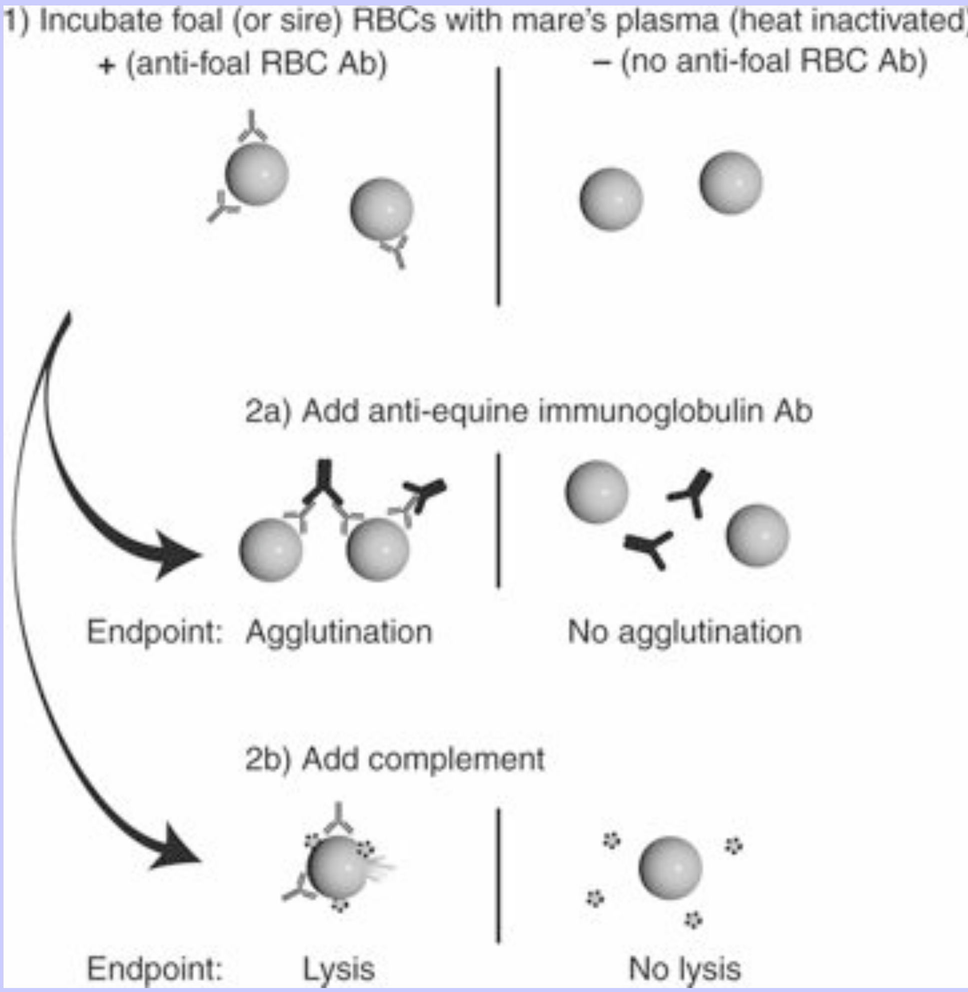
1.2.2.4 IMMUNOGLOBULIN G–MEDIATED DISEASES

These conditions, which broadly correspond to type II and III hypersensitivities, also have been termed *immune-complex diseases* in the horse.[3] The examples described subsequently are distinguished from other immediate hypersensitivities of the horse described previously in that no evidence exists for the involvement of IgE in their pathogenesis.

1.2.2.4.1 Neonatal Isoerythrolysis and Alloimmune Thrombocytopenia

Neonatal isoerythrolyis is a common condition of foals and is reviewed extensively elsewhere in this text. The condition results from the passive transfer of maternal antibodies in colostrum that recognize allogenic foal erythrocyte antigens principally of the Aa and Qa haplotype inherited from the sire. A similar condition occurs in mules because of inheritance of a donkey-specific erythrocyte antigen.[32,33] A severe, potentially life-threatening anemia results as the antibody-positive erythrocytes are removed by the reticuloendothelial system or, less commonly, are lysed by complement. A similar condition less commonly affects platelets, causing severe neonatal thrombocytopenia in horses[34] and mules.[35] These conditions are typical of type II hypersensitivities and are mediated by circulating IgG recognizing cell surface antigens on erythrocytes. Diagnosis can be performed using a variation of the Coombs' test (Figure 1.2-3).

Figure 1.2-3 Neonatal isoerythrolysis test. *Ab,* Antibody; *RBCs,* red blood cells.



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1.2.2.4.2 Purpura Hemorrhagica

Purpura hemorrhagica is an acute disease of the horse characterized by edema of the head and limbs; leukocytoclastic vasculitis; petechial hemorrhages in mucosae, musculature, and viscera; and sometimes glomerulonephritis. The condition usually is associated with *Streptococcus equi* infection of the upper respiratory tract. Serum of affected horses contains immune complexes of *S. equi*–specific antigens with equine IgA.[36] The glomerulonephritis sometimes seen with purpura has been attributed to deposition of similar immune complexes containing streptococcal antigens and IgG.[37]

1.2.3 Delayed Hypersensitivity Diseases

Documented immunologic characterization of delayed hypersensitivity conditions of the horse are lacking, although contact hypersensitivities have been reported in horses.[3] One well-characterized example of this type of condition is recurrent uveitis.

Equine recurrent uveitis, also known as moon blindness or periodic ophthalmia, is the most important cause of blindness in horses.[38] The disease results in acute and chronic ocular inflammatory disease, and chronic sequelae include development of posterior and anterior synechiae, cataracts, lens opacities, secondary glaucoma, and blindness. Eyes of affected horses contain IgG antibodies and autoreactive T cells specific for retinal anti gens.[39] A specific cause has not been identified. However, sensitization to a variety of pathogens, in particular to *Leptospira* spp.,[40,41] is thought to induce the immune-mediated pathologic response that is central to the disease.[42] Treatment with corticosteroids and other antiinflammatory agents is essential to avoid visual debility or blindness. However, treatment failures are common, and the disease frequently recurs with further ocular damage months after the initial event, commonly leading to euthanasia.[38]

The understanding of the immunological basis of equine recurrent uveitis has been extended by studies of the immunoregulatory events in the eyes of affected horses. The T lymphocytes that invade the iris and ciliary body during this disease produce a pattern of interferon-γ cytokine production typical of a TH1 response.[43] These studies indicate that equine recurrent uveitis is an example of a type IV hypersensitivity disease mediated by TH1 cells.

1.2.4 Autoimmunity

Although a number of equine diseases are considered to be autoimmune in origin, few have been extensively studied.[44] Much of the explanation given previously for the immunopathologies involved in hypersensitivity disease can be applied to autoimmune disease. Well-described equine autoimmune disorders include neonatal isoerythrolysis, alloimmune thrombocytopenia, and immune-mediated anemia of adults, which have characteristics of type II hypersensitivities.[45] Less well-described entities include systemic lupus erythematosus[46] and pemphigus foliaceus,[47] which have characteristics of type II and III hypersensitivity diseases. As described previously, equine recurrent uveitis appears to represent a type IV hypersensitivity, and some morphological and immunological evidence exists for similarly classifying cauda equina syndrome (polyneuritis equi).[48–50]

With the exception of a few conditions, such as neonatal isoerythrolysis and penicillin-associated hemolytic anemia, the cause of few autoimmune conditions is well understood. One exception, however, is the anemia that can develop following administration of human recombinant erythropoietin to horses.[51,52] Substantial evidence indicates that horses mount an antibody response to the exogenous erythropoietin that cross-reacts with the endogenous hormone, resulting in erythroid hypoplasia. The lesson of this example may be that in the modern world, with increasing availability of recombinant drugs that mimic natural biological compounds, one would do well to remember that the immune system has an exquisite ability to distinguish what is foreign and to reject it vigorously.

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1.3

1.3—Immunodeficiency

D. Paul Lunn

David W. Horohov

Immunodeficiencies occur in primary and secondary forms and recently have been reviewed extensively.[1,2] Primary immunodeficiencies have a genetic basis, whereas secondary immunodeficiencies result from failure of passive transfer in foals, immunosuppressive infections or drug treatments, neoplasia, or malnutrition. Immunodeficiencies

can affect specific components of the immune system, such as the lymphoid or phagocytic system. Typically immunodeficiency is suspected in any of the following circumstances[3]:

1. Onset of infections in the first 6 weeks of life
2. Repeated infections that respond poorly to therapy
3. Infections caused by commensal organisms or organisms of low pathogenicity
4. Disease resulting from the use of attenuated live vaccines
5. Failure to respond to vaccination
6. Marked neutropenia or lymphopenia that persists for several days

Equine immunodeficiency is suspected most commonly for the first three reasons, that is, because of increased susceptibility to infections. The most common immunodeficiency recognized in clinical practice is failure of passive transfer in foals.[4–6] Other causes of immunodeficiency vary from well-defined clinical entities, such as severe combined immunodeficiency of Arabian foals,[7] to cases in which immunodeficiency is suspected on clinical grounds but the specific cause or nature of the problem is difficult or impossible to define.[8] Regardless of their cause, immunodeficiencies result in increased susceptibility to infections that respond poorly to appropriate therapy. Defects in antibody production tend to predispose horses to pyogenic infection, whereas deficiencies in cell-mediated responses lead to infections with organisms normally not pathogenic in horses, such as *Candida albicans, Cryptosporidium* spp., or adenovirus. When any immunodeficiency is suspected, specific diagnostic tests are indicated to define the deficiency. The aim of the next section is to identify tests clinicians can apply practically in such cases and to explain their merits and limitations.

TABLE 1.3-1 Components of the Immune System and Tests for Quantitative or Functional Analyses[*]

COMPONENT	QUANTITATIVE TESTS	FUNCTIONAL TESTS
Immunoglobulin	**Radial immunodiffusion, membrane ELISA,[†] electrophoresis, precipitation tests[‡]**	**Response to vaccination**
Lymphocytes	**Complete blood cell count, DNA-PK$_{CS}$ genetic evaluation,** [§] FACS analysis of lymphocyte subsets using monoclonal antibodies	**Response to vaccination, intradermal PHA test,** in vitro lymphoproliferation assays
Neutrophils and macrophages	**Complete blood cell count**	Chemiluminesence and bactericidal assays; flow cytometric evaluation of phagocytosis and oxidative burst
Eosinophils and basophils	**Complete blood cell count**	No commonly available tests
Complement	No commonly available tests	No commonly available tests
Acute phase proteins	Electrophoresis	No commonly available tests

* The table lists components of the immune system that can be evaluated in horses and appropriate tests for quantitative or functional analyses of each component. The list is not exhaustive and is restricted to tests of likely practical value for which normal data is available. Tests in boldface type are routinely available to clinicians.

† *ELISA,* Enzyme-linked immunosorbent assay; *DNA-PK*$_{cs}$, DNA-protein kinase catalytic subunit; *FACS,* fluorescence-activated cell sorter; *PHA,* phytohemagglutinin.

‡ For example, zinc sulfate turbidity and glutaraldehyde coagulation.

§ See the section on severe combined immunodeficiency for a description of DNA-protein kinase catalytic subunit genetic testing.

1.3.1 Tests of Equine Immune Function

Tests of components of the immune system (e.g., lymphocytes and immunoglobulins) generally can quantitate that component or measure its functional capacity. Table 1.3-1 identifies the components of the immune system that currently can be analyzed in this manner and lists the corresponding quantitative and functional tests. Table 1.3-1 also identifies those tests that are likely to be commercially available. Few of the functional tests described are available unless the clinician is able to identify a sympathetic and capable equine immunologic research 37
laboratory. Despite these limitations, the available tests permit the identification of many of the well-defined 38
causes of immunodeficiency in horses.

1.3.1.1 TESTS OF ANTIBODY-MEDIATED IMMUNITY

Some assays of B lymphocyte function and number are described next; however, the principal tests of humoral immunity are quantitative assays of immunoglobulin concentration and measurements of specific antibody responses to vaccination. The variety of classes of immunoglobulins in the horse is complex and was reviewed earlier in this chapter.[9] For practical purposes, this section generally focuses on immunoglobulin G (representing the combination of two subclasses: IgGa and IgGb), IgG(T), IgA, and IgM.

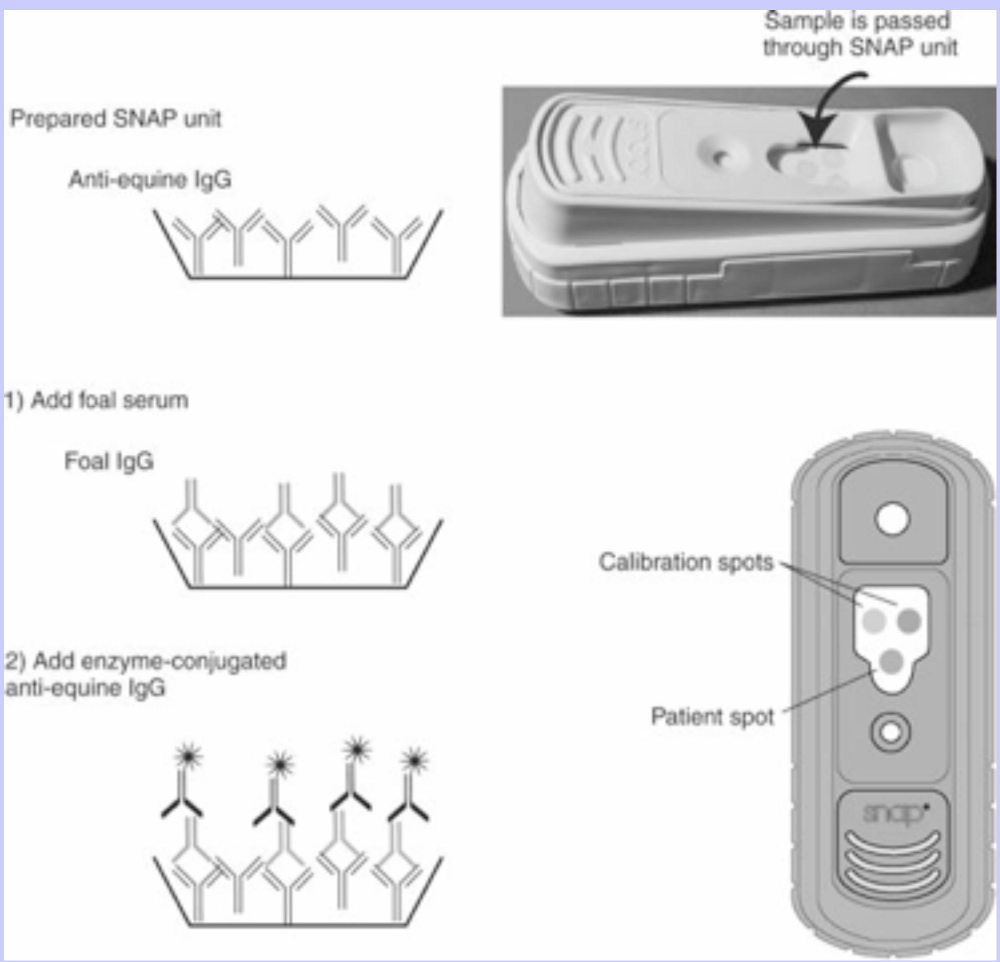
The current gold standard for measurement of concentrations of immunoglobulin classes is the radial immunodiffusion (RID) assay. The disadvantages of this test are cost and time required to perform the assay (24 hours), which makes it generally unsuitable for screening for failure of passive transfer of immunity in foals. Nevertheless, this form of test remains the single most valuable assay available to the clinician trying to measure total antibody concentrations in the horse. Currently, test kits are available for IgG, IgG(T), IgA, and IgM (VMRD Inc., Pullman, Washington). Specific IgG subclass RID tests—including IgGa, IgGb, IgGc, and IgG(T)—are available for research use (Bethyl Laboratories, Montgomery, Texas). The RID test is based on the ability of antigen and antibody to precipitate at equivalence when combined in proportion in agar gel plates. The serum being tested is added to punched-out wells in agar impregnated with antibody to the specific immunoglobulin class being measured and is allowed to diffuse outward and bind with the anti–class-specific antisera. A precipitate forms when equivalence is reached and the area within the precipitate ring is directly proportional to the concentration of the patient's immunoglobulin class. Normal ranges of serum immunoglobulin concentrations are typically provided with commercial kits, and normal serum, milk, and colostrum concentrations of equine immunoglobulins have been described in numerous published studies. These results have been summarized and are available in tabular form in two sources[10,11] and at the VMRD Web site *(http://www.vmrd.com/RID/Eridinf.htm)*. However, one should note that the original literature cited in developing these normal ranges typically was published 15 to 20 years ago. More recent studies of foal and

adult horse serum IgG and IgM concentrations using currently available RID assays measured considerably higher normal values in some instances.[12,13] In addition, an extensive study of immunoglobulin concentrations in adult and foal serum and nasal secretions and in colostrum and milk using an experimental monoclonal antibody–based system has been reported.[14]

The most common question that a clinician seeks to answer regarding the immune status of a horse is whether a foal has achieved adequate passive transfer of immunity. Whatever test is chosen should be able to distinguish serum concentrations of IgG of less than 200 mg/dl, 200 to 400 mg/dl, 400 to 800 mg/dl, and greater than 800 mg/dl to permit diagnosis of total or partial failure of passive transfer. The test should be rapid to allow early initiation of therapy. A variety of tests have been used for this purpose: zinc sulfate turbidity, latex bead agglutination tests, enzyme-linked immunosorbent assay (ELISA), turbidometric analysis, or glutaraldehyde coagulation.[15,16] Addition of serum to zinc sulfate solution causes precipitation of immunoglobulins, principally IgG. Although the degree of resultant turbidity is usually proportional to the IgG concentration, turbidity may be increased by hemolysis in the sample, poor operating conditions, and poor-quality reagents. In the glutaraldehyde coagulation test, glutaraldehyde forms insoluble complexes with basic proteins in the serum.[17] Gel formation in 10 minutes or less is equated with a serum IgG concentration of 800 mg/dl or greater, whereas a positive reaction in 60 minutes indicates at least 400 mg IgG/dl serum. Like the zinc sulfate turbidity test, hemolysis may cause overestimation of the IgG concentration. In the latex agglutination test (Foalcheck, Haver Mobay Corp., Shawnee, Kansas), the patient's serum is mixed with the antiequine IgG absorbed in latex particles. Macroscopic agglutination is proportional to serum IgG. Currently, for rapid diagnosis the most convenient test system may be membrane-filter–based ELISA systems (e.g., SNAP, Idexx, Westbrook, Maine; Figure 1.3-1). This test can be performed on site with whole blood. Tests such as the glutaraldehyde coagulation test are simpler and cheaper, although they have the disadvantage that serum is required. Although data suggests that the glutaraldehyde coagulation test may be more sensitive than membrane-filter ELISAs in detecting failure of passive tranfer,[16,18] particularly in differentiating normal foals (>800 mg/dl IgG) from partial failure of passive transfer (400 to 800 mg/dl), specificity can be poor. The latter problem affects many of the rapid diagnostic tests for failure of passive transfer, and a more extensive discussion of test selection for this condition is presented in the section covering this disease.

Alternative available tests that give information about serum immunoglobulin content include electrophoresis and immunoelectrophoresis. Immunoelectrophoresis can demonstrate the presence of all currently recognized equine immunoglobulin classes. However, the test has a poor sensitivity and gives no quantitative information, such as might be obtained from rocket electrophoresis.[19] Serum electrophoresis gives quantitative information 38
about albumin, α-, β-, and γ-globulin concentrations (Figure 1.3-2), and its utility is demonstrated in detecting 39
the monoclonal gammopathies that accompany plasma cell myelomata.[20] Nevertheless, in the diagnosis of immunodeficiencies, electrophoresis should be viewed as an adjunct to RID assays, which are superior in terms of specificity and sensitivity.

Figure 1.3-1 Membrane-based enzyme-linked immunosorbent assay system (SNAP, Idexx) for measuring serum immunoglobulin G concentration. The diluted test equine serum sample is applied to a "patient spot" on a membrane impregnated with a capture antibody recognizing equine IgG. Calibration spots corresponding to specific concentrations of equine IgG (400 and 800 mg/dl) are adjacent to the patient spot. An enzyme-conjugated second antibody against equine IgG is applied to the entire membrane, and finally the device is triggered to release an enzyme substrate that produces a colored reaction corresponding to the amount of enzyme-conjugated antibody on the membrane. By comparison with the calibration spots, the concentration of IgG in the test sample may be estimated.



1.3.1.2 TESTS OF CELLULAR IMMUNITY

The simplest test of the cellular arm of the immune response is a total and differential white blood cell count, and this should be the starting point for any evaluation. Identification of an absolute lymphopenia, for example, is a critical finding in a suspected case of severe combined immunodeficiency (SCID) in an Arabian foal, although the result must be repeatable in a series of tests, given the variability of blood lymphocyte counts. Such a finding logically would lead to genetic testing to confirm the diagnosis.[7] The evaluation of a lymph node biopsy for normal architecture, including the presence or absence of cells in the B lymphocyte– or T lymphocyte–dependent areas, is another powerful test of the immune system. However, in profound immunodeficiencies such as SCID, lymphoid organs may be impossible to locate ante mortem. Beyond these readily available conventional techniques, three other more complex types of analysis can be valuable: flow cytometric analysis (primarily of lymphocytes, although other cell types can be analyzed), lymphocyte function testing, and functional analysis of phagocytic cells. 39

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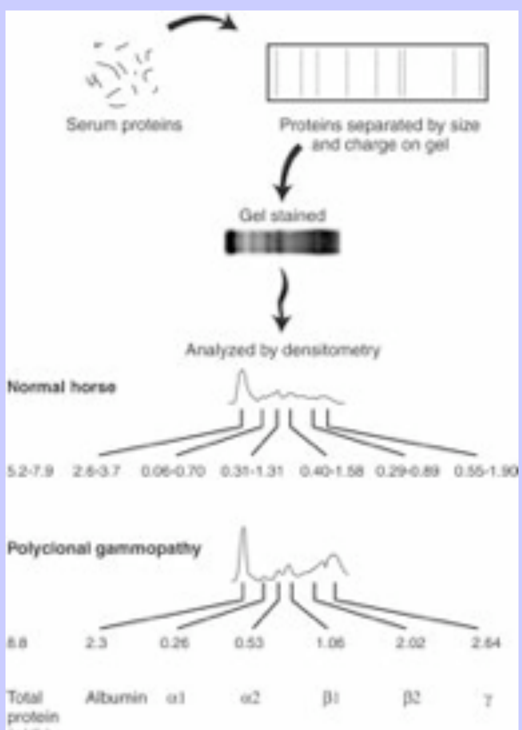
Figure 1.3-2 Serum protein electrophoresis. The complex mixture of serum proteins is separated by migration through an agarose gel slab in response to an electric field. Proteins are stained, and the intensity of staining of different bands is measured by densitometric scanning. These measurements are used to identify different types of globulins and albumin corresponding to stained bands.



1.3.1.2.1

Flow Cytometry

Taking the equine differential white blood cell count a step further is currently feasible because monoclonal antibodies are now available that can differentiate the morphologically identical equine lymphocyte family into distinct subsets with specific functions.[21] Many of these reagents and their sources are listed on the Equine Immunology Resources Web page: *http://www.vetmed.wisc.edu/research/eirh/home.html*. Flow cytometry allows rapid measurements to be made of individual cells in a fluid stream. Flow cytometers use lasers to measure multiple parameters including light scatter and fluorescence characteristics of cells and are complex instruments to construct, but the principles of their operation are simple (Figure 1.3-3). The fluidics system of the flow cytometer delivers cells one-by-one to a point in space intersected by a laser beam. The laser beam emits light of a defined wavelength to illuminate the cell, which results in scattered light of the same wavelength and fluorescent light of a different wavelength that is collected by photodetectors and converted into electronic signals.

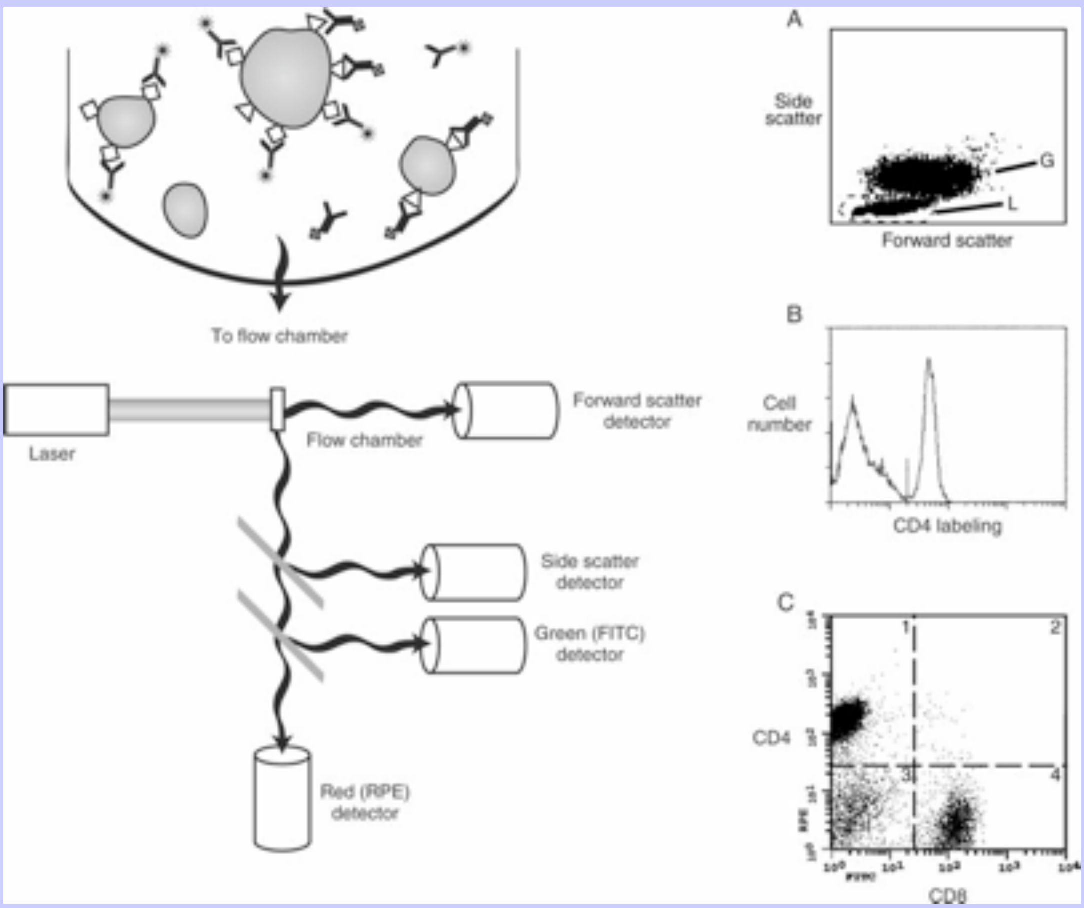
A forward collection lens collects light on the side of the flow chamber opposite the laser source. Light scattered from 1 to 20 degrees from the laser beam axis is collected as “forward scatter,” and the amount depends on the size of the cell being analyzed. Light scattered at 90 degrees (orthogonal) to the laser beam path is collected for the purpose of measuring “side-scatter” and fluorescent emission. Optical filtration separates scattered light and fluorescent light to permit independent measurement. Side scatter light depends on the granularity of cells. Fluorescent light can be detected independently for a number of fluorochromes of different wavelengths; typical examples include fluorescein and phycoerythrin.

Signals from the different detectors can be processed directly or after logarithmic amplification. The advantage of logarithmic amplification for the fluorescent signals is amplification of weak signals and compression of strong signals allowing their simultaneous display. By this means, signals with a 10,000-fold difference in intensity can be displayed. Signals typically are displayed as histograms or dual parameter correlated plots (dot plots), and statistical analysis is completed by computer. Histograms are analyzed by setting markers in particular channels. Dot plots are generated by drawing rectangular or polygonal boxes around data points. The software also allows the setting of gates for determining which events are collected or which events are to be included in later analyses. Typically these gating techniques use forward and side scatter to differentiate cell types, such as lymphocytes, monocytes, and granulocytes. The final key characteristic of flow cytometers is their capacity to analyze large numbers of events (cells) in a short time, making it possible to analyze many thousands of cells in a matter of seconds.

Figure 1.3-3 shows an example of such an analysis. In this instance the goal was to identify lymphocytes expressing the equine homologues of the CD4 and CD8 molecules using two monoclonal antibodies independently labeled with the fluorescein isothiocyanate (CD4) and phycoerythrin (CD8) fluorochromes. For this analysis a peripheral blood leukocyte population was prepared by simply lysing the erythrocytes in a blood sample using distilled water. Subsequently, the whole leukocyte population was stained in solution using the monoclonal antibodies. Alternatively, lymphocytes could have been purified from blood using
differential centrifugation techniques before staining. However, identical analytic results are obtained in the 40
horse using the more rapid technique of whole leukocyte preparation and exploiting the capacity of the flow 41
cytometer to distinguish lymphocytes from other cells.[22] During the flow cytometric analysis, the first step was to identify the lymphocytes using the forward and side scattering characteristics of different leukocyte populations. Part A of Figure 1.3-3 shows a dot plot of forward scatter versus side scatter, with each dot representing a cell. Granulocytes *(G)* can be distinguished from lymphocytes *(L)* by their greater granularity (side scatter) and size (forward scatter). The dotted line is a gate drawn around the lymphocyte population.

Subsequent analysis of fluorescence is directed only toward cells that fall within this gate. After establishing the physical characteristics of the cells to be analyzed, their fluorescence can be examined. In part B of Figure 1.3-3 a histogram depicts CD4 (fluorescein isothiocyanate) staining. A vertical marker identifies a gate set based on staining by a negative control antibody. Therefore all cells to the right of this marker are staining positively for CD4. For two-color staining, dot plots are again used. In part C of Figure 1.3-3, each dot represents a cell, and its position relative to the two axes illustrates its staining characteristics; the dotted lines represent the cutoff points for negative or positive staining. Therefore all cells in quadrants 1 and 2 are positively stained for CD4 and all cells in quadrants 2 and 4 are positively stained for CD8. Effectively, quadrant 1 contains all the helper T lymphocytes ($CD4^+$), quadrant 4 contains all the cytotoxic T lymphocytes ($CD8^+$), quadrant 3 contains the B lymphocyte population, and quadrant 2 is empty because no double-positive T lymphocytes are present in the blood of horses. The sum of the cells in quadrants 1 and 4 represents the T lymphocyte population.

Figure 1.3-3 Flow cytometric analysis (see text for key). *FITC,* Fluroscein isothiocyanate; *RPE,* R-phycoerythrin.



Flow cytometry has revolutionized immunobiologic studies in recent years, finding its most obvious clinical application in enumerating human $CD4^+$ lymphocytes in cases of acquired immune deficiency[23] and in 41
classifying leukemia and lymphoma.[24,25] A large number of antibodies are now available for use in the 42

horse,[21] and recent reports describe the use of flow cytometry to chart the response to microbial infection[12,26,27] and to differentiate equine leukemia.[25,28] Several recent studies provide examples of normal values for peripheral blood analysis.[12,13,29,30]

1.3.1.2.2 Lymphocyte Function Testing

Unfortunately, tests of lymphocyte function generally are limited in their availability in the field. In vitro tests of lymphocyte function include lymphocyte proliferation responses to mitogens such as pokeweed (B cell dependent), phytohemagglutinin (T cell dependent), or concanavalin A (T and B cell dependent). These assays are generally not commercially available, although they commonly are performed by immunologic researchers. In addition, some caution should be used because inferences about the intact animal drawn from the results of these in vitro tests may not always be valid.[3] Because of significant variability in results, performing parallel studies on suitable age-matched control horses is essential. The end point of these tests usually is read by determining the incorporation of radioactive tritium-thymidine into the total population of proliferating cells. Nonradioactive alternatives exist, and one strategy uses intracellular labeling of lymphocytes with 5-carboxyfluorescein diacetate-succinimidyl ester. Labeled cells fluoresce, and after subsequent divisions in response to mitogens, this fluorescence decreases by half for each cycle of cell division. Labeling allows for measurement of equine lymphocyte proliferation using flow cytometry, and simultaneous two-color staining allows for measurement of proliferation in specific lymphocyte subsets.[31]

Two tests that can be valuable and are readily available in practice are response to vaccination, as measured by rising serum titers, and response to intradermal phytohemagglutinin, which depends on a delayed-type hypersensitivity T lymphocyte response and develops in normal animals without prior sensitization.[32] A 50-μg dose of phytohemagglutinin in 0.5 ml of phosphate-buffered saline is injected intradermally, while 0.5 ml of phosphate-buffered saline is administered intradermally at a distant site. At the phytohemagglutinin site, an increase in wheal size of 0.6 mm or less indicates a defect in cell-mediated immunity. Response to vaccination has proved to be a potent means of identifying immunodeficiency in conditions such as juvenile llama immunodeficiency syndrome.[33] Similarly, the equine immune response to a polyvalent inactivated bovine vaccine has been used to document the immunosuppressive effects of corticosteroid administration.[34] For practical purposes, response to rabies or tetanus vaccination may be the most suitable available test provided that no routine vaccination had been administered in the immediate past. Equine rabies or tetanus antibody titer determination is typically commercially available, and the majority of available vaccines are sufficiently potent to provoke a fourfold increase in titer in normal horses.

1.3.1.2.3 Phagocyte Function Testing

Testing of equine neutrophil migration, phagocytic function, and bactericidal activity has been reported by several investigators.[35–38] The techniques used are typically only available in research laboratories and are not well adapted to investigations of individual animals unless adequate age-matched control animals also are examined. More quantitative information may be obtained by adapting assay systems to flow cytometric analysis. Two reports describe flow cytometric analysis of neutrophil phagocytosis of fluorescent microspheres[39] or yeast cells.[40] More recently, Raidal, Bailey, and Love[41,42] have described testing of alveolar macrophage and blood neutrophil phagocytic function using fluorescent-labeled bacteria and oxidative burst activity using oxidation of dichlorofluorescein. These flow cytometric approaches have great

promise, and have been applied to various studies including measurements of the effect of age[43–45] and of exercise[46] on neutrophil function.

1.3.2 Tests of Innate Immunity

Components of the innate immune response that have been measured in the horse include the numbers of granulocytes and monocytes in peripheral blood and their phagocytic function (see the previous discussion), natural cytotoxicity in terms of lymphokine-activated killer cell activity,[36,47,48] and measurement of soluble factors including several acute phase proteins[49–53] and complement. Equine complement activity can be measured using a hemolytic assay in which antibody-sensitized chicken erythrocytes are used as target cells, and the amount of serum required to lyse 50% of these targets is expressed as CH_{50} units.[54] More recently a flow cytometric assay has been described.[55] These tests have been used in some instances to detect relative immunodeficiency in terms of lymphokine-activated killer cell activity in exercised horses[48] or complement activity in foals.[56] Currently, these techniques have limited availability.

1.3.3 Primary Immunodeficiencies

1.3.3.1 SEVERE COMBINED IMMUNODEFICIENCY

SCID is a lethal primary immunodeficiency, affecting Arabian foals, human beings, mice, and dogs; characterized by failure to produce functional B and T lymphocytes; and resulting in lack of any antigen-specific immune responses.[1,57,58] The majority of affected foals are Arabians, in which the condition is 42
inherited as an autosomal recessive trait and results from a lack of DNA protein kinase activity that prevent 43
V(D)J recombination.[7,59] In studies conducted in the United States and reported in 1977, the incidence of SCID among Arabian foals was at least 2% to 3%,[60] suggesting a carrier prevalence rate between 25% and 26%. However, in more recent studies conducted in the United States using a precise molecular diagnosis of the carrier state, carrier prevalence was consistently 8%.[61,62]

1.3.3.1.1 Clinical Signs and Laboratory Findings

Affected foals are clinically normal at birth but develop signs of infection during the first 1 to 3 months of life. The age of onset of infection depends on the adequacy of passive transfer and degree of environmental challenge. As maternal antibodies are catabolically eliminated, foals with SCID are increasingly susceptible to infections with bacterial, viral, fungal, and protozoan agents. Bronchopneumonia is a prominent disease, often caused by adenovirus (which is the most significant pathogen of foals with SCID, affecting two thirds of all animals),[63] *Pneumocystis carinii,* or *Rhodococcus equi*. Enteritis, frequently caused by *Cryptosporidium parvum,*[64] arthritis, and omphalophlebitis are common. Adenoviral infection frequently extends to the gastrointestinal and urogenital systems and causes pancreatic disease leading to loss of endocrine and exocrine tissue and possibly contributing to the impaired growth and weight loss observed in foals with SCID.[1]

Clinical signs include nasal discharge, coughing, dyspnea, diarrhea, fever, and weight loss. Although antibiotics, plasma, and supportive care prolong the course of disease, death invariably occurs before 5 months of age. The only exception to this rule was a single foal experimentally treated with a bone marrow

transplant from a histocompatible donor. The foal lived until 5 years of age before dying of an unrelated cause.[65] A consistent hematologic finding is absolute lymphopenia (<1000 μ/L), with neutrophilia being a variable finding resulting from bacterial infection. Total serum globulins and serum IgG can be normal in the first weeks of life if passive transfer is adequate but decline as maternal immunoglobulins are catabolized. Normal foals synthesize IgM from 180 days of gestation and have detectable IgM at birth in presuckle samples, whereas foals with SCID have no IgM.[66] After colostral ingestion, SCID foals also have IgM, although with its relatively short half-life, IgM is undetectable by 2 to 4 weeks of age.[1]

1.3.3.1.2 Cause and Pathogenesis

Normal maturation of T and B cells requires rearrangement of a series of germ line genes to create genetic sequences that can encode the wide diversity of antigen receptors required by these lymphocytes (the T cell receptor or immunoglobulin antigen-binding sites, respectively, as described in Chapter 1.1). These genes are called the V, D, and J genes, and the overall process is called V(D)J recombination. This process depends on two groups of enzymes, the recombinase activating gene products, and DNA-protein kinase (DNA-PK), which is critical for DNA double-stranded break repair. In foals with SCID a component of DNA-PK called DNA-PK catalytic subunit (DNA-PK_{cs}) is defective, and consequently V(D)J recombination events do not occur, lymphocytes do not mature, and immature lymphocytes are eliminated.[7,59] The defect is a five nucleotide deletion in the DNA-PK_{cs} gene that produces a premature stop codon and prevents formation of the complete enzyme.

1.3.3.1.3 Diagnosis

Previously, antemortem diagnosis of SCID was suggested by appropriate clinical signs in a foal of Arabian breeding with persistent marked lymphopenia (usually <500/μL) and the absence of serum IgM by RID. If presuckle serum is unavailable for testing, serum IgM cannot be used as a diagnostic aid until the foal is older than 3 weeks. All suspected cases required confirmation by the necropsy finding of hypoplasia of the spleen, thymus, and lymph nodes with the absence of any normal lymphoid architecture. With the identification of the genetic defect causing SCID, the current standard for definitive diagnosis is demonstrating that the foal is homozygous for the defective SCID gene. This test depends on polymerase chain reaction amplification of a specific region of the DNA-PK_{cs} gene, and evaluation of the amplicon in a Southern blot using probes specific for normal and mutant sequences.[7] This test is commercially available (VetGen, Ann Arbor, Michigan), requires whole blood or cheek swabs, and can identify homozygous affected, heterozygous carriers, and normal animals.

1.3.3.1.4 Treatment

Supportive care may prolong the course of disease, but affected foals die by 5 months. Immunologic reconstitution is currently impractical and an ethically questionable procedure.

1.3.3.1.5 Client Education

Arabian mares and stallions intended for breeding should be tested for SCID carrier status. When two heterozygous carriers are bred, the progeny will include 25% foals with SCID, 50% carriers, and 25% homozygous normal foals. Therefore prevention of SCID requires identification of carriers and their removal

from the breeding population or breeding exclusively with homozygous normal animals and subsequent testing of progeny before their own breeding future is planned. 43

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1.3.3.2 ANEMIA, IMMUNODEFICIENCY, AND PERIPHERAL GANGLIONOPATHY: FELL PONY IMMUNODEFICIENCY SYNDROME

In the late 1990s a new syndrome of anemia, immunodeficiency, and peripheral ganglionopathy was described in Fell pony foals.[67,68] Affected foals became ill within 2 to 3 weeks of birth and died by 3 months of age.

1.3.3.2.1 Clinical Signs and Laboratory Findings

Clinical signs include ill thrift, anemia, respiratory infection, glossal hyperkeratosis, and diarrhea. Anemia can be severe and is normochromic and normocytic to macrocytic, with small numbers of late erythroid precursors in bone marrow.[68,69] Some foals are affected by cryptosporidial enteritis and adenoviral bronchopneumonia and pancreatitis. Plasma protein concentrations and blood lymphocyte counts may be normal or decreased. T cell subsets measured by flow cytometry are normal, although expression of major histocompatibility complex II is low. In vitro lymphoproliferative responses are normal.[70] On necropsy, lymphoid organs can be small, and secondary lymphoid follicles and plasma cells are absent.[68] Neuronal changes are characterized by neuronal chromatolysis in the cranial mesenteric, dorsal root, and trigeminal ganglia.

1.3.3.2.2 Cause and Pathogenesis

Preliminary evidence indicates that Fell pony syndrome is a genetic disease inherited as an autosomal recessive trait, although the underlying defect is unknown (M. Holmes, personal communication, 2002).

1.3.3.2.3 Diagnosis

Confirmation of diagnosis depends on the presence of clinical signs in Fell pony foals and histologic confirmation of erythroid hypoplasia in the bone marrow, neuronal chromatolysis in peripheral nerve ganglia, absence of secondary lymphoid follicles, and low numbers of plasma cells in spleen and mesenteric lymph nodes.

1.3.3.2.4 Treatment

Treatment of specific infections is of limited efficacy in affected foals, particularly those affected by severe anemia and diarrhea, and all die by 3 months.

1.3.3.2.5 Client Education

Because the underlying basis of this disease is unknown, giving specific advice is difficult. If the condition is proved to be an autosomal recessive trait, then the dam and sire of affected foals will be confirmed as carriers and breeding should be avoided.

1.3.3.3 SELECTIVE IMMUNOGLOBULIN M DEFICIENCY

Selective IgM deficiency is characterized by substantially reduced or absent serum IgM with normal or increased concentrations of other immunoglobulins and no other evidence of immunodeficiency.[1,71] Serum IgM concentrations are more than two standard deviations below the mean of age-matched control animals. All other immunologic parameters are normal, although in one case a lack of response to lipopolysaccharide, a B cell mitogen, was reported.[72] The syndrome has been described most frequently in Arabians and Quarter Horses, although the diagnosis has been made in other breeds.

1.3.3.3.1 Clinical Signs and Laboratory Findings

Two clinical syndromes have been described. The first condition affects foals 2 to 8 months of age, which develop severe pneumonia, arthritis, and enteritis with or without septicemia. Many affected foals die before 10 months of age. Gram-negative bacterial infections are common (especially with *Klebsiella* species), and age at onset of signs is generally older than in foals with combined immunodeficiency. Some affected foals survive but have a history of repeated bacterial infections that respond temporarily to therapy but recur once antimicrobial therapy is discontinued. These foals grow poorly and generally die within 2 years. Foals rarely can recover from IgM deficiency, suggesting that such cases actually may be secondary rather than primary immunodeficiencies.[73]

The second presentation involves horses between 2 and 5 years of age, many of which have or ultimately develop lymphosarcoma. These individuals may have external or internal lymphadenopathy or both. Chronic weight loss, depression, and other nonspecific signs usually accompany lymphosarcoma. In cases associated with lymphosarcoma, the IgM deficiency is presumed to be a secondary rather than a primary immunodeficiency.

Routine laboratory findings are not diagnostically specific. Hematologic abnormalities consistent with chronic inflammatory disease, such as anemia, neutrophilia, and hyperfibrinogenemia, are commonly present. The total plasma protein and serum globulin concentrations are usually normal.

1.3.3.3.2 Cause and Pathogenesis

Although a genetic basis is suspected, the pathogenesis of selective IgM deficiency is unknown.[1] Primary and secondary forms of this syndrome seem likely to exist.

1.3.3.3.3 Diagnosis

Definitive diagnosis of selective IgM deficiency is made by measuring the major serum immunoglobulins by RID and determining the absolute lymphocyte count. Horses with selective IgM deficiency have serum IgM concentrations persistently less than two standard deviations below that of age-matched controls (<15 mg/dl at 4 to 8 months; <25 mg/dl at >8 months) coupled with normal concentrations of IgG (≥400 mg/dl) and a 44
normal lymphocyte count. Because seriously ill foals may have transiently depressed serum IgM 45
concentration, suspected cases of deficiency should be tested at least twice to document that IgM concentrations remain low. All other immunoglobulin concentrations are normal.

1.3.3.3.4

Treatment

Other than supportive care and antimicrobial therapy, no treatment is effective for selective IgM deficiency. Transfused plasma concentrations of IgM are low and the half-life is short, thus any benefit would be only temporary.

1.3.3.3.5

Client Education

The prognosis must be guarded; however, recovery has been reported.[73] Because primary immunodeficiency is likely in foals affected during the first year of life, remating the sire and dam may be inadvisable.

1.3.3.4

OTHER PRIMARY IMMUNODEFICIENCIES

Transient hypogammaglobulinemia and agammaglobulinemia are reported as established primary immunodeficiency syndromes in horses. However, these conditions have been reported infrequently and consequently remain poorly defined. The available information follows. Another form of immunodeficiency affecting humoral immunity was described by Boy, Zhang, Antczak et al.[74] in a 10-month-old Arabian colt. The horse exhibited an absence of serum IgM, IgA, and IgG(T) and a normal concentration of IgG. In vitro testing of peripheral blood mononuclear cells with T cell mitogens elicited normal responses, whereas responses to B cell mitogens were weak. On postmortem examination lymphoid organs showed generalized lymphocyte depletion.

To increase the understanding of these and other currently unidentified immunodeficiency syndromes of horses, it is critical that every effort be made to identify such cases and thoroughly investigate them. Newly available immunologic resources may make further defining of these diseases possible and increase the diagnostic and prognostic resources, provided case material is identified.

Transient hypogammaglobulinemia has been reported in only two foals, an Arabian and a Thoroughbred, and is characterized by delayed onset of immunoglobulin synthesis.[73,75] Affected foals manifest signs consistent with bacterial and viral infections when passively acquired immunoglobulins are catabolized to nonprotective concentrations. For unknown reasons the onset of autologous immunoglobulin production, which generally occurs at birth, is delayed until these foals are approximately 3 months of age. Hematologic studies may suggest chronic infection, although total plasma protein is normal or slightly reduced. Diagnosis is based on the presence of low serum IgG (<200 mg/dl) and IgG(T) (<20 mg/dl) at 2 to 4 months of age, with low-normal serum IgM (>15 mg/dl) and IgA (>20 mg/dl). Lymphocyte counts are normal. Antimicrobial therapy and plasma transfusions are necessary to minimize infections. Affected foals usually survive if they have not suffered failure of passive transfer concomitantly and they receive appropriate support between 2 and 4 months of age. The fact that foals spontaneously recover from this condition raises the question of whether the immunodeficiency may be secondary.

Agammaglobulinemia is characterized by absence of B lymphocytes and failure to produce immunoglobulins in the presence of normal cell-mediated immunity.[1] The disease has been described in five colts of Thoroughbred, Standardbred, or Quarter Horse breeds.[73,76,77] Clinical signs commence between 2 and 6 months of age and result from bacterial infections such as pneumonia, enteritis, and arthritis. Multisystemic infections that respond poorly to therapy are common, and laboratory changes reflect chronic inflammatory disease. The fact that this

syndrome has been described only in colts suggests an x-linked mode of inheritance, as occurs in a similar disease of human beings. A maturation defect from stem cells to B cells has been suggested.[1] Affected foals have persistently subnormal serum concentrations of all immunoglobulin classes and normal lymphocyte counts. Serum IgM and IgA are generally absent at the time of evaluation, and maternally derived IgG and IgG(T) decline with time. At 2 months of age, IgG is less than 300 mg/dl, declining to less than 100 mg/dl by 6 months. No serologic response to immunization occurs, and B lymphocytes, as determined by immunofluorescence, are absent. Tests of cell-mediated immune function such as intradermal phytohemagglutinin and in vitro blastogenesis are normal. Plasma and antimicrobial therapy only result in transient improvement. Affected horses die from disseminated infection between 1 and 2 years of age.

1.3.4 Secondary Immunodeficiencies

1.3.4.1 FAILURE OF PASSIVE TRANSFER

Failure of passive transfer is the most common immunodeficiency disorder of horses and recently has been reviewed extensively.[2] The condition occurs in all breeds secondary to inadequate absorption of colostral antibodies. Failure of passive transfer is correlated significantly with increased susceptibility to infectious disease and death in neonatal foals.[78,79] The newborn foal is capable of mounting a normal immune response, as described in Chapter 1.1. However, neonatal foals are immunologically naïve and thus have not yet developed memory responses or produced antigen-specific antibody and other forms of adaptive immunity. 45
During the first 1 to 2 months of life, foals depend on passively transferred immunity for protection from 46
infectious disease. The diffuse epitheliochorial nature of the equine placenta does not allow for in utero immunoglobulin transfer to foals. Although minor concentrations of some immunoglobulins can be detected at birth, the foal is born essentially agammaglobulinemic and acquires passive immunity by the ingestion and absorption of colostrum from the dam.[80,81] Colostrum is a specialized form of milk containing immunoglobulins that are produced during the last 2 weeks of gestation under hormonal influences. Colostrum contains primarily IgGa, IgGb (IgGa plus IgGb is the equivalent of IgG), and IgG(T), with smaller quantities of IgA and IgM, all of which have been concentrated into mammary secretions from the blood of the mare.[14,82] Colostrum is produced only one time each pregnancy and is replaced by milk that contains negligible immunoglobulins within 24 hours of the initiation of lactation.[14,82,83] Normal foals suckle within 1 to 3 hours of birth. The absorptive capacity of the foal's gastrointestinal tract for immunoglobulins is greatest during the first 6 hours after birth and then steadily declines until immunoglobulins can no longer be absorbed when the foal is 24 hours old. The incidence of failure of passive transfer is highly variable among groups of horses and seems to depend primarily on management factors that ensure early colostral ingestion.[84] The reported prevalence of at least partial failure of passive transfer ranges from 3% to 37%.[18,73,78,84]

1.3.4.1.1 Clinical Signs and Laboratory Findings

Failure of passive transfer does not cause any clinical signs of disease directly. Failure of passive transfer is suspected when signs of generalized or localized bacterial infections such as septicemia, pneumonia, enteritis, and arthritis develop during the first 3 weeks of life. Routine laboratory findings may suggest sepsis, but the presence of infection in the neonatal period is not pathognomonic for failure of passive transfer. Common abnormalities include neutropenia or neutrophilia, hypoglycemia, and hyperfibrinogenemia. The total plasma protein may be low, normal, or increased in foals with failure of

passive transfer because of the wide variation in normal presuckle total plasma protein and the confounding effects of dehydration secondary to sepsis.

1.3.4.1.2 Cause and Pathogenesis

Causes for failure of passive transfer in foals include (1) failure of the foal to ingest an adequate volume of colostrum in the early postpartum period; (2) loss of colostrum via premature lactation; (3) inadequate immunoglobulin content of the colostrum; and (4) insufficient immunoglobulin absorption via the intestine.[2,85] A high negative correlation exists between foal serum IgG concentration and the incidence of severe infections[78]; however, the minimum amount of IgG necessary for protection of a foal from infection varies with the amount and virulence of environmental pathogens, concomitant stress factors, and colostral antibody titer against specific pathogens. Although a serum IgG concentration of at least 400 mg/dl has been considered evidence of adequate passive transfer, most normal foals attain values more than twice this high,[78,84] and serum IgG greater than 800 mg/dl may be required for adequate immunity.[79] Numerous other colostral factors may be important for the immune protection of foals. Colostrum has been shown variously to regulate cell-mediated immunity, activate granulocytes, promote intestinal absorption of macromolecules, decrease intestinal colonization by pathogens, and contain constituents of innate immunity (e.g., lactoferrin and complement) and leukocytes that have a local protective role in the neonatal digestive tract and may be absorbed systemically.[86,87] At this time the significance of these various phenomena for the health of neonates and their immunologic development is largely unknown. The one exception is the finding that colostral ingestion suppresses de novo antibody responses in foals in a nonspecific and an antigen-specific manner.[83,88]

Neonatal weakness and lack of maternal cooperation (common in maiden mares) are common reasons for the foal to ingest an inadequate volume of colostrum. If colostral ingestion is delayed beyond 6 hours, the absorption of immunoglobulins is reduced significantly. Lactation before parturition is another common reason for failure of passive transfer, because colostrum is only produced one time each gestation. The causative factors for premature lactation are unknown at this time, but foals from mares that “leak” milk hours to days before parturition are likely to suffer failure of passive transfer.[85]

Subnormal colostral immunoglobulin content (<3000 mg/dl) is rare in mares that do not prelactate,[84] but wide individual variation in colostral concentration of immunoglobulins does occur.[84,89–91] Poor-quality colostrum undoubtedly may cause failure of passive transfer. Colostral immunoglobulin content can be estimated by specific gravity or quantitated by RID.[85]

Malabsorption is implicated as a cause of failure of passive transfer when foals are known to have ingested an adequate volume of good-quality colostrum within 12 hours of birth. Because glucocorticoids hasten the maturation of specialized enterocytes, stress-causing endogenous corticosteroid release may cause reduced immunoglobulin absorption. However, obvious stress factors often are not found in foals with apparent impaired ability to absorb IgG.[6]

1.3.4.1.3 Diagnosis

Subnormal serum IgG concentration 24 hours after birth is the basis for diagnosis of failure of passive 46
transfer. Serum IgG of less than 200 mg/dl indicates complete failure of passive transfer, whereas 200 to 800 47
mg/dl should be considered partial failure of passive transfer. Many foals under good management

conditions may remain healthy if the serum IgG is at least 400 mg/dl, and consequently this cutoff point is measured by several rapid diagnostic tests. The most quantitatively accurate method to determine serum IgG is the single radial immunodiffusion test (VMRD Inc.); however, this assay is time-consuming and expensive and thus inappropriate for the diagnosis of failure of passive transfer when timely therapeutic intervention is paramount.[92] Numerous field screening procedures for IgG have been evaluated.[2,16,18,92] Criteria for selecting a screening test for equine failure of passive transfer must include accuracy, the time necessary to perform the test, ease of performance, and cost. Although the zinc sulfate turbidity and glutaraldehyde coagulation tests are inexpensive and provide results within 1 hour, the ease and reported accuracy of membrane-based ELISAs frequently make them the test of choice in many practice situations. However, results of a recent study suggest that the choice of screening test is not so straightforward. McClure, DeLuca, and Miller[18] evaluated the recently withdrawn CITE test (Idexx), the SNAP test (Idexx) (both membrane-based ELISA systems), a latex agglutination test (Foalcheck), the glutaraldehyde coagulation test, and the modified zinc sulfate turbidity test; all results were compared with an RID test. Serum from 203 foals at 24 to 72 hours of age was evaluated. The prevalence of failure of passive transfer at serum IgG concentration of less than 400 mg/dl was 22% and at less than 800 mg/dl was 37%. The withdrawn CITE test and the latex bead agglutination test were judged unsuitable for use as screening tests because of sensitivities of 52% and 53% for IgG less than 400 mg/dl. The most sensitive tests were the modified zinc sulfate turbidity and glutaraldehyde coagulation tests (95% and 89%, respectively, for IgG <400 mg/dl; 100% and 97%, respectively, for IgG <800 mg/dl). The modified zinc sulfate turbidity and glutaraldehyde coagulation tests were specific for complete failure of passive transfer but were less so for partial failure (80% and 91%, respectively, for IgG <400 mg/dl; 59% and 57%, respectively, for IgG <800 mg/dl). In comparison, the SNAP test was less sensitive (76% for IgG <400 mg/dl; 88% for IgG <800 mg/dl), but more specific (95% for IgG <400 mg/dl; 91% for IgG <800 mg/dl). These data highlight the difficulty faced by equine clinicians in selecting a rapid diagnostic test for failure of passive transfer. Because failure to diagnose and treat the condition could result in death of many foals, a sensitive test is required. However, specificity is also important, particularly given the cost of treatment (plasma transfusion) and the fact that treatment is not without its own inherent complications. These data suggest that no clearly superior test exists, comparing the modified zinc sulfate turbidity, glutaraldehyde coagulation, and SNAP tests. Individual clinician judgment is therefore important in test selection, although at this time the relatively good performance of the SNAP test and its convenience may continue to make it a popular choice. These data confirm that in general all screening tests are accurate in identifying foals with complete failure of passive transfer; however, variation exists in their ability to detect marginally deficient foals.[2]

1.3.4.1.4 Treatment

If failure of passive transfer is anticipated because of premature lactation, neonatal weakness, dam death, or low-specific-gravity colostrum, an alternative colostral source may be given orally. A minimum of 2 L of equine colostrum given in 500-ml increments during the first 8 hours after birth is optimal. Bovine colostrum may be substituted safely if equine colostrum is not available[56,93]; however, foals given bovine colostrum also may require plasma transfusion because bovine immunoglobulins have a short half-life in foals and are not specifically directed against equine pathogens.

If a foal is more than 12 hours old when failure of passive transfer is suspected or diagnosed, an intravenous plasma transfusion is indicated. Numerous commercial sources of equine plasma are available (Lake Immunogenetics, Ontario, New York; Veterinary Dynamics Inc., Chino, California; Ameri-Vet Labs, Addison, Illinois). Use of these products is convenient, saves time, and is safe because donors are free of alloantibodies and negative for infectious diseases. The only potential drawback to the use of commercial

plasma is that antibodies specific for pathogens in the environment of the foal may be lacking. Optimal plasma would be obtained from a local blood-typed donor, known to lack serum alloantibodies and alloantigens Aa and Qa. The volume of plasma necessary to bring serum IgG into an acceptable range cannot be predicted accurately because the volume depends on the severity of failure of passive transfer, the immunoglobulin content of the plasma, and on concomitant diseases, which may hasten immunoglobulin catabolism. Generally, 1 L of plasma increases the serum IgG concentration of a 50-kg foal by 200 to 300 mg/dl,[38] thus 2 to 4 L may be necessary to achieve a serum IgG concentration greater than 800 mg/dl. A therapeutic dose of plasma should be administered, and then foal serum IgG should be remeasured. If the desired concentration has not been attained, more plasma is necessary. Some foals with partial failure of passive transfer (IgG >400 and <800 mg/dl) may do well without plasma therapy if no preexisting infections exist and exposure to pathogens is minimized. These foals should be monitored closely for the development of infections. 47

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1.3.4.1.5

Client Education

The prognosis for foals with failure of passive transfer depends on the degree of failure, the environment to which the foal is exposed, the age of the foal at the time of diagnosis, and the presence and severity of secondary infections. Management factors that ensure the ingestion of at least 2 L of high-quality colostrum within 6 hours of birth are paramount to prevent failure of passive transfer. Foaling should be witnessed so that any mispresentations can be corrected, and foals that do not nurse readily within 3 hours can be given colostrum via nasogastric tube. The evaluation of colostral specific gravity with a hydrometer (Lane Manufacturing, Denver, Colorado) may aid in predicting failure of passive transfer.[85] A colostral specific gravity of 1.060 corresponds to approximately 3000 mg/dl IgG, which is the minimum acceptable value. When dam colostral specific gravity is less than 1.060, some degree of failure of passive transfer should be suspected in the foal and corrected. Routine screening of foal serum IgG at 24 to 48 hours after birth allows necessary plasma therapy before the onset of infections.

Foals that are born prematurely, weak, or from prelactating mares should be provided with an alternative colostral source within 6 hours of birth. A colostrum bank can be established by collecting and freezing (−20°C) 250 ml of colostrum from mares that have not prelactated within 6 hours of foaling, once their own foals have suckled. Ideally, banked colostrum should be screened for alloantibodies, although they are unlikely if the mare's own foal remains healthy. The immunoglobulins in banked frozen colostrum are stable for at least 1 year.

1.3.4.2

EXERCISE

When conducted at a stressful level, exercise can affect equine immune function significantly.[94] Strenuous exercise significantly suppresses lymphoproliferative responses and increases lymphokine-activated killer cell activity.[48,95] In race horses, decreased lymphoproliferative responses can be demonstrated 12 to 16 hours after racing.[96] Protracted high-intensity training results in decreased phagocytosis and oxidative burst activity in neutrophils and lymphocytes, although pulmonary alveolar macrophage function is unaffected.[46] Other studies have demonstrated decreased neutrophil and pulmonary alveolar macrophage function in response to single bouts of intense exercise.[97,98] In unconditioned ponies, strenuous exercise increases susceptibility to experimental infection with equine influenza virus.[99,100] In an influenza infection study in trained horses, moderate exercise led to increased signs of clinical disease, although duration of disease was unaffected.[100]

Overall these various studies have demonstrated a clear immunomodulatory effect of exercise, with some specific evidence for increased susceptibility to infectious disease. Although this remains an active area of investigation with much to be learned, clearly the potentially immunosuppressive effect of high-intensity exercise, particularly of protracted duration or in unconditioned animals, needs to be recognized.

1.3.4.3 AGE

The high incidence of *respiratory infections in foals and weanlings,* which frequently relapse on cessation of antibiotic treatment,[101] has led to speculation that this condition results from immunodeficiency.[8] Although this possibility exists, another possibility is that the normal level of immunocompetence in this age group leads to increased susceptibility to pyogenic respiratory infections, particularly under group housing conditions. Features of the immune system of the foal that may predispose to such infections are discussed elsewhere in this chapter.

Old age has the potential to result in relative immunodeficiency, although few equine studies have investigated this possibility. Older horses display decreased lymphoproliferation to mitogens,[102] and in an exercise study they demonstrated reduced immunologic response to exercise.[95] The implications of such phenomena for disease risk in older horses remains uncertain. The high incidence of pituitary dysfunction in older horses is a risk factor for infectious disease, possibly as a consequence of high steroid levels.

1.3.4.4 LEUKOPROLIFERATIVE DISEASE–ASSOCIATED IMMUNODEFICIENCY

Lymphosarcoma often is associated with IgM deficiency[2] and also can be associated with decreased lymphocyte blastogenesis.[103,104] Affected horses were diagnosed with bacterial pneumonia in some instances, and in another report a horse suffering from myelomonocytic leukemia was found to be suffering from pulmonary aspergillosis.[105] These cases demonstrate the importance of considering leukoproliferative disease, and particularly lymphosarcoma, in cases of persistent infections that are refractory to treatment.

1.3.4.5 DRUG-INDUCED IMMUNODEFICIENCY

The most common iatrogenic cause of immunosuppression is corticosteroid treatment, often given with the specific aim of treating a hypersensitivity disorder. The mode of action and the effects of corticosteroids are reviewed in Chapter 1.4, but evidence exists for the capacity of corticosteroids to induce recrudescence of viral diseases such as equine infectious anemia[106] or equine herpesvirus 1 infection[107] and to lead to development of life-threatening bacterial infection.[108] Evidence also indicates that corticosteroid treatment can bias adaptive immune responses to vaccination in horses, specifically suppressing IgGa and IgGb responses without affecting 48
IgG(T) responses.[34] This phenomenon may be consistent with the capacity of corticosteroids specifically to suppress T helper cell type 1 immune responses without affecting T helper cell type 2 responses.[109,110] 49

1.3.4.6 INFECTIOUS DISEASE

Several infectious diseases have been associated with immunodeficiency in horses. The best characterized example may be *perinatal equine herpesvirus 1 infection.*[111] Foals infected with equine herpesvirus 1 late in gestation often are born weak with interstitial pneumonia and develop a variety of bacterial diseases.[112]

Affected foals have profound lymphopenia and generally die, despite therapy. The immunodeficiency is thought to be caused by viral-induced lymphoid damage, because necropsy reveals significant necrosis of lymphoid tissue in the thymus, spleen, and lymph nodes.

1.3.4.7 UNDIFFERENTIATED IMMUNODEFICIENCIES

A group of foals with *oral candidiasis* and *bacterial septicemia* between 2 weeks and 4 months of age had laboratory or histologic evidence of immunodeficiency that did not fulfill diagnostic criteria for any of the recognized primary immunodeficiencies.[113] Oral lesions ranged from focal white plaques on tongue margins to a generalized thick, white pseudomembrane covering the tongue and gingiva. Affected foals exhibited bruxism, ptyalism, fever, and depression in addition to pneumonia, arthritis, and diarrhea. The lymphocyte counts of affected foals were usually normal. Several foals had IgM deficiency coupled with depressed blastogenesis, suggesting cellular immune dysfunction. Many of the foals had low or marginally decreased serum IgG in addition to IgM deficiency or reduced blastogenesis. Whether the immunologic defects were primary or secondary was not determined. All foals died despite extensive therapy with parenteral antimicrobials, topical antimycotics, and intravenous plasma.

Acquired immunodeficiency was identified in a 7-year-old Appaloosa gelding with no history of previous illness.[114] Clinical signs included lethargy, anorexia, and dyspnea. Pneumonia and septicemia caused by *Rhodococcus equi* were confirmed by tracheal wash and blood culture, respectively. Immunologic evaluation revealed significant lymphopenia, subnormal serum IgG and IgA with marginally low IgG concentrations, failure to respond serologically to immunization, and reduced in vitro lymphocyte blastogenesis. Histologic examination of lymph nodes and spleen revealed lymphoid atrophy.[97,98]

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1.4

1.4—IMMUNOMODULATORS

D. Paul Lunn

David W. Horohov

Clinicians frequently seek to increase normal, restore deficient, and temper overexuberant host immune responses. For these reasons, modulation of the immune system, which provides critical defense against many types of disease, has become an area of intense interest in clinical medicine. Immunostimulants and immuno depressants are considered immunomodulators. A variety of ways exist to classify immunomodulators beyond this distinction, but for practical purposes they may be classified best based on their origin, that is, physiologic products (actual normal components of the immune response), microbial products, and chemically defined agents. Immunomodulation also can result from modes of therapy not considered in this chapter, including bone marrow transplantation and irradiation.

Although a scientific rationale for the use of immunomodulators exists, a major limitation is the complexity of the immune response to be modulated. Clinicians face several major problems in the rational clinical application of immunomodulators. Current diagnostic methods do not allow precise identification of the in vivo defects, deficiencies, or excesses of substances or regulators present within the immunoregulatory network. Consequently, attempts to intervene with immunomodulators often are crude and nonspecific. Some of the information on which the use of immunomodulators is based has come from controlled experimental studies, some performed in vitro and others in vivo. In clinical patients, expectations based on these types of studies frequently are not realized. Reasons

for this include the timing of the administration of the immunomodulator during the course of disease and the fact that observation of a single immunological phenomenon resulting from use of an immunomodulator, for example, an increase in lymphocyte count or lymphoproliferative responses, does not necessarily translate into improved clinical performance with an infectious disease. Because of the complexity of the immune network, the rational use of immunomodulators can be considerably more difficult than the use of antimicrobial agents. An example of the importance of timing in immunomodulatory intervention is the success of immunosuppressive therapy in the context of organ allografts compared with the frequent treatment failures experienced in treating autoimmune disease. In the former case, therapy is planned in advance of introducing the allograft, whereas in the latter case the immune response and resulting disease are well established before they are detected and therapy is initiated.

Immunomodulators have been embraced by clinicians with great enthusiasm, and the concept of their use remains appealing.

1.4.1 Immunosuppressors

1.4.1.1 CORTICOSTEROIDS

Corticosteroids are classic examples of immunosuppressive agents. Corticosteroids exhibit an extensive range of effects on elements of the innate (inflammatory) and adaptive immune responses. The mechanism of action of corticosteroids is illustrated in Figure 1.4-1. Corticosteroids, acting through their cytoplasmic steroid receptors, directly regulate as many as 1% of genes in the genome, usually resulting in induction of transcription.[1] Table 1.4-1 summarizes the useful antiinflammatory effects of corticosteroids. The effect on the adaptive immune response is complex.[2] For example, in cases of autoimmune disease such as autoimmune hemolytic anemia, corticosteroids may act by reducing phagocytosis of antibody-coated cells by the 52
reticuloendothelial system rather than decreasing antibody production. Nevertheless, corticosteroids have been 53
shown to have effects on antibody production, and in the horse they can suppress de novo antigen-specific immunoglobulin Ga (IgGa) and IgGb responses while sparing IgG(T) responses.[3] Such suppression may be consistent with the action of corticosteroids to suppress type 1 T helper cell responses while sparing type 2 T 53
helper cell responses.[4–7] Cell migration is affected significantly by corticosteroids, and in the horse, 54
corticosteroids suppress neutrophil migration and phagocytic and bactericidal activity.[8] The mechanism whereby migration is decreased involves decreased expression of integrin molecules including selectins and integrins.[9]

Figure 1.4-1 Lipid-soluble corticosteroid molecules diffuse across the plasma membrane into the cytosol and bind to steroid receptors. This displaces a heat-shock protein (Hsp90) normally bound to the nascent steroid receptor, exposing a DNA-binding region. The new complex enters the nucleus and binds to specific regulatory DNA sequences, resulting in modulation of transcription of a wide variety of genes.

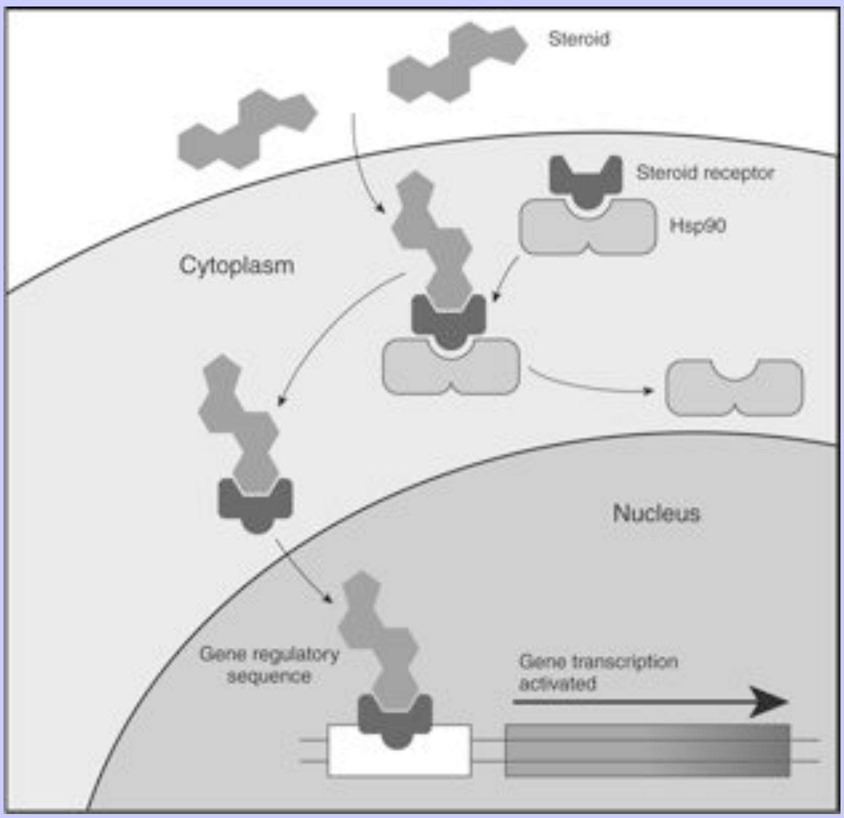

TABLE 1.4-1 Antiinflammatory Effects of Corticosteroid Therapy Mediated by Regulation of Gene Transcription

ACTION OF CORTICOSTEROIDS	
DIRECT EFFECT	**PHYSIOLOGIC EFFECTS**
Decrease IL-1, TNF-α, GM-CSF, IL-3, IL-4, IL-5, and IL-8.	Decrease inflammation caused by cytokines.
Decrease NOS.	Decrease NO.
Decrease phospholipase A_2 and cyclooxygenase type 2. Increase lipocortin-1.	Decrease prostaglandins and leukotrienes.
Decrease adhesion molecules.	Decrease emigration of leukocytes from vessels.
Increase endonucleases.	Increase apoptosis in lymphocytes and eosinophils.

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IL, Interleukin; *TNF,* tumor necrosis factor; *GM-CSF,* granulocyte-macrophage colony-stimulating factor; *NOS,* nitric oxide synthase; *NO,* nitric oxide.

Although corticosteroids are powerful immunosuppressive agents, they also may predispose patients to life-threatening opportunistic infections[10] or recrudesence of viral infection.[11–13] Additional extensive undesirable side effects include fluid retention and decreased wound healing. For this reason, prolonged or high-dose corticosteroid therapy must be used judiciously.

1.4.1.2 CYTOTOXIC DRUGS

The two commonly used immunosuppressive cytotoxic drugs, *azathioprine* and *cyclophosphamide,* interfere with DNA synthesis and act primarily on dividing cells.[1] This activity is useful for treating cancer and for suppressing dividing lymphocytes. Toxicity limits the use of these drugs, although at lower doses they can be used in combination with corticosteroids. Two case reports describe the use of these drugs, with some success, in horses suffering from immune-mediated hemolytic anemia or thrombocytopenia when corticosteroid therapy alone had failed.[14,15]

1.4.1.3 BACTERIAL AND FUNGAL DERIVATIVES

This class of drugs includes relatively nontoxic alternatives to immunosuppressive cytotoxic drugs. *Cyclosporine* is a fungal derivative and has emerged as a major immunosuppressive agent for allograft survival. The drug selectively inhibits proliferation, cytotoxicity, and lymphokine production of T cells by binding to intracellular proteins known as immunophilins and by interfering with signaling pathways that are important for clonal expansion of lymphocytes.[1] Cyclosporine is efficacious in suppressing specific immune responses with minimal nonspecific toxic effects on polymorphonuclear leukocytes, monocytes, and macrophages. Thus immunosuppressed patients suffer fewer severe secondary infections. The drug is not hazard free; in addition to suppressing lymphocyte responses in general, cyclosporine is also toxic to the kidneys and other organs.

In horses the use of cyclosporine has been limited to topical therapy for ocular inflammatory disease, including keratitis[16] and uveitis.[17] In the treatment of uveitis, cyclosporine treatment suppressed the numbers of infiltrating T lymphocytes and the interleukin-2 and interferon-γ levels.[18]

1.4.2 Immunostimulants

Although the clinical value of immunosuppressive drugs such as corticosteroids and immunostimulant adjuvants in vaccines clearly is established, evidence for the clinical value of many immunostimulants is sparse. Looking critically at the immunomodulatory drugs available and making attempts to evaluate the effects of therapy objectively is important. Many agents that have been discarded for use in human medicine are now being resurrected for veterinary use. Is it realistic to expect better results in veterinary patients? This discussion is limited to drugs currently marketed for use as immunomodulators or for which published evidence of activity exists. Advances in genetic engineering have made available recombinant forms of many cytokines with immunomodulatory potential, but only those with some established value are discussed.

1.4.2.1 PHYSIOLOGIC PRODUCTS

1.4.2.1.1 Immunoglobulins

The use of immunoglobulins for treatment of failure of passive transfer of immunity is discussed in Chapter 1.3, and antigen-specific passive immunoglobulin therapy is discussed subsequently. Other forms of immunoglobulin immunomodulation include the use of monoclonal or polyclonal sera directed against components of the immune system. This type of therapy still has not found practical application in the 30 years since the advent of monoclonal antibody technology, largely because of the immunogenicity of the antibodies themselves. Although recombinant technology now has the prospect of resolving this problem, no current examples of clinical applicability in the horse exist.

Another mode of immunoglobulin therapy is polyclonal intravenous immunoglobulin (PIVIG) therapy using pools of immunoglobulin derived form several thousand donors. PIVIG administration was observed serendipitously to be useful for treatment of autoimmunity,[19,20] and mechanisms proposed include idiotype-antiidiotype network regulation,[21] cytokine regulation, specific antibody interactions, Fc receptor blockade, inhibition of activated complement deposition, and long-term selection of immune repertoires.[22] Although the explanation for the mechanisms underlying the effects of PIVIG are fascinating, good examples of therapeutic success also exist in the case of immunothrombocytopenic purpura.[23] However, diseases such as autoimmune diabetes mellitus are resistant to therapy.[24] No publications describe PIVIG therapy in horses, although this treatment remains an intriguing possibility in this species.

1.4.2.1.2 Cytokines

Given the central role of cytokines in immunoregulation (see Chapter 1.1), their potential as immunomodulators is obvious, and a discussion of the possible use of cytokines as therapeutic agents would 54
be extensive although largely hypothetical. Therefore consideration is limited to the two cytokines that have 55
found clinical application in horses to date.

1.4.2.1.2.1

Interferon-α

The clinical application of interferon-α in the horse has been reviewed extensively.[25,26] Interferon-α has antiviral and immunostimulant properties, and oral administration of interferon-α has been shown to reduce inflammatory airway disease[27] in racehorses.[28] The treatment used was low-dose (50 to 150 IU) natural human interferon-α and resulted in decreased bronchoalveolar lavage cell counts and a noninflammatory cytologic profile.[29] Higher doses (450 IU) were less effective, consistent with results in other species.[26] The efficacy of oral therapy probably depends on effects mediated through oropharyngeal lymphoid tissue because the agent is destroyed in the stomach.

1.4.2.1.2.2

Granulocyte Colony-Stimulating Factor

The clinical application of granulocyte colony-stimulating factor in the horse recently has been reviewed extensively.[26] Granulocyte colony-stimulating factor treatment in neonatal foals results in a sustained, dose-dependent increase in neutrophil count and a left shift.[30,31] Treatment increases neutrophil production in the bone marrow and shortens the time to release into the circulation. Neutrophil half-life remains unchanged at 8 hours. In horses, canine recombinant granulocyte colony-stimulating factor (Amgen) has been used to treat sepsis and endotoxemia with some success.[26]

1.4.2.2

BACTERIAL, VIRAL, AND PLANT PRODUCTS

A variety of bacterial and fungal microorganisms or microbial products have been identified that have immunomodulating effects on the immune system. A common feature of many of these products is a nonspecific immunostimulant effect, purportedly caused by macrophage activation and release of cytokines including interferons, interleukin-1, tumor necrosis factor, and interleukin-6. Consequently, mild fever and malaise may be associated with this form of treatment. In the horse, these treatments most commonly are used in cases of respiratory infection or sarcoids. An extensive review of the use of these and other immunostimulants in the horse has been published recently.[26]

1.4.2.2.1

Mycobacterial Products

A wide range of mycobacterial fractions have been identified that have immunomodulating ability.[26] The minimal structure with immunologic (adjuvant) activity is muramyl dipeptide, which is a potent adjuvant.[32] Preparations commercially available for use in horses include BCG (bacille Calmette-Guérin), a modified live human tuberculosis vaccine, and protein-free mycobacterial cell wall extracts (Nomagen, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa; Equimune, Vetrepharm Inc., London, Ontario). Efficacy is claimed for treatment of equine respiratory disease. Adverse reactions have been reported after multiple intravenous treatments in horses, resulting in significant interstitial lung infiltration and progressive pulmonary fibrosis. As a result, caution should be exercised in selecting this treatment modality. In horses the major successful application of these products has been in the intralesional treatment of sarcoids, particularly periocular sarcoids.

1.4.2.2.2 *Propionibacterium acnes (Corynebacterium parvum)*

Propionibacterium acnes is a gram-positive anaerobe marketed for use in horses as a killed preparation under the trade name EqStim (Immunovet, Inc., Tampa, Florida) for treatment of equine respiratory disease. The preparation is recommended for use prophylactically before weaning, transport stress or co-mingling, or for the therapy of chronic infectious respiratory disease. In a blind randomized clinical trial, *P. acnes* treatment resulted in early resolution of spontaneously occurring respiratory disease.[26] In healthy horses, *P. acnes* resulted in increased $CD4^+$ T lymphocyte numbers and lymphokine-activated killer cell activity in peripheral blood and bronchoalveolar lavage fluid, increased nonopsonized phagocytosis in peripheral blood leukocytes, and decreased pulmonary cellularity.[33] Overall, this agent is one of the most popular immunostimulants currently marketed for horses, although an extensive literature to confirm its value still is lacking.

1.4.2.3 CpG DNA

In recent years an extensive literature has developed describing the immunomodulatory effects of certain bacterial DNA motifs.[34] The specific immunostimulatory DNA motifs are called CpG sequences, and appropriate sequences for immunostimulation of domestic species have been identified.[35] Much work has been done examining the efficacy of CpG sequences as vaccine adjuvants,[36] as immunomodulators for hyposensitization (see the following discussion), or cancer therapy.[37] However, the value of CpGs as nonspecific immunostimulants currently is being evaluated in horses as a potential new therapy for the future.

1.4.2.3.1 Acemannan

Acemannan is an extract of the aloe vera plant predominately used for treatment of fibrosarcomata in dogs and cats.[26] Acemannan also can be used as a vaccine adjuvant and has antiviral activity. Anecdotal reports describe efficacy for treatment of equine respiratory disease (intravenous) and sarcoids (intralesional), although both forms of treatment are associated with side effects including syncope, tachycardia, tachypnea, and sweating. Controlled studies of efficacy in the horse are lacking. 55

1.4.2.3.2 *Parapoxvirus ovis* 56

Marketed under the trade name Baypamun (Bayer Animal Health, Research Triangle Park, North Carolina), inactivated *Parapoxvirus ovis* has been used extensively in Europe for prophylaxis and treatment of infectious disease in companion animals (including horses) and pigs. This product will be available shortly in the United States. The immunostimulant properties depend on a component of the viral envelope, an increase in natural killer cell activity, and macrophage activation. Efficacy has been demonstrated against viral and bacterial disease in several species,[26] and some evidence indicates that in horses prophylactic administration before weaning reduces signs of respiratory disease after weaning.[38] Studies of treatment of sarcoid found no evidence of efficacy.[39]

1.4.2.3.3 Echinacea

Extracts of *Echinacea angustifolia* have been reported to be an immunostimulant. One study in horses evaluated the effect of an experimental oral echinacea extract on neutrophil number and phagocytosis and on lymphocyte count.[40] Findings provided limited support for changes in cell count and behavior consistent with immunostimulation, but evidence was limited.

1.4.2.4 CHEMICALLY DEFINED AGENTS

Levamisole is a synthetic anthelmintic used for treating nematode infections and also has been reported to restore impaired host immune defenses.[26] Levamisole appears to have little effect on the normal immune system but may stimulate a subnormal response and suppress hyperactive responses. The effects are dose-related, and low doses are reported to enhance responses, whereas higher doses suppress responses. In cattle, levamisole enhances lymphoproliferative responses in vitro, although in vivo co-administration with a vaccine had no immunostimulant effect.[41] Similarly, levamisole did not prevent corticosteroid-mediated immunosuppression in cattle[42] or enhance postpartum lymphoproliferative responses in pigs.[41] Other than anecdotal reports, no published controlled studies of the value of levamisole in horses are available.

1.4.3 Antigen-Specific Immunomodulation

1.4.3.1 VACCINATION AND ADJUVANTS

Vaccination is a critically important tool in preventing infectious disease in human beings and animals, and passive and active vaccination are used extensively in the horse. Specific equine vaccination strategies are presented elsewhere in this book, and the scientific principles and practice of equine vaccination have been reviewed extensively.[43] Active vaccination implies provocation of an antigen-specific immune response by administration of antigen in a dead, live, or DNA vaccine form. Success of all of these forms of vaccination frequently depends on use of an effective adjuvant, a compound capable of amplifying and directing immune responses.[44] As such, adjuvants are one of the most important forms of immunomodulating agents in use in equine medicine.

Passive vaccination is accomplished by administering preformed antibodies as a plasma transfusion or in a concentrated form, as in commercially available tetanus antitoxin. This strategy can be highly effective in diseases for which no product is available for active vaccination (e.g., *Rhodococcus equi*) or in high-risk situations when time is inadequate for protection to be generated by active vaccination. Generally, passive vaccination should be avoided when possible because of the risk of transmission of infection in serum-derived products. An example of this is the association of acute hepatic necrosis with administration of tetanus antitoxin.[45]

1.4.3.2 HYPOSENSITIZATION

Because horses suffer from a number of hyposensitivity diseases, attempts have been made to perform antigen-specific immunosuppression; for example, for sweet itch and recurrent airway obstruction. The principle of this type of therapy is that the immune response to an allergen can be redirected to reduce hypersensitivity disease.

For example, type 1 hypersensitivity disease may depend on a type 2 T helper cell immune response, and treatments that can change this to a type 1 T helper cell immune response may eliminate or control the hypersensitivity disease by changing the immune response from one dominated by IgE to one dominated by IgG.[46] Typically, hyposensitization treatments use injections of the allergen itself, starting with small doses and gradually increasing the dose over time. This form of treatment depends on correct identification of the allergen against which the hypersensitivity disease is directed. The difficulty in identifying these allergens using available intradermal testing methodologies[47,48] may provide an explanation for the mixed success of hyposensitization treatment in horses.[49] Prospects for hyposensitization therapy may be improved by new techniques to produce large numbers of recombinant allergens.[50,51] These allergens are defined far better than conventionally prepared allergen extracts. In initial experimental studies of recurrent airway obstruction, these allergens were found to be far superior in terms of specificity and sensitivity for detection of allergen-specific IgE. Such developments, together with the development of DNA vaccination strategies incorporating CpG immunomodulation for hyposensitization,[52–54] mean that a good chance exists that new and effective therapies will be developed in the future. 56

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1.4.4 Conclusion

The properties and efficacy of immunosuppressive agents generally are well documented, as are the effect of antigen-specific immunostimulant adjuvants. Of all the non specific equine immunostimulants described in the literature, only interferon-α and *P. acnes* are supported by well-designed and well-performed studies.[28,29,33] Given the interest in this modality of therapy, this lack of experimental information is regrettable and is evidence of the difficulty in designing studies to establish immunostimulant efficacy, possible lack of efficacy of such products in some instances, and the dire need for additional scientific research.

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2 # CHAPTER 2 MECHANISMS OF INFECTIOUS DISEASE

2.1 ## 2.1—Mechanisms of Establishment and Spread of Bacterial and Fungal Infections

Maureen T. Long

2.1.1 ### Normal Flora

Dermal and mucosal surfaces provide a life-preserving protective barrier composed of physical, chemical, and microbial defenses. Normal flora contributes to this protective barrier against pathogens while paradoxically providing a source for potential opportunistic invasion. Commensal bacteria are those that benefit from living on or within a host without mediating harm. For a relationship to be commensal, it must be mutually beneficial, and the interruption of this association results in abnormal host development or overt disease.[1] A pathogen is any disease-producing organism; thus a commensal organism has the potential to be pathogenic. Colonization is infection without disease. Skin, gastrointestinal, respiratory, and urogenital colonization occurs early in life and persists unless disrupted.

2.1.1.1 #### SKIN FLORA

The combination of normal flora and mucosal immunity provides an effective barrier against infectious colonization of nondisrupted skin surfaces. Specific bacteria are stratified by site, and certain bacteria multiple and colonize depending on associated adnexa.[2] Most bacteria and fungi on the surface of the skin are not associated with disease; however, yeast and bacteria within hair follicles are more likely to be related to a disease process.[3] Even though horses inhabit an environment heavily contaminated with fecal flora, normal dermal flora in the horse is surprisingly devoid of members of the Enterobacteriaceae.[4] Normal inhabitants include mixed populations of bacteria of species of *Acinetobacter, Aerococcus, Aeromonas, Bacillus, Corynebacterium, Flavobacterium, Micrococcus, Nocardia*, coagulase-negative *Staphylococcus, Staphylococcus aureus, Streptomyces*, and nonhemolytic *Streptococcus* generae.[2] Certain *Staphylococcus* spp. have been associated with skin disease in the horse and these include *S. aureus, S. intermedius*, and *S. hyicus*, whereas species such as *S. xylosus* and *S. sciuri* more often are associated with normal skin.[5] More than 30 species of fungi can inhabit the skin, and *Alternaria, Aspergillus, Candida, Fusarium, Rhizopus*, and *Trichophyton* spp.[2] commonly are present.

2.1.1.2 #### ORAL, PHARYNGEAL, AND RESPIRATORY FLORA

Oral and pharyngeal mucosal flora is associated with health and disease of the upper and lower respiratory system. The oral and pharyngeal mucosa is populated richly with many bacteria, including obligate aerobes, anaerobes, and facultative anaerobes.[6] Aerobic and facultative anaerobic populations are comprised mainly of *Streptococcus equi*, *Pasteurella* spp., *Escherichia coli*, *Actinomyces* spp., and *Streptococcus* spp. Anaerobes actually predominate in the mouth of the normal horse, and colonization of the oropharynx and mouth consists of members of several bacterial genera. Gram-positive and gram-negative anaerobic species inhabit the pharyngeal tonsillar area, and *Bacteroides fragilis* and *Bacteroides* spp. predominate. *Fusobacterium* spp., 59
Eubacterium spp., *Clostridium* spp., *Veillonella* spp., and *Megasphaera* spp. are also common. These same 60
genera are found consistently in horses with lower respiratory infections, indicating that pharyngeal flora is a

likely source of contamination.[6] Contamination of the trachea of the horse occurs frequently, as evidenced by the fact that transtracheal aspiration yields positive bacterial cultures in approximately 30% of normal adult horses and foals.[7] As with skin flora, normal horses have multiple fungal species inhabiting conjunctival, nasal, and oral mucosae. Stabling increases the frequency of ocular fungi in normal horses.[8]

2.1.1.3 INTESTINAL FLORA

In animal models and chronic human conditions, normal flora is considered important for intestinal maturity and containment of disease. Changes in cecal weight, villus-to-crypt ratio, development of gut immunoglobulin A responses, and volatile fatty acid production are affected by suboptimal cecal colonization in germ-free animals.[9] A relationship between severity of mucosal disease and normal flora also has been demonstrated in models of inflammatory bowel disease of human beings.[10]

Bacteria are present in all parts of the intestinal tract of the horse, and the microbial fauna increases in complexity and density aborally.[11] The stomach of the horse is not a sterile environment. A dense population of gram-positive bacterial rods, primarily composed of *Lactobacillus* spp., colonizes the nonsquamous portion of the equine stomach. Substantial colonization of the duodenum occurs with a large population of proteolytic bacteria, and this colonization increases tenfold in the ileum.[12]

Microbial degradation and fermentation of plant material in the large intestine is an important component of nutrient acquisition in the horse. The consumption of cellulose and starch results in the production of volatile fatty acids.[13] The major cellulolytic bacterial species of the large intestine are similar to those of Bovidae, but the strains in the horse produce different arrays of fermentation products.[14] As in cows, *Ruminococcus flavefaciens* is one of the predominate cellulolytic bacteria of the equine cecum. Overall, gram-negative rods are the most populous bacteria, comprising 50% of flora. Gram-positive rods and cocci each represent around 20% of the bacterial population. Predominant species include members of the Enterobacteriaceae, *Butynvibrio* spp., *Streptococcus* spp., *Bacteroides* spp., *Lactobacillus* spp., *Selenomonas* spp., *Eubacterium* spp., *Propionibacterium* spp., *Staphylococcus* spp., and many unclassified rods and cocci.[15] Yeasts and fungi of the order Mucorales have been identified in the cecum of normal horses and are capable of digesting cellulose and starch.[16]

The latest investigations demonstrate a lack of intestinal pathogens in the flora of normal horses as detected by routine culturing of feces. In the largest study to date, fecal shedding of *Salmonella* in normal horses from farms without evidence of salmonellosis was 0.8% in resident horses.[17] Based on limited investigations, the carriage rates of *Clostridium difficile* in normal horses and foals also appears to be low (<1.5%).[18] Intestinal flora in the horse is an important source for extraintestinal pathogens. In studies examining the carriage rate of *Rhodococcus equi*, all horses cultured carried the bacteria regardless of age.[19,20] Furthermore, if the farm had endemic *R. equi* and respiratory isolates contain the 90-kd plasmid, which has been associated with disease, fecal isolates also contained this plasmid.

2.1.1.4 UROGENITAL FLORA

By far most of the work that characterizes equine normal flora has focused on urogenital flora in an effort to gain understanding of the role of uterine contamination or infection in fetal loss. Although the vaginal and vestibular mucosae of mares are colonized with normal mucosal flora, the uterus is considered sterile.

However, typical culturing techniques result in frequent isolation of what might be considered pathogens, and cytologic examination and bacterial counts are important supplemental tests for detecting true infection. Colony counts less than 10 colony-forming units and lack of inflammatory cells indicate uterine or technical contamination.[21] Many bacteria inhabit the external genitalia of stallions, including those considered to be associated with metritis in mares. The predominant aerobe isolated is coagulase-negative *Staphylococcus* spp., followed by *Corynebacterium* spp., α-hemolytic *Streptococcus* spp., and *Lactobacillus* spp. Pathogens such as β-hemolytic *Streptococcus* spp., *Pseudomonas aeruginosa*, and *Klebsiella* spp. can be found frequently in servicing stallions.[22,23] Pregnancy rates appear to be the same in mares bred to stallions with semen infected with *P. aeruginosa*.[24]

2.1.1.5 FUNGAL FLORA

Essentially the same principles apply regarding normal flora, host immunity, and specific virulence factors for the pathogenesis of fungal infection. Fungal infections can be divided into primary or opportunistic pathogens. True pathogens are less dependent on host status than are opportunistic pathogens, although even a true pathogen may require some degree of alteration of normal flora or host immunity to become established. Long-term antibiotic use, immunosuppression, and compromised organ function especially involving the pulmonary or endocrine system are three primary host factors highly associated with establishment of opportunistic fungal infection. Fungi often can adapt to the mammalian environment over a short time. Adaptation usually requires a change in thermal range, oxygen requirements, and resistance to host defenses. 60

61

2.1.2 Population Biology of Bacterial Infections

Inoculum size, virulence of the organism, and microbial resistance are three important determinants in the outcome of infectious challenge. Understanding the importance of inoculum size in bacterial pathogenesis in general and for a particular bacterium has profound implications for prevention and management of disease. Minimizing the size of bacterial challenge by controlling environmental contamination is the ultimate goal of any management strategy for animals in their natural environment and in hospital settings. Control of environmental challenge dose is especially important in the control of nosocomial and iatrogenic disease.

2.1.2.1 INOCULUM SIZE

Although inoculum size and development of disease are studied frequently in equine viral diseases, comparatively little dose-response work is done to demonstrate a clear relationship between frequency or severity of disease and dose for many equine bacterial pathogens. In general, for systemic infections, inoculum size is the most important determinant of disease in hematogenously disseminated infections such as meningitis and, in rodent models, osteomyelitis.[25,26] Failure of passive transfer, immunodeficiency, infection in multiple joints, and infection with *Salmonella* spp. are important determinants in survival regarding equine neonatal osteomyelitis, but actual data on bacterial characteristics are limited. For skin infections, colonization of experimentally induced wounds with *Staphylococcus* spp. depends on inoculum size.[27] Thus control of surgical and wound hygiene depends on agents to which *Staphylococcus* is sensitive. The occurrence of diarrhea has been demonstrated to be dose-dependent in salmonellosis in calves,[28] but little is known regarding challenge dose in the foal or the adult horse. Minimal inoculum size is required for certain pathogens that form toxins, such as *Escherichia coli* and *Clostridium* spp. For instance, disease in human

beings caused by *E. coli* 0157:H7 requires a small inoculum, and other factors such as diet or antibiotics promote colonization of a few organisms, resulting in severe disease.[29]

2.1.2.2 VIRULENCE

Virulence is the ability of an organism to cause disease.[1] Virulence of an organism frequently is tested by inoculation of different strains of a pathogen into groups of a rodent species and evaluation of lethality or invasiveness. Using this particular methodology, the severity of many diseases frequently is found to be strain-dependent, and virulence commonly is associated with certain phenotypic characteristics of a particular strain. For example, severity of pulmonary disease resulting from *Klebsiella pneumoniae* depends on differences in capsular size and colony morphology.[30] These characteristics become the focus of future investigations and eventually may be defined as virulence factors.

2.1.2.3 ANTIBIOTIC RESISTANCE

Widespread use of antibiotics in animal and human infection occurred following World War II.[31,32] Within 30 years, resistance of gram-positive organisms already was occurring in human pneumococcal infections.[33] From the 1960s to the 1980s, staphylococcal resistance progressed from initial methicillin-resistant organisms to vancomycin-resistant organisms.[32] As a result, for gram-positive and gram-negative organisms, progressive resistance has resulted in less effective means for therapeutic intervention.

Although specific types and mechanisms of bacterial resistance are discussed elsewhere, high-grade and intermediate resistance occur, which are speculated to have arisen from different processes. In terms of bacterial pathogenesis, intermediate resistance occurs in geographically defined isolates in a stepwise fashion resulting from genetic changes under antibiotic pressure. Thus proper dose and length of antibiotic exposure is important in preventing the development of these isolates.[33,34] High-grade resistance is associated with multidrug resistance.[35] In high-grade and multidrug forms of antibiotic resistance, clonal expansion of small numbers of isolates occurs.[35] Individual carrier animals and persons are important for high-grade and multidrug resistance in addition to selective antibiotic pressure. Of note is the fact that commensal or noninvasive pathogens have higher reportage rates for resistance, whereas more highly pathogenic or invasive organisms have less resistance. Thus virulence and antibiotic resistance are not synonymous. Because commensal organisms are ubiquitous, they have a higher likelihood of contact with bacteria that have multiresistance genes. Thus commensal organisms eventually become a reservoir for resistance genes.[36]

2.1.3 Development of Disease and the Role of Normal Flora

2.1.3.1 DISRUPTION OF NORMAL FLORA

As stated earlier, development of disease is caused by disruption of normal flora and invasion by a pathogen or the conversion of a common commensal organism into a pathogen. The pathophysiology of certain types of
equine colitis and pleuropneumonia provide examples consistent with each of these two circumstances. 61
Disruption of normal gastrointestinal flora is likely the underlying pathogenic mechanism for development of 62
acute enterocolitis. Development of colitis in the horse, presumably from disruption of fecal flora, has been associated with feed change, antibiotics, surgery, nonsteroidal antiinflammatory drugs, and transport.[37–39]

Rapid change from a roughage diet to concentrate results in increased numbers of anaerobes, decreased numbers of cellulolytic bacteria, decreased cecal protozoa diversity, and decreased pH in the equine cecum.[37] Isolation of *Clostridium difficile* increases in horses administered antibiotics, and *C. difficile* diarrhea associated with ampicillin, erythromycin, penicillin, and potentiated sulfonamides has been reported in adult horses.[38,40] In ponies infected with *Salmonella* spp., transport and surgery reactivated infection and diarrhea, and antibiotics (oxytetracycline) prolonged shedding.[39] In a case-control study, potentiated sulfonamides were not associated significantly with development of diarrhea in hospitalized horses; however, overall antibiotic use was associated highly with the occurrence of diarrhea.[41]

Several mechanisms exist by which antibiotics disrupt normal gastrointestinal flora and intestinal function. These mechanisms include disruption of carbohydrate metabolism, decreased metabolism of bile acids, direct effects on intestinal motility, and alterations in intestinal mucosa. Change in carbohydrate metabolism is a large intestinal event following decreased microbial reduction of carbohydrates to short-chain fatty acids (SCFA). Because SCFA metabolism and absorption results in fluid and electrolyte absorption, a sudden decrease in SCFAs leads to osmotic diarrhea with intraluminal accumulation of organic acids, cations, and carbohydrates. In human studies, reduced SCFAs have been demonstrated with many antibiotics, including ampicillin, metronidazole, and erythromycin. Bile acids, reduced in the colon by dehydroxylating bacteria, are potent colonic secretogues. Increases in fecal bile acids have been demonstrated in human beings with the use of ampicillin and clindamycin. Erythromycin and amoxicillin directly affect colonic motility.[42] Erythromycin is a motilin receptor agonist that stimulates contraction of antral and duodenal smooth muscles.[43] In the horse, erythromycin results in a dose-dependent increase in ileocecal emptying.[44] Motility-enhancing effects also have been observed in human patients treated with amoxicillin.[42]

2.1.3.2 DISEASE CAUSED BY COLONIZATION OF COMMENSAL FLORA

Occurrence of infectious lower respiratory disease in the adult athlete is an example of the conversion of several commensal bacteria into pathogenic organisms because of contamination of a normally sterile site. Changes in upper respiratory mucosal flora and transportation are two important elements that contribute to the development of pleuropneumonia in horses. The tonsillar mucosa of the oropharynx is colonized heavily with *Streptococcus equi*, and necrosis of this tissue occurring during viral infection is associated with spread to the lower respiratory tract.[6] The association of transport as a risk factor for the development of pleuropneumonia in horses was demonstrated in a retrospective study where transport of greater than 500 miles was associated highly with disease.[45] Elevation of the head for an extended duration is an important initiating event. Natural feeding behavior of horses, which results in a lowered head for several hours, is likely an important method for tracheal clearance. Under experimental conditions, elevation of the head for prolonged periods results in an increase in types and multiplication of oral/pharyngeal commensal bacteria within the trachea.[46,47] *Pasteurella, Actinobacillus*, and *Streptococcus* spp. are the most frequent and prolific colonizers of the trachea after prolonged head elevation. In addition to prolonged head elevation imposed by transport, decreased phagocytosis of equine peripheral neutrophils occurs in horses exposed to extended travel.[47] As a result, common commensal bacteria of the oropharynx and upper airways become opportunistic pathogens.

2.1.3.3 NOSOCOMIAL INFECTIONS

Nosocomial infections are defined by the Centers for Disease Control and Prevention as "an adverse reaction to toxin or infection that was not present or incubating at the time of admission to a hospital."[48] The Centers for Disease Control also have case definitions for each body site and particular infection in human nosocomial disease. No central mechanism exists for detecting and documenting nosocomial infection in veterinary medicine, and frequently many such infections go unrecognized. Nosocomial infections are becoming a major problem for large animal veterinary teaching and private referral hospitals. Infections with *Serratia marcescens, Acinetobacter baumannii, Staphylococcus aureus*, methicillin-resistant *Staphylococcus* spp., *Enterococcus* spp., and various *Salmonella enteritidis* serovars have been reported in association with nosocomial infection in equine patients.[49–59] Surgical incision infection, joint sepsis, catheter phlebitis, wounds, and diarrhea represent the common clinical nosocomial syndromes reported in horses.[49,51–59] When nosocomial infection involves the acquisition of isolates from the hospital environment, these isolates are more difficult to treat because they frequently undergo high-level antibiotic pressure and attain multiresistance. These organisms also have been shown to cross species lines. Reports of nosocomially transmitted salmonellosis in equine hospital wards are increasing, and outbreaks of *S. enteritidis* serotypes 62
Krefeld, Saint Paul, DT104, and Anatum have demonstrated attainment of multiple antibiotic resistance over 63
the course of the outbreak.[50–54] Only one study of a nosocomially transmitted *S. enteritidis* (serotype Heidelberg) did not demonstrate significant acquisition of multiple antibiotic resistance over time.[60]

2.1.4 Pathogenesis of Bacterial Infections

The ability of bacteria to gain entry and cause disease results from a combination of factors possessed by the agent itself, environmental conditions, and status of host defenses. Bacteria gain entry through a body surface by direct inoculation or colonize and damage a dermal or mucosal barrier to cause disease. Environmental or risk factors specific for individual diseases increase the probability of successful penetration or colonization and are discussed for specific diseases in various chapters. Innate and specific immunity, which alter host susceptibility to disease, also are discussed elsewhere in this text. Mechanisms that are specific to bacteria and enhance disease are virulence factors. Virulence factors may allow bacteria an advantage to gain entry and disseminate or may cause damage to the host directly once entry has been gained.

2.1.5 Factors That Enhance Entry of Bacteria

2.1.5.1 ADHESION AND ENTRY

Fibrillar adhesins, nonfibrillar adhesins, and membrane ruffling are virulence factors for bacterial invasion because they aid in colonization of host surfaces. The most common type of adhesin found in gram-positive and gram-negative bacteria are lectins.[61–63] These proteins are highly conserved in bacteria and are important targets for immunoprophylaxis. Although attachment is thought to be the primary role of these proteins, attachment itself results in an intracellular change including actin rearrangements, cell signaling regulation, or actual secretion of bacterial substances into the host cell.

2.1.5.2

FIBRILLAR ADHESINS

Multiple types of fimbria occur in gram-positive and gram-negative bacteria, with gram-negative papillae the most well characterized.[64] Fimbriae are filamentous appendages that are not flagella and do not function in bacterial conjugation.[62,65] Pili or fimbriae are rod-shaped structures composed of an orderly array of a single protein usually arranged helically to form a cylinder. The tip of the fimbria mediates attachment to carbohydrate moieties on cell surfaces and is integral to bacterial invasion and colonization. Bacteria also can contain multiple types of pili. The bacterial pili themselves and the cellular pathways bacteria use for secretion and formation of pili are targets for pharmacologic intervention.[62] Type I fimbria are distributed in the periplasmic space and translocated to the cell surface by a chaperone/usher pathway. These pili are made up of multiple major pilin subunits compiling a rigid shaft with minor pili proteins composing the flexible tip. The chaperone is a special bacterial cell protein that prevents the pilus from achieving its final figuration while in the periplasmic space located between the inner and outer membrane before assuming its position on the outer membrane. *Salmonella* bacteria and uropathogenic *Escherichia coli* commonly possess type I pili. The K88 and K99 fimbriae of enteropathogenic (EPEC) *E. coli* are also type I pili. *Haemophilus* spp. and *Klebsiella* spp. contain type I pili. Type IV pili are located at the pole of the cell and assembled via the type II secretion system that is called the general assembly pathway.[63,65] These pili are flexible fibers of variable length that can aggregate, are important in the formation of microcolonies, and are responsible for the twitching motion of bacteria. The proteins are secreted into the periplasmic space but remain anchored to the inner membrane. The protein then is assembled and passed through a pore formed in the outer membrane. This type of pilus commonly is found on *Pseudomonas aeruginosa*, enterotoxigenic *E. coli*, and EPEC. Curli fimbriae are solid surface structures that use the extracellular nucleation/precipitation pathway.[63] This pilus is secreted as a soluble protein extracellularly with a second protein, the nucleator, for stability.

In gastrointestinal and urinary tract infections, fimbriae are likely one of the most important virulence factors for successful invasion.[64] Although the importance of *E. coli* adhesive fimbriae is questionable in equine disease, they nonetheless provide a model by which these organelles assist bacteria. Once receptor-mediated attachment occurs, intracellular calcium concentration increases in the host cell.[64] Proteins and protein kinases involved in the breakdown of actin are activated, resulting in the disruption of microvilli. A change occurs in the cytoskeleton of the cells and permeability to ions and water. Ions are secreted, resulting in the classic secretory diarrhea. The specificity of the receptor determines the host species susceptibility to the bacteria itself and the ability of the bacteria to colonize a specific body site or surface.

The classic example of pili-mediated attachment is EPEC; however, receptors for EPEC have not been identified in the horse. Enterotoxigenic *E. coli* have been isolated from foals.[66] Although this bacterium is able to cause gastritis and enteritis in experimental infection, adhesion to foal intestinal mucosa was not demonstrated despite the fact that this same organism could adhere and colonize swine epithelium. The antiphagocytic M-protein of *Streptococcus* spp. is actually a fibrillar protein and also assists, but is not essential for, adhesion. In anaerobic bacteria, antibodies to flagellar proteins FliC and FliD can block 63
Clostridium difficile adhesion. These proteins are thought to mediate intestinal adherence and colonization.[67] 64

2.1.5.3

AFIMBRIAL ADHESINS

Afimbrial adhesins are cell proteins that enhance the binding of bacteria to host cells. They also are called "microbial surface components recognizing adhesive matrix molecules."[68–70] Gram-positive organisms

possess afimbrial proteins on their surfaces that presumably aid in binding to host cells. The three most commonly studied afimbrial adhesins are those that bind salivary glycoprotein, bind fibronectin, or are composed of lipoteichoic acid.[70,71] Salivary-binding proteins commonly are found in pathogens and commensals of the oral cavity. *Streptococcus* spp. and *Actinomyces* spp. possess these proteins. Fibronectin-binding protein (FBP) is necessary for *Staphylococcus aureus* invasion and binds fibronectin and collagen to form a bridge between the FBP and the host cell integrin (fibronectin integrin $\alpha_5\beta_1$).[72,73] FBPs are essential for invasion of epithelial and endothelial cells as demonstrated by the fact that *Streptococcus pyogenes* FBP mutants do not bind to epithelial cells in vitro.[68–70] Heterologues of FBP have been demonstrated in *S. pneumoniae* of human beings, *S. equi equi*, and *S. equi zooepidemicus*. Other potential equine pathogens that have FDPs on their surface include *Actinomyces* spp., *Enterococcus faecalis*, and *Listeria monocytogenes*.[71,74] Lipoteichoic acid is a common binding factor found in *Streptococcus* group A bacteria, and antibodies to this protein inhibit adhesion of the bacteria to cells.[75] This protein is also important in stimulation of cytokine secretion from the cells during infection, and lipoteichoic acid has been demonstrated in group B *Streptococcus*, including *S. equi equi*.[76] A less commonly described afibrillar adhesin is composed of surface polypeptide chains in *Corynebacterium* that bind to lectin.[77] Binding of these proteins can be abolished by trypsin treatment. Afibrillar adhesins are also present in gram-negative organisms, the most commonly studied are conserved high-molecular-weight adhesion proteins of *Haemophilus influenzae* and *Bordetella pertussis*.[75]

2.1.5.4 CYTOSKELETAL CHANGES

Binding of bacteria frequently results in cytoskeletal changes within the host cell to enhance susceptibility to invasion. Membrane ruffling is a virulence factor that results in internalization or breakdown of intracellular components to allow for invasion of tissues. The two models of membrane transformation commonly are referred to as zipperlike or triggerlike, and bacteria commonly studied in this phenomenon include *Yersinia* spp., *Listeria monocytogenes*, *Salmonella* spp., and *Shigella flexneri*. Binding results in actin rearrangement and engulfment of these intracellular bacteria by phagocytic and nonphagocytic cells. *Yersinia* and *Listeria* form a zipperlike relationship in which the tightly adhered bacteria result in actin polymerization and formation of a phagocytic cup.[78] This three-stage process is controlled by different proteins, the first of which initiates actin nucleation and branching of filaments. A second protein stimulates actin polymerization and depolymerization, which is followed by activation of a third protein that ends the process. *Salmonella* and *Shigella* bacteria adhere and secrete proteins that are translocated into the host cell cytoplasm and trigger actin polymerization. Bacterial proteins are secreted into the cell,[79] and an actin-binding protein forms a complex with the cell protein, T-plastin. In the presence of F-actin, an increase in actin bundling of T-plastin occurs primarily in the vicinity of bacterial-host cell contact, resulting in enhanced *Salmonella* invasion. In addition to membrane ruffling, *Mycobacterium avium* and *Salmonella* spp. rely on activation of intracellular guanosine triphosphatases leading to phagocytosis.[80,81]

2.1.6 Factors That Enhance Spread of Bacteria

Once colonization occurs, multiplication and spread of bacteria is enhanced through virulence factors. These factors assist bacteria in evasion of immune defenses, use of the host environment, and breakdown of tissue barriers. Many of these defenses overlap, but the end result is avoidance of destruction by the host and sublimation of host tissues into a new bacterial niche.

2.1.6.1 EVASION OF IMMUNE DEFENSES

2.1.6.1.1 Capsule

One of the most common and potent strategies for avoidance of phagocytosis is the presence of a capsule. The polysaccharide components of capsule are important targets for control of bacterial infections. Immunization strategies against capsular components, such as with vaccination against *Haemophilus influenzae* infection in human beings,[82,83] has been successful in the control or elimination of this disease.

Bacteria are diverse and many have a capsule, but assembly and structure of the capsule is remarkably similar between bacteria. The *Escherichia coli* capsule is representative of the majority of gram-negative capsule types.[84] Three genetic regions control the development of capsule, and all are targets for intervention. The first region controls transport and assembly of the mature polysaccharide through the outer membrane. The second genetically encoded region controls formation and polymerization of 64
polysaccharide itself. The complexity of the resulting protein product depends on the size of this region. 65
The third genetic locus contains genes that are involved in translocation of the polysaccharide through the inner membrane.

The capsule of bacteria is composed mainly of polysaccharide, which is inherently a poor immunogen. Ultrastucturally, the capsule in *E. coli* and *Klebsiella pneumoniae* is a fine fibrillar meshwork covering the bacteria surface; however, the capsule of *Klebsiella* is approximately 20 times thicker than that of *E. coli*.[85] In Enterobacteriaceae, the K antigen is one of the major antigens of this complex and expression is temperature dependent.[86] The presence of certain K antigens and a large capsule, as studied in *Klebsiella* virulence, is associated highly with increased pathogenicity and resistance to phagocytosis.[87,88] The other major antigen is the lipopolysaccharide without the lipid A component of the molecule. A third minor component, colanic acid, appears to exhibit antiphagocytic activity in some *E. coli*. Classically, the presence of capsules on *E. coli* have been shown to prevent complement-mediated phagocytosis.[89] Lipopolysaccharide itself activates complement, and the capsule prevents immune activation by concealing the lipopolysaccharide molecule. Introduction of the genes associated with production of a more viscous capsule into a bacteria change an avirulent phenotype into a virulent phenotype.[90]

As early as 1928 the engulfment and digestion of *Streptococcus pneumoniae* was observed to be associated with lack of capsule.[91] By 1940 this virulence factor was understood to be genetically encoded.[92] Early studies with *S. equi equi* demonstrated that resistance to phagocytosis was associated with an increase in capsule and M protein,[93] and in a model of *S. equi zooepidemicus* infection in mice, enhancement of virulence was associated with increased capsule and resistance to phagocytosis.[94] Although colonization of the guttural pouch occurs with nonencapsulated *S. equi equi* strains, induction of lymphadenopathy is correlated more to the capsular strains. Recent studies have shown that when M protein content is kept constant, the amount of capsule actually is correlated with resistance to phagocytosis.[95] Resistance to in vitro phagocytosis can be abolished with treatment with hyaluronidase. In support of this, phagocytosis of *S. equi equi* and *S. equi zooepidemicus* can be enhanced significantly in a murine model by induction of specific immunity against the hyaluronan component of this capsule.[96]

The two most important underlying components of abscess formation are (1) resistance to phagocytosis and (2) failure of bacterial clearance. Capsules of anaerobic bacteria are unique, and their role in virulence may account directly for the formation of abscesses within the host. The capsule of *Bacteroides fragilis* has two distinct polysaccharides composed of repeating subunits with oppositely charged groups (zwitterion).[97] This polysaccharide complex injected alone promotes the induction of abscess. Development of antibody against these polysaccharides prevents abscess formation.

Infection of rodents with the encapsulated form of *Bacteroides* spp. and *Fusobacterium* spp. results in the formation of intraperitoneal abscesses, whereas nonencapsulated bacteria do not cause abscessation.[98–100] Higher mortality is associated with encapsulation in these models. Synergism of capsular anaerobes with other bacteria occurs; nonencapsulated bacteria have enhanced survival in abscesses and produce capsules when cultured or inoculated with encapsulated bacteria.[99]

2.1.6.1.2 Anticomplement Factors

Similar and overlapping with the function of capsules are structural proteins that block complement. The O side chain of lipopolysaccharide on gram-negative bacteria is an anticomplement factor.[101] The longer the side chain, the farther the distance between phagocytes and the bacteria. Many capsules contain sialic acid, the interaction of which with O antigen prevents the formation of C3 convertase.[102] Bacterial enzymes are formed by *Streptococcus* spp. and other organisms that damage the polymorphonuclear cell chemoattractant C5a.[103–106] Production of a protein in *Salmonella* spp., encoded by the *rck* gene, prevents insertion of the C9 fragment of complement into the bacterial membrane.[9]

The M proteins of *Streptococcus* spp. are considered important for resistance to phagocytosis. Specifically, for *S. equi equi*, the M protein appears to decrease deposition of complement on the surface of the bacteria. The hypervariable region of the M protein of *S. pyogenes* and *S. pneumoniae*[107,108] confers this resistance to phagocytosis.[109] The M protein of *S. equi zooepidemicus* is 90% homologous in the hypervariable region and likely mediates the same function.[93] This mechanism for complement resistance appears to be through enhancement of binding of fibrinogen to the bacteria in the presence of M protein.[110–112] When fibrinogen is present on the outer surface of the bacteria, phagocytosis is blocked.

2.1.6.1.3 Phagolysosomal Survival

The intracellular environment should be inhospitable for bacteria, yet many organisms are ingested by phagocytic cells and use the intracellular environment to multiply and disseminate. In normal phagolysosomal fusion the phagocytic vesicle first becomes fused with a host cell endosome. Shortly thereafter, fusion with the lysosome occurs. Several digestive proteins are released within the lysosome and a drop in pH occurs, resulting in inactivation and digestion of a foreign protein or microorganism. *Shigella* 65
spp. and *Listeria monocytogenes* are bacteria that escape the phagocytic vesicle to multiply in the host cell 66
cytoplasm.[113]

Extended survival (and replication) in the phagosome itself is an important strategy for dissemination of bacteria. The two main mechanisms for phagosome survival include (1) inhibition of phagolysosomal fusion and (2) blockage of the formation of lysosomal enzymes once phagocytosis occurs. Before escape from the phagosome, *Listeria* modulates maturation of the phagosome by delaying fusion with the

lysosome.[114] One of the earliest observations of *Mycobacterium tuberculosis* infection in cell culture was uninhibited multiplication within macrophages.[115] Later studies determined that *Mycobacterium* and *Legionella* cause a change in the maturation of the phagosome.[116] Once the phagosome is infected, many lysosomal proteins appear in it, yet others do not, indicating a manipulation of the environment after fusion. Engulfment of *Salmonella* in the phagosome appears to induce formation of an actual phagolysosome, but the organism survives nonetheless.[117] Acidification occurs, but production of reactive oxygen species is decreased. In addition, *Salmonella* appears resistant to reactive oxygen, which is controlled by a number of *oxy* genes that are upregulated in response to oxygen stress. This occurs rapidly, within 20 minutes of intracellular uptake of the bacteria.[118] Many bacteria that are resistant to reactive oxygen are able to make catalase and superoxide dismutase.

Inhibition of phagolysosomal fusion itself is an important protective mechanism of bacteria. *Rickettsia*, *Neorickettsia* (formerly *Ehrlichia*), and *Rhodococcus* spp. bacteria appear to inhibit phagolysosomal fusion. More detail regarding mechanism of this phenomenon is given in the section on equine *Ehrlichia. Rhodococcus equi* also inhibits phagolysosomal fusion, and although the mechanism of inhibition is unknown, opsonization by *R. equi*–specific antibody results in enhanced fusion and killing.[119,120] Resistance to phagolysosomal fusion appears to depend on the presence of the 90-kd virulence plasmid.[121]

2.1.6.2 ADAPTATION TO HOST ENVIRONMENT

2.1.6.2.1 pH Resistance

The acid tolerance response is important primarily for intracellular bacteria as a way to survive the acidification within a mature phagolysosome. Many bacteria including *Listeria monocytogenes, R. equi*, and *Salmonella* spp. are able to withstand highly acidic environments.[120,121] Genes that control virulence in *Salmonella* actually are upregulated by an acidic environment.

2.1.6.2.2 Bacterial Nutrition

Nutrition of bacteria is associated intimately with cellular and tissue environments. Although bacteria use iron for many enzyme systems, the relationship between bacteria and iron is more complex. The proper level of iron is important because iron is required for the production of reactive oxygen intermediates. The *fur* gene in *Escherichia coli* was identified first as the major regulator of iron acquisition.[122] Homologues of this gene have been identified in many other bacteria, including *Salmonella* spp., *Vibrio* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacteroides fragilis*.[122–127] This gene regulates the amount of iron handled by the bacterial cell depending on the host stores. In times of iron stress, the gene is downregulated to allow for more iron uptake from the environment by the bacterial cell. In addition, bacteria possess siderophores, which are potent chelators of iron.[128] These siderophores are secreted outside of the bacterial cell, where they take up iron, and are taken back into the cells through a receptor-mediated process. After internalization, iron is cleaved and used by the bacteria. Resistance to carbon and nitrogen starvation are two other means assisting bacterial survival in inhospitable environments.

2.1.6.3 DAMAGE TO HOST TISSUES

2.1.6.3.1 Toxins

Exotoxins are virulence factors secreted by bacteria generally to aid spread of infection. Gram-positive and gram-negative organisms secrete an array of exotoxins. Classically, three types of exotoxins occur[129]: (1) the A-B form with two distinct parts for binding and for enzymatic action, (2) toxins that form pores within host membranes, and (3) superantigens that form a bond between the major histocompatibility complex class II receptor of macrophages, resulting in release of various T helper cell–mediated cytokine cascades. Several examples of common equine pathogens follow.

Toxins of *Staphylococcus aureus* are well characterized and are associated directly with the pathogenesis of disease.[130] Toxins that may be important for disease in the horse include the four hemolysins, toxic shock syndrome toxin 1 (TSST-1), exfoliative toxins, and leukocidin. Few overt syndromes are recognized in the horse that are caused specifically by *S. aureus*. However, *S. aureus* is the most common bacteria isolated from cellulitis and joint infections in adult horses. Cellulitis induced by *S. aureus* in the horse may be similar to wound-associated toxic shock in human beings. Toxic shock syndrome is a disease of increased capillary permeability characterized by hypotension, hypoalbuminemia, and edema. The toxins of *S. aureus* work in concert to (1) induce massive release of cytokines, (2) increase sensitivity to cytokines, and (3) damage endothelial cells directly. The superantigen toxin of *S. aureus* forms a bridge between the major histocompatibility complex receptor on macrophages and the T cell receptor resulting in a massive release 66
of interleukin-2 (IL-2). The downstream effect is T cell proliferation and induction of type 1 cytokines that 67
stimulate release of proinflammatory cytokines from macrophages. Exfoliative toxin also induces T cell proliferation. TSST-1 and exfoliative toxin of *S. aureus* enhance the effects of endotoxin in cell culture and live animal experiments. In rabbits the lethal dose of endotoxin is decreased by 100-fold. The TSST-1 binds directly to endothelial cells and in endothelial cell culture demonstrates cytotoxicity. A dose-dependent induction of leakage across monolayers also occurs. Exfoliative toxin and TSST-1 have been identified in equine *S. aureus* isolates associated with severe phlegmon and metritis.[131,132]

Alpha-toxin has been studied intensely and is directly toxic to many cell types.[129] This toxin can damage erythrocytes, skin, and nerve cells and is lethal.[130] At high concentrations, molecules of the toxin polymerize and form pores in monolayers of cells resulting in permeability of the cells. The arachadonic acid cascade is activated, resulting in thromboxane- and prostacyclin-mediated vasoconstriction in the face of edema formation. Alpha-toxin also appears to act directly on platelets, resulting in release of procoagulant factors. Increased permeability and swelling of cells may be important in the pathogenesis of dermal and neural damage. Myelin sheath destruction is the lesion observed in rabbits. The toxin, β-hemolysin or sphingomyelinase C, is produced in high concentrations in many *S. aureus* isolates, and the degree of pathogenicity to erythrocytes likely is determined by sphingomyelin content of erythrocytes of different strains and species. This toxin has high amounts of phosphorylase C activity in the presence of Mg^{2+}. The presence of this toxin appears to be correlated with enhanced growth characteristics. The toxins γ-hemolysin and leukocidin appear to cause degranulation of leukocytes, with leukocidin being the more potent of the two. Finally δ-toxin is toxic to erythrocytes and cellular organisms and also is lethal at high doses.

2.1.6.3.2

Clostridial Infections

Clostridrium spp. mediate clinical disease by producing exotoxins. The clostridial bacteria causing botulism and tetanus excrete neurotoxins to which horses are highly susceptible. The main toxins of botulism and tetanus are remarkably similar even though they exert effects that appear to be opposites, with botulism causing a flaccid paralysis and tetanus causing a spastic paralysis.[133] Both toxins share amino acid sequences of metalloproteinases that are most similar to zinc-requiring endopeptidases. With botulism, at least six serologically different neurotoxins occur, with most reports in horses caused by type B toxin, although A, C, and D also have been identified.[133–137] Only one tetanus neurotoxin is responsible for the clinical signs of spastic paralysis.

Neurotoxin is secreted as a progenitor and must be cleaved by proteases (trypsin) to the derivative toxin to produce clinical signs. The active toxin consists of a light and heavy chain (A-B type toxin) that mediates paralysis by blocking acetylcholinesterase in a three-step process.[129,138] The first step is rapid and involves recognition and binding of the neurotoxin to its receptor on the nerve. In the second step, translocation occurs as the active site of the molecule is internalized in the nerve cell ending by endocytosis. The acidified vesicle induces a conformational change in the toxin so that it can be translocated to the cytosol. The third and final step is the slow step in which cell neuroproteins called synaptopeptidases are cleaved, preventing acetylcholine release. The proteins that are cleaved by the neurotoxin include synaptobrevin, synaptosome-associated membrane proteins, and syntaxin. The protein synaptobrevin is a component of the neurotransmitter cell membrane, is essential for neurotransmitter release, and is involved in exocytosis of the neurotransmitter vesicle and axonal growth. Syntaxin is important for vesicle and cell membrane fusion. The inactivation of one or more of these proteins accounts for the prevention of release of synaptic vesicles. The different botulinum neurotoxins exhibit variable specificity to these proteins. Types B, D, F, and G are active against synaptosome-associated membrane proteins, whereas A, C, and E are active against synaptobrevin, and C is the only form that cleaves syntaxin.

The difference in clinical signs noted between *C. tetani* and *C. botulinum* is a reflection of the binding and level of activity of the respective toxin. *C. botulinum* binds to peripheral nerves, and *C. tetani* binds to cells within the central nervous system. Furthermore, *C. botulinum* toxin binding results in prevention of downstream release of other neurotransmitters, such as γ-aminobutyric acid. Injection of *C. tetani* toxin intravenously in low doses results in flaccid paralysis.

The main pathologic features of disease cause by *C. perfringens* are edema, necrosis, and death, regardless of the site of action.[138] Toxins elaborated by these clostridia include lethal and nonlethal toxins that cause necrosis and hemolysis and frequently contain lecithinases and lipases. *C. perfringens* has four major lethal toxins, an enterotoxin, and three minor toxins. Recent genetic evaluation of equine isolates associated with diarrhea indicate the majority of isolates are type A, the toxic affects of which are mediated by α-toxin.[139] The *cpe*-toxin or enterotoxin has been detected by enzyme-linked immunosorbent assay in 16% to 19% of equine isolates, so disease has been linked to enterotoxigenic type A and nonenterotoxigenic type A strains.[18,140] Individual case reports exist of neonatal diarrhea associated with type B (β- and ε-toxins), type C (β-toxin), and type D (ε-toxin) strains, which also carry the α-toxin.[141,142] 67

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Classic experiments demonstrate that injection of purified α-toxin into mice results in hemolysis and death, and recent evidence confirms the role of α-toxin as the major virulence factor of gas gangrene.[138] α-Toxin is one of several zinc metalloproteinase enzymes of bacteria including that of *Listeria monocytogenes,*

Bacillus cereus, and other *Clostridium* spp. One enzyme is phospholipase C, which is able to hydrolyze phosphatidylcholine (lecithin) and sphingomyelin.[143,144] This toxin is responsible for cardiovascular collapse and death, likely by increasing vascular permeability. At the cellular level, this toxin can cause platelet aggregation and hemolysis and is cytotoxic and myotoxic. Vascular activity is mediated by upregulation of adhesion molecules in leukocytes and endothelial cells. Endothelial cells and macrophages produce platelet-activating factor in response to toxin exposure. Muscle fibers are damaged by the formation of intravascular aggregates within the muscle.[143]

Recent work with β-toxin and ε-toxin indicates that these toxins act as pore formers.[145,146] Early research into the mechanism of action of β-toxin indicated a role for catecholamine release because of the sudden increase in heart rate and drop in blood pressure when the toxin was injected into experimental subjects.[138] β-Toxin polymerizes and forms pores that act as selective ion channels, the generation of which within cell membranes effectively creates a neurotoxin.[145] Work with ε-toxin indicates that this toxin is able to form large pores within membranes, resulting in a flux of potassium outward and chloride and sodium inward. Later ion flux is dominated by movement of calcium into the cell.[146] This flux results in irreversible morphologic changes in the cell membrane and death of the cell.

Enterotoxin binds directly on the cell membrane, causing a change in ion movements that affects cellular metabolism.[138] Similar to the actions of these other toxins, most of the damage is mediated by increased calcium levels within the cell, affecting cell membrane function and permeability. The newly recognized β_2-toxin has been associated with intestinal disorders in horses; however, the exact mechanisms of action of this toxin are unknown.[147]

C. difficile has five toxins, of which toxins A and B are thought to be most important in development of disease.[148,149] The evidence to date indicates that toxins A and B mediate the pathogenesis of *C. difficile* diarrhea.[149] Research suggests that the formation of the classic pathologic lesion, the pseudomembrane, results from the combined actions of toxin A, toxin B, and IL-8. Classically, toxin A is considered an enterotoxin and lethal, whereas toxin B is a virulent cytotoxin. Toxin A appears to be the primary mediator of fluid accumulation.[150,151] The toxin is thought to bind to an oligosaccharide receptor and is internalized immediately. After a lysosomally mediated acidification step, a rapid fall in intracellular adenosine triphosphate concentration occurs, followed by release of mitochondrial cytochrome *c* and generation of reactive oxygen intermediates. Injection of toxin A results in fluid accumulation, cell necrosis, and recruitment of inflammatory cells. One mechanism of fluid accumulation appears to be by interruption of actin filaments and destruction of tight junctions. Toxin A also results in neutrophil recruitment possibly mediated by the direct action of the toxin on macrophages to stimulate release of IL-1, tumor necrosis factor α, and leukotrienes. In cell culture, toxin A can cause IL-8 secretion (a potent recruiter of neutrophils), cell detachment, and apoptosis of separated cells. Direct activation of macrophages and secretion of IL-8 is through a calcium and calmodulin–dependent mechanism that results in direct nuclear upregulation after nuclear translocation of transcription factors NF-κβ and AP-1.[150] Toxin B is more cytotoxic than toxin A, especially to human epithelial cells.

2.1.6.3.3 Cell Death

Apoptosis is a distinctive morphologic process that results in cleavage of nuclear material and scavenging of unwarranted cells without immune activation.[152] Apoptosis or programmed cell death is an important

pathway for complex organisms to deal with damaged and diseased tissue. Apoptosis avoids the release of the tissue damaging enzymes and nonspecific elimination of tissue that occurs in cellular necrosis. Several bacteria modulate the host apoptotic pathways to enhance survival.[153] *Shigella flexneri, Salmonella typhimurium*, and toxins of *Staphylococcus aureus, Pseudomonas* spp., and *Corynebacterium diphtheriae* have demonstrated programmed cell death as a consequence of cellular infection or exposure.[152,154] A protein of *Shigella flexneri*, IpaB, induces apoptosis by binding to and activating the cellular enzyme caspace 1, which induces apoptosis of macrophages.[113] *Staphylococcus aureus* α-toxin, presumably escapes the macrophage after engulfment and induces host cell apoptosis.[130] The TSST of *S. aureus* induces B cell apoptosis and blocks immunoglobulin production.

2.1.7 Pathogenesis of Fungal Infections

Of the 250,000 species of fungi, fewer than two hundred are true pathogens.[155] Superficial mycoses affect the hair shaft and the superficial epidermis. Cutaneous mycoses (dermatophytosis) infect the epidermis, dermis, hair, and nails of animals, and *Microsporum, Trichophyton*, and *Epidermophyton* are the most commonly associated pathogenic genera. Subcutaneous tissues can become infected with *Sporothrix, Conidiobolus, Basidiobolus*, and 68
69 members of the Dematiaceae fungi, which cause the chromoblastomycosis, mycetoma, and phaeohyphomycosis infections. Most of these infections are introduced by penetration through skin or opportunistic invaders of damaged skin surfaces. *Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis*, and *Paracoccidioides brasiliensis* are the four most important systemic fungal pathogens. The most common opportunistic infections include *Candida albicans, Aspergillus* spp., *Cryptococcus neoformans, Mucor* spp., and *Pneumocystis carinii*.

2.1.8 Factors That Enhance Entry of Fungi

Fungal virulence factors may be more complex than those of bacteria because of the higher degree of opportunism that occurs primarily because of a change in host status. Subtle factors may combine with host status to result in a certain fungus attaining a virulent state. For example, the typical fungal wall is composed of three major polysaccharides: mannose, glucans, and chitin. Chitin mutants of *Candida albicans* are less virulent when tested in rodent models than wild-type fungi.[156] A mutant *C. albicans* that cannot synthesize complex mannose oligosaccharides does not adhere to other yeast and epithelial cells and has lost virulence in a guinea pig model.[157] Both of these mutants can proliferate in vitro normally, and whether this is an actual virulence factor is unclear.

As in bacteria, adherence is an important characteristic for infection and colonization of the host. Adhesins have been identified in *C. albicans* and *B. dermatitidis*. Two genes have been associated with adhesion in *C. albicans*. The first is a glycoprotein that has sequences consistent with agglutinating activity. Transformation of this gene into other nonadherent fungal species results in adhesion of the transformed yeast to cells.[158] *C. albicans* also has integrin-like proteins, the disruption of which results in diminished hyphal growth, adhesion to cells, and loss of virulence in mice.[159,160] The *B. dermatitidis* adhesin mediates binding to human monocyte-macrophages through the CD14 receptor.[161]

2.1.9 Factors That Enhance Spread of Fungi

2.1.9.1 EVASION OF IMMUNE DEFENSES

Many fungi have polysaccharide capsules that help them resist phagocytosis and immune activation. The capsule of *Cryptococcus neoformans* inhibits leukocyte accumulation, cytokine secretion, and macrophage phagocytosis.[162] Mutants without capsules are highly infective and avirulent. As indicated earlier, many fungi are engulfed by macrophages, and intracellular survival is mediated by virulence factors. Macrophages are capable predators of *Candida albicans*,[163] *H. capsulatum*,[164] and *B. dematitidis. H. capsulatum* is primarily a yeast in vivo, and this form infects macrophages. Phagolysosomal fusion occurs at a normal rate,[165] but blockage of acidification of the phagolysosome occurs.[166]

2.1.9.2 ADAPTATION TO HOST ENVIRONMENT

2.1.9.2.1 Morphology and Temperature

Adaptability to the host environment is also a trait that enhances fungal virulence. Fungal dimorphism, which is the ability to adopt another morphologic state, clearly is tied to virulence. Mutants of *C. albicans* that cannot switch to the hyphal form are avirulent in certain in vivo models, although both forms likely contribute to pathogenesis.[167] In a second adaptation, phenotypic switching, colonies of fungi change during in vitro growth. *Candida albicans*, *Cryptococcus neoformans*, and *H. capsulatum* display phenotypic switching, and different phenotypes are associated with degrees of virulence.[168–171] *H. capsulatum* spontaneously gives rise to mutants that have less capsule, are less virulent, and are not cytotoxic to macrophages.[170] The signal for dimorphic fungi to change form is usually temperature. Many fungal species germinate within the host with increased temperature, allowing for dissemination within the host. Calcineurin is found in many yeast and mammalian cells and in *C. neoformans*. This protein mediates the ability of the fungus to grow at 37°C.[172] Temperature and heat shock also are mediated by the calcium-dependent protein cyclophilin B in *Aspergillus*.[173] Adaptation to mammalian pH is controlled genetically in *Candida albicans*. Mutants display abnormal cell morphology at physiologic pH ranges.[174]

2.1.9.2.2 Nutrients

Nutrient requirements that affect virulence include melanin, iron, and calcium. Melanins are present in the wall of *Cryptococcus neoformans*, and melanin can scavenge reactive oxygen intermediates, making the organism resistant to the oxidative burst of neutrophils.[175] Pathogenic fungi have siderophores and high-affinity ferric iron reductase to acquire iron from low-iron environments.[176,177] *H. capsulatum* secretes a calcium-binding protein that enhances calcium uptake from calcium-poor environments.[178,179] Without this protein, *H. capsulatum* cannot form colonies and does not survive in cultured macrophages.

2.1.10 Damage to Host Tissues

2.1.10.1 TOXINS

Exposure to pathogenic and saprophytic fungi is an everyday occurrence. Respiratory contamination and 69
infection is important for many species, but skin penetration and dissemination from necrotic gut are important 70
portals for large animals also. After initial infection, dissemination depends on previous damage to host tissues, deeper mechanical penetration, or actual invasion of new tissues. *Candida albicans* can grow through and replace cell membranes.[163] True molds invade blood vessels and grow along the intima of the vessels. Fungi secrete many degradative enzymes, including proteinases, phosphatases, and DNAses to surmount structural barriers.[180] Aspartyl proteinase genes allow more persistent colonization of host surfaces and deeper penetration.[181]

When *Coccidioides immitis* invades the host, the fungi form endospores. Endospores secrete proteinase and urease, which likely aid in the breakdown of pulmonary tissues.[182–184] The two proteinases of *Aspergillus fumigatus* break down elastin, a major component of lung tissue.[185,186] Phospholipase activity has been demonstrated in *Candida albicans, Cryptococcus neoformans*, and *A. fumigatus*.[187] Strains of *Candida* spp. with high amounts of this enzyme have enhanced virulence,[188] and abolishing this activity results in decreased adherence of the organism.[189] Host eicosanoids enhance fungal colonization. Recent evidence demonstrates production of eicosanoids by dermatophytes and systemic fungi.[190]

2.1.10.2 APOPTOSIS

Fungi induce apoptosis by a direct effect of a fungal toxin or following host cell cytoskeleton rearrangements.[191] The gliotoxin of *A. fumigatus* can induce DNA fragmentation and apoptosis in macrophages.[192] This toxin also has many other immunosuppressive qualities, including inhibition of the neutrophil respiratory burst and T cell activation.

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2.2

2.2—Mechanisms of Establishment and Spread of Viral Infections

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Viral infections are responsible for some of the most medically and economically important diseases of horses. A few notable examples include influenza, equine rhinopneumonitis and abortion (Herpesviridae), African horse sickness (Reoviridae), equine infectious anemia (Retroviridae), various encephalitis viruses (Alphaviridae), and most recently in the United States the West Nile virus (Flaviviridae). Specific therapy of viral infections remains a significant challenge because antiviral drugs are generally ineffective, impractical, or cost-prohibitive for the treatment of horses. Treatment of most viral infections focuses on supportive care of the affected organ system(s) and control of secondary complications such as bacterial infection. Currently, control of most clinically significant viral diseases in horse populations relies on vaccination, quarantine, or even destruction of infected animals. 74 75

Despite the great significance of some viral infections, recognizing that many equine viruses are ubiquitous, weakly pathogenic, or not associated with any known disease under normal circumstances is also important. Some examples include equine adenovirus,[1] respiratory and enteric reoviruses[2] (the term *reo* is derived from the acronym for *r*espiratory *e*nteric *o*rphan, indicating that these isolates have not been associated with disease), and equine herpesvirus (EHV) types 2 and 5.[3] Some host-virus relationships may be mutualistic in that virally derived genetic elements are theorized to benefit the host by facilitating genetic variability and evolution.[4] Thus many viruses are of no practical clinical significance, and no control efforts are warranted. For this reason, one should never assume that the recovery of a virus from a clinical specimen indicates significance without proof that the virus can cause the disease in question.

Veterinary virology is a rapidly changing field. New diseases continue to emerge or be discovered; a dramatic example is the Hendra paramyxovirus that appeared in Australia in 1994, killing horses and human beings.[5] More recently, another paramyxovirus, the Salem virus, has been identified in the United States, although the clinical importance of this virus is unclear.[6] Less dramatic but of more relevance to most equine veterinarians is the association of bovine papillomavirus with sarcoids.[7,8] A number of equine diseases occur in which viral involvement has yet to be excluded, including Theiler's hepatitis[9] and lymphosarcoma.[10] Viral origins also likely will be discovered for diseases in which viruses were not suspected previously to play a role; in human beings and mice, viruses have been proposed to have a role in everything from diabetes to obesity.[11,12] However, the greatest advances in veterinary virology are in understanding the molecular biology of viral replication, virus-cell interactions, and virus-host interactions. Related advances in molecular biology techniques, such as the polymerase chain reaction and immunohistochemistry, also are providing sensitive, specific, and rapid tools for the diagnosis of viral infections. In the realm of antiviral drugs, aggressive searches are ongoing for effective and economical drugs for treatment of human beings, and in the near future, antiviral drugs likely will be a realistic therapeutic option for equine veterinarians.[13,14] Finally, significant advances in the design of vaccines likely will improve the efficacy of immunization.

This chapter outlines the general ways that viruses cause disease and describes the most important virus-cell and virus-host interactions that result in pathologic conditions. Although the focus is on equine viruses where possible, the principles described are generally not species dependent, and no attempt is made to limit the discussion to recognized equine viral pathogens or diseases. Discussion of a particular mechanism or virus also should not be taken to suggest that this mechanism or type of virus has been documented in horses. Where possible, supporting references have been selected to include review articles or texts for additional information about key concepts.

2.2.1 Viruses and Virus-Cell Interactions

An in-depth discussion of viral structure, taxonomy, and replication is beyond the scope of this chapter, and the reader is referred to textbooks of veterinary or human virology for more detailed information.[15,16] However, a brief overview is presented to emphasize those features that have clinical relevance.

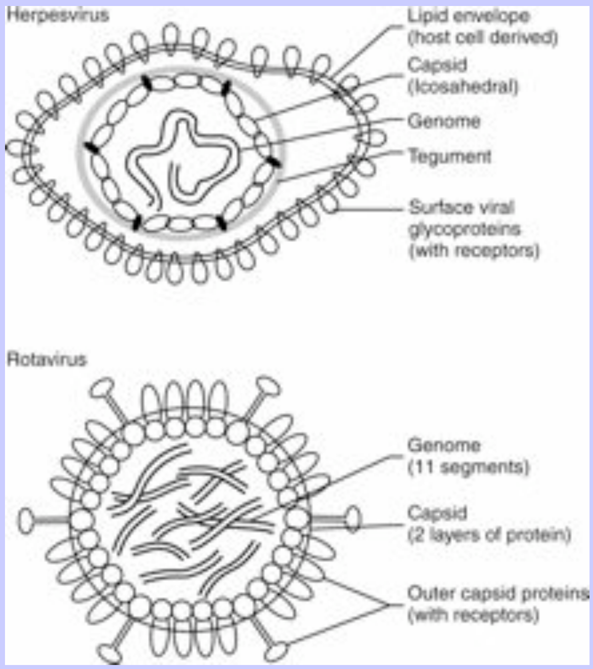
2.2.1.1 VIRUS STRUCTURE, TAXONOMY, AND REPLICATION

The fundamental structure of all viruses is a DNA or RNA genome enclosed by a coat of protein called the capsid (Figure 2.2-1). For viruses that are enveloped, the capsid is enclosed further by a host cell–derived lipid membrane into which viral proteins have been incorporated. In addition to protecting the viral genome, the capsid and other associated structural proteins (e.g., matrix proteins) are important for virus assembly,

packaging the viral genome, releasing the genome into a target cell, and for non-enveloped viruses, providing receptors that bind to host cells. For enveloped viruses the receptors are incorporated into the lipid membrane. The primary clinical significance of these features is that enveloped viruses, because of their fragile lipid membrane, are highly susceptible to inactivation by heat, desiccation, or detergents, and transmission typically requires direct exchange of body fluids, short distance aerosols, or arthropod vectors. In contrast, nonenveloped viruses (e.g., equine rotavirus) are resistant to physical inactivation, and environmental contamination is more likely to be a significant factor in their transmission.

The composition of the viral genome is an important basis for virus classification (Figure 2.2-2). The type of viral genome also determines the strategies required to replicate the genome and transcribe messenger RNA (mRNA) (Figure 2.2-3). Viral genomes may be single-stranded RNA, double-stranded RNA, single-stranded 75
DNA, or double-stranded DNA. The genomes of single-stranded RNA viruses may be of "positive" polarity in 76
which the genome also serves directly as mRNA for translation of viral proteins. To replicate genomes, these viruses must first synthesize a strand of complementary RNA that can be used as a template to replicate genomes and transcribe new mRNA. Retroviruses are a subset of single-stranded, positive polarity RNA viruses that use their RNA genome as template to produce double-stranded DNA, which in turn is used for the transcription of mRNA and new viral genomes. Single-stranded RNA viruses may also be of "negative" polarity in which the genome is antisense to mRNA, and synthesis of a complementary RNA strand is required to serve as mRNA and as template for new genomes. The clinical significance of these types of replication strategies is that they require polymerases and other enzymes not normally found in eukaryotic host cells. Eukaryotic cells, which exclusively use DNA as genetic material and as templates for mRNA transcription, use DNA-dependent DNA polymerases and DNA-dependent RNA polymerases for these functions, respectively. RNA viruses require RNA-dependent RNA polymerases to replicate or transcribe RNA, or RNA-dependent DNA polymerase (reverse transcriptase) to produce DNA from an RNA template. These unique viral enzymes are important targets for antiviral drugs because they can be used selectively to inhibit viral replication. The most common antiviral drugs currently available are nucleotide analogs that are used selectively by viral but not cellular enzymes and result in defective DNA or RNA.[17,18] Although most DNA viruses follow the eukaryotic pattern of replication and transcription, in many cases these viruses produce homologs of host cell enzymes that are sufficiently unique to make them selectively susceptible to nucleotide analogs. Additional targets for current and future antiviral agents are viral proteins used for translation and posttranslational protein processing.[17]

Figure 2.2-1 Schematic representations of basic viral structure. The basic structure of an enveloped virus is shown by the drawing of a herpesvirus. The basic structure of a non-enveloped virus is shown by the drawing of a rotavirus.



Viral polymerases and the genetic organization of viruses are also important for rapid antigenic variation and immunologic evasion. Viral RNA polymerases are low fidelity and lack proofreading functions and thus randomly will introduce errors into new RNA at an average rate of about one nucleotide mismatch per 10,000 bases copied.[19] Therefore in a population of viruses, virtually every individual virus differs slightly, and this population is referred to as a quasi-species. Although many of these mutations are neutral or even deleterious, in the face of selective pressures such as the host immune responses or antiviral drugs, this genetic plasticity allows rapid development of resistant virus populations.[20] Secondary structures or certain sequences in the viral genome may facilitate polymerase errors further at selected regions that are important for immune evasion, such as in sequences that code for neutralizing epitopes.[19] The genomes of some viruses, such as influenza, are comprised of separate segments, which allows reassortment of entire gene segments and sudden and dramatic changes in antigenicity.[21]

2.2.1.2

VIRUS LIFE CYCLE

All viruses are obligate intracellular parasites. Viral replication can occur only within living cells, and all viruses to some extent depend on the host cell synthetic machinery. The life cycle of all viruses includes the following steps: attachment to the target cell, entry into the cell, uncoating and release of the viral genome, transcription and translation of viral proteins, replication of the viral genome, assembly of new virions, and

release of progeny virions[15,16] (Figure 2.2-4). Although the biochemistry of these steps is beyond the scope of this discussion, recognizing that they are all specific, energy-requiring interactions between the virus and the host cell is important. The inability of the virus to interact appropriately with a cell at any of these steps prevents replication in that cell type and defines the tropism of the virus. Any of these steps are also important potential targets for antiviral drugs and host immune responses.

One of the most critical virus-cell interactions is attachment and entry. This initial step is one of the most important determinants of species susceptibility, host cell tropism, and is an important target for antiviral 76
antibodies that neutralize infectivity. Attachment and entry requires a specific interaction between a viral 77
receptor and a cell-surface protein that acts as the host cell receptor. Many viruses also require interaction with additional cell surface molecules (co-receptors) for successful attachment and entry. Influenza provides a good example of this process. The influenza virus hemagglutinin molecule binds to sialic acid residues of cell-surface glycolipids or glycoproteins. Binding induces a conformational change in the hemagglutinin protein, exposing a cleavage site to a host cell protease. Cleavage of hemagglutinin induces fusion of the viral envelope to the host cell membrane and release of the capsid into the cytosol.[22,23] Equine influenza does not infect human beings because the hemagglutinin molecules do not recognize human sialic acid molecules.[24] Within the horse, equine influenza infection is restricted to respiratory epithelial cells by the distribution of the appropriate host cell protease.[22] Viruses may infect other cell types by using a different host cell receptor for attachment and entry. Highly pathogenic strains of avian influenza are able to spread systemically because of mutations in the hemagglutinin that allow cleavage by host cell proteases found on cells outside of the respiratory tract.[22] Other ways in which a virus can infect multiple cell types are using a host cell receptor that is present on different cell types or infecting a single cell type that is present in different tissues (e.g., macrophages and vascular endothelium). Host cell receptors also may be present in an age-dependent fashion, 77
accounting for age-related differences in susceptibility to diseases such as polioencephalomyelitis and 78
rotaviral enteritis.[25,26]

Figure 2.2-2 Virus family classification based on genome composition and presence of envelope or absence of envelope. The relative size of the viruses to each other is also shown.

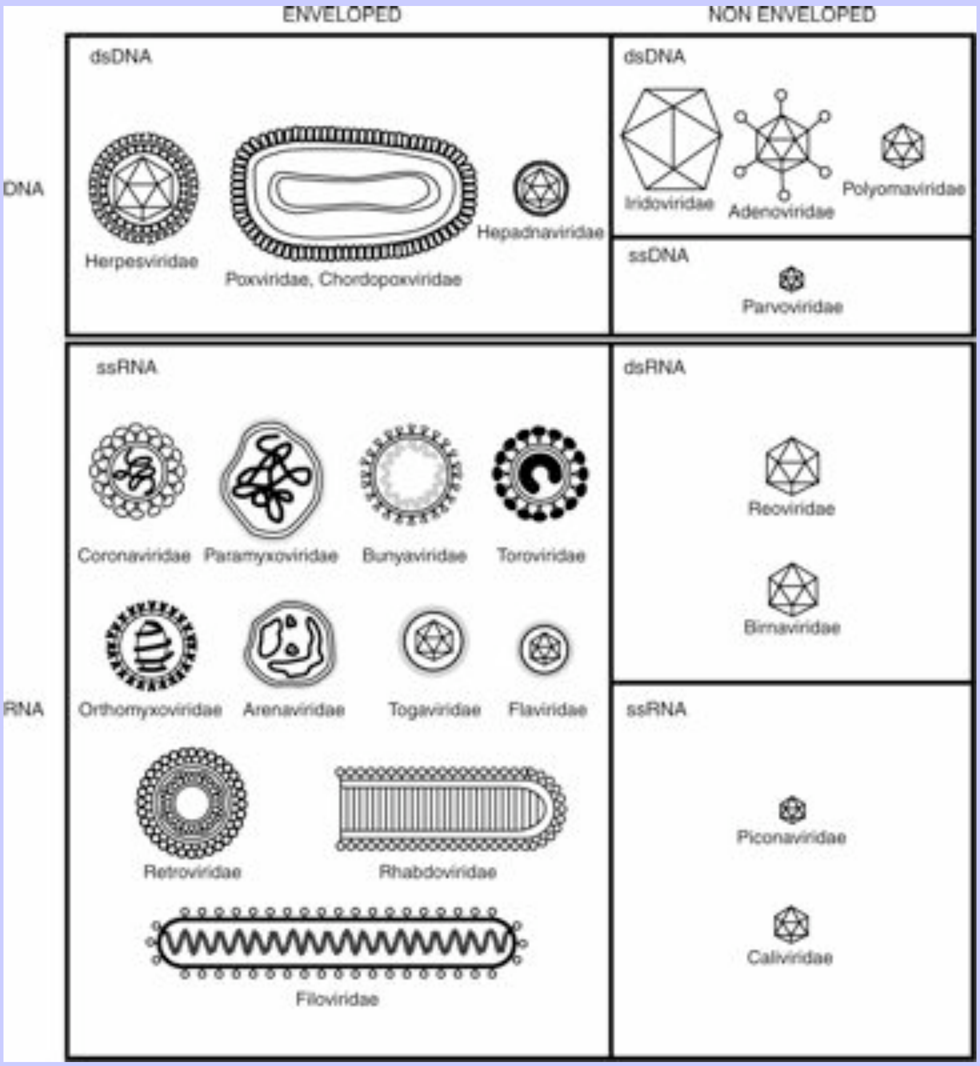
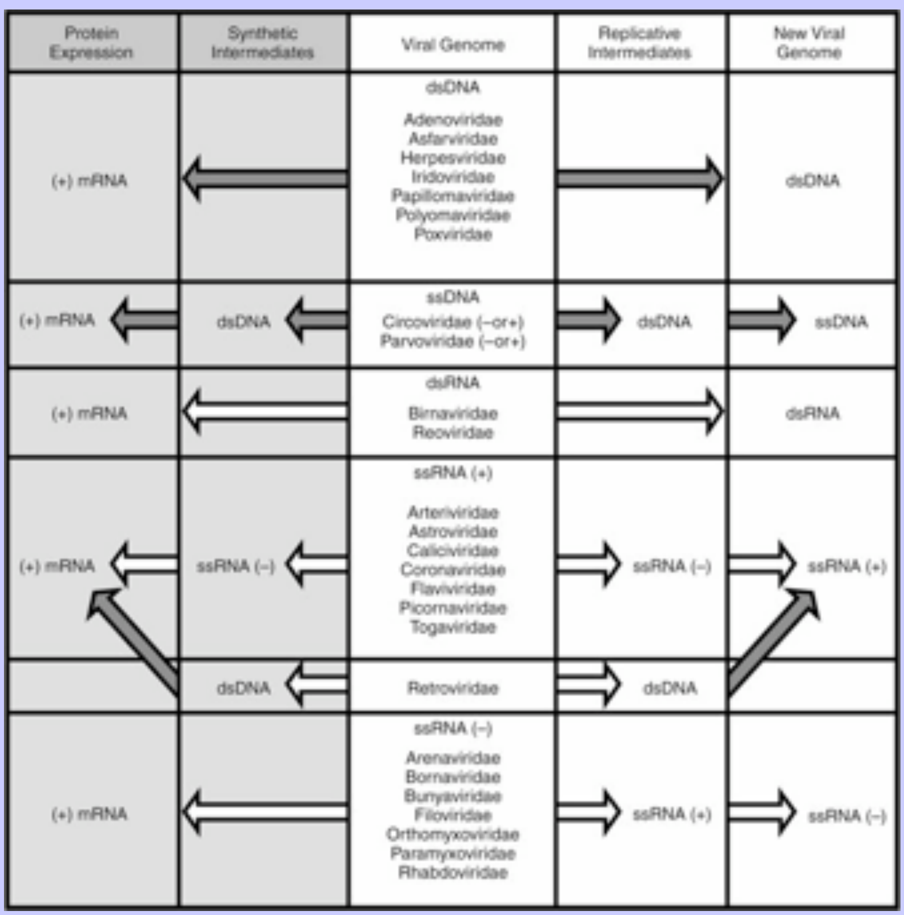
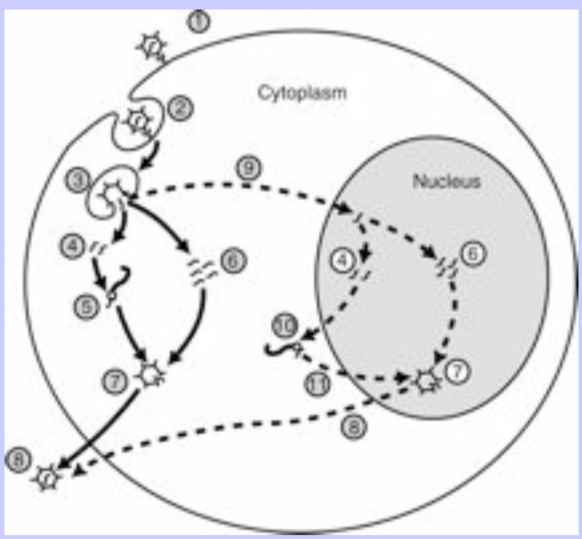


Figure 2.2-3 Summary of the main virus families that infect vertebrates, and general strategies employed by these viruses to produce mRNA for protein expression and to replicate genomes. Required intermediate molecules are indicated. *White arrows* indicate the need for unique viral polymerases, including RNA-dependent RNA polymerase and RNA-dependent DNA polymerase (reverse transcriptase). *Dark arrows* indicate the use of cellular polymerases or viral homologues of cellular polymerases. *ds* = Double-stranded; *ss* = single-stranded; *(+) for RNA* = positive polarity, polarity of RNA used for protein translation; *(+) for DNA* = coding strand, sequence same as for (+) RNA; *(– or +) DNA* = contains single strands of DNA of both polarities. (Modified from Baltimore D: Expression of animal virus genomes, Bacteriol Rev 35:235-241, 1971.)



Once the viral nucleocapsid gains entry to the cytosol of the cell, the viral genome is released through the process of uncoating. After uncoating, depending on the genetic composition of the virus, the viral genome localizes to the appropriate regions of the cell for replication and mRNA transcription. DNA viruses typically replicate genomes and transcribe mRNA in the nucleus and then transport the mRNA to the cytoplasm for translation. RNA viruses typically replicate, transcribe mRNA, and translate viral proteins in the cytoplasm. These sites of replication account, respectively, for the location of viral inclusion bodies that are diagnostically useful in histopathologic sections. In general, the first viral proteins expressed are regulatory factors, enzymes, and polymerases required for initiation of viral gene expression and diversion of host cell resources to viral 78
replication. Subsequently, viral structural proteins are expressed and assembled with new viral genomes into 79
virions. Enveloped viruses require the additional step of acquiring a lipid envelope by budding the nucleocapsid through areas of the nuclear or plasma membrane into which viral proteins have been incorporated. Viruses then are released, usually in association with death of the host cell, into the extracellular space and blood. Some viruses such as herpesviruses and paramyxoviruses also may transfer progeny virions directly into an adjacent cell by inducing fusion between the two cells, a strategy that allows the virus to avoid contact with neutralizing antibodies in the extracellular space.

Figure 2.2-4 Schematic representation of the general virus life cycle. Both RNA and DNa viruses share the initial steps, including attachment *(1)*; entry/fusion *(2)*; and uncoating and release of viral genome into the cell *(3)*. *Solid arrows* indicate remaining steps for RNA viruses, which all occur in the cytoplasm, including transcription of mRNA *(4);* translation of virual proteins *(5)*; replication of the viral genome *(6)*; assembly of new virions *(7)*; and release of progeny virions *(8)*. *Dashed arrows* indicate steps for a DNA virus. The life cycle is similar except that the DNA genome is translocated to the nucleus *(9)* for transcription *(4)* and replication of viral genomes *(6)*. Viral mRNA is then translocated back to the cytoplasm for translation *(10)*, and newly synthesized viral proteins are translocated back to the nucleus *(11)* for assembly *(7)*; new virons are then released from both nuclear and cytoplasmic membranes *(8)*.



2.2.1.3 VIRUS-CELL INTERACTIONS

After a cell is infected, the process of viral replication may have a number of effects on the host cell. Viruses have evolved a number of strategies and host cell interactions to facilitate their own replication and circumvent host responses that may eliminate the infected cell. In general, virally driven effects favor viral replication, transmission, and persistence and often result in death or dysfunction of the infected cell. Cellular

or host responses generally favor control and elimination of the virus from the host. These responses may block viral replication, may initiate innate and specific host immune responses, and are frequently evident clinically as inflammation.

2.2.1.3.1 Cell Cycle and Viral Replication

Viral replication requires that the host cell support high levels of nucleic acid replication, mRNA transcription, and protein translation. The growth and division phase of the cell cycle (S phase) is most suited for this purpose. However, many cells infected by viruses already are differentiated fully and in the resting phase (G_0 or G_1 phase). Some of the more complex viruses, such as herpesviruses, poxviruses, and adenoviruses, have virally encoded proteins that activate the cellular systems for growth and division, thus manipulating the cell to create an environment favorable for viral replication. This ability allows tropism for cells that otherwise might not be able to support viral replication. The "immediate early" genes expressed by herpesviruses, which initiate viral replication in differentiated cells, induce progression of the cell cycle from G_0 to the S phase.[27,28] Other viruses, particularly the genetically simpler viruses such as parvoviruses, lack the ability to activate resting cells.[27,29] Thus their tropism is restricted to cells that naturally go through a growth and division phase, such as intestinal crypt cells and hematopoietic cells. Consequently, parvoviral diseases such as feline panleukopenia typically involve replication in the rapidly dividing cells of the intestine (diarrhea) and bone marrow (pancytopenias). If infection occurs in utero during development of tissues such as the cerebellum, neurologic disease also may be observed.

2.2.1.3.2 Cell Killing

Cells that replicate virus often are killed as a direct consequence of infection. One mechanism by which viruses kill cells is lysis, often associated with the release of progeny virions. Insertion of viral proteins into cell membranes, budding, direct toxicity of viral proteins, and diversion of normal host cell homeostatic processes to viral replication may result in death of the cell.[15,16,30] Viruses also may activate the cellular self-destruct mechanism of programmed cell death (apoptosis). Although cells may induce apoptosis to try to prevent completion of the virus life cycle, viruses also may use this mechanism to kill the cell and facilitate release of virions.[31]

2.2.1.3.3 Neoplasia

Viral infection can cause neoplastic transformation of infected cells. The most common examples in horses include warts (equine papillomavirus) and sarcoids (bovine papillomavirus). Unlike other species, virally induced invasive neoplastic diseases such as leukemia or lymphosarcoma have not been recognized in the horse. However, these types of diseases may be identified in the future. 79 80

Viral proteins that activate the cell cycle into the growth and division phases may lead to neoplastic transformation if expressed in a cell that is not killed by the infection. Papillomavirus infections induce epithelial neoplasms (fibropapillomata) using a virally encoded protein (E5 oncoprotein) that induces proliferation of normally quiescent cells and that presumably is needed for viral replication.[8] The E1A oncoproteins and immediate early proteins of adenoviruses and herpesviruses, respectively, are highly oncogenic in cells that do not allow full virus expression.[32,33] Adenoviral and herpesviral infections in horses currently are not recognized as oncogenic. The lack of oncogenic transformation by these viruses is most likely because abortive infections may not occur in vivo, transformed cells are always removed by the

immune system, or the requisite co-factors for transformation are not present. As an example of the role of co-factors, infection of human beings with human herpesvirus 4 (Epstein-Barr virus, the cause of infectious mononucleosis) is not associated commonly with neoplasia in North America or Europe, whereas infection with this virus in Africa is associated strongly with Burkitt's lymphoma and in China with nasopharyngeal carcinoma.[34]

Oncogenic retroviruses, including leukemia and sarcoma viruses, induce neoplastic transformation in several ways but in all cases do so by integration into the host cell genome and activation of cellular oncogenes.[35] Specifically, a retrovirus and its viral promoter sequences may insert upstream from and activate a normally quiescent cellular oncogene in the process of viral gene expression. Other retroviruses, such as the bovine leukemia virus, produce a virally encoded protein, *tax*, that transactivates and upregulates the expression of cellular oncogenes. These are believed to be the primary mechanisms of transformation by the leukemia viruses. Integration of the retroviral genome also may inactivate by insertion host cell repressors required to actively suppress the expression of cellular oncogenes. Some retroviral genomes, such as those of the rapidly transforming sarcoma viruses, may transform the cell by acquiring cellular oncogenes that are then co-expressed along with other viral gene products during replication.

2.2.1.3.4

Interference With Differentiated Cell Function

Although not described in horses and still poorly documented in other species such as human beings, some viral infections are theorized to play a role in chronic diseases such as diabetes and obesity. For example, the hepatitis C virus may infect the B cells of the pancreas and interfere with the production of insulin.[11] In experimental infections of rodents, canine distemper virus and Borna disease virus cause obesity, possibly by infection of the hypothalamus and downregulation of leptin receptors.[12] Although the role of viruses in these types of infections is still controversial, advances in the detection of viruses are likely to uncover unusual and previously unsuspected roles for viruses in chronic diseases, including those of horses.

2.2.1.3.5

Interactions With Host Immune Responses

One of the main obstacles to successful viral replication in vivo is the host immune response. In response to infection, cells can react in a number of ways to block viral replication, initiate the expansion of specific antiviral immune responses, and target the infected cell for immunologic recognition and destruction. As a result, many viruses have developed counterstrategies to block cell signals that promote these host cell responses.

Interferons induce the expression of a number of cellular proteins that inhibit viral replication in the cell. Secreted interferons similarly impart resistance to viral replication in adjacent cells, and are an important mechanism for controlling the local spread of infection. Some viruses, including paramyxoviruses, adenoviruses, and herpesviruses, produce proteins that interfere with the cell signaling mechanisms required for the expression of interferon-induced proteins.[36,37]

One of the most important systems for recognition of virally infected cells by cytotoxic lymphocytes is endocytic processing of viral proteins followed by expression of the resultant viral peptides on the cell surface complexed with major histocompatibility complex (MHC) class I molecules. Viral products may interfere directly with the processing, transport, or cell surface expression of MHC I molecules or viral

peptides, thereby preventing recognition by cytotoxic, $CD8^+$ T lymphocytes.[36,38] In antigen-presenting cells, cell surface expression of viral peptides with MHC II molecules to immune regulatory cells, mainly $CD4^+$ helper T lymphocytes and B cells, is required for initiation and upregulation of antibody and cell-mediated antiviral immune responses. Similar interference with the processing, transport, or cell surface expression of MHC II molecules or viral peptides can interfere with antiviral immune responses.[36] In addition, some herpesviruses also have been shown to interfere with recognition of virally infected cells by natural killer cells, an early, nonspecific cell-mediated immune response.[39]

An interesting and more recently recognized viral mechanism for interference with host immune responses is the use of virally encoded cytokine mimics. Cytokines are critical cell signaling molecules that coordinate and regulate the development of host immune responses. Some viruses, including poxviruses, herpesviruses, and adenoviruses, express cytokine homologs that mimic and interfere with the activity of interferons, interleukin-1, interleukin-8, interleukin-10, tumor necrosis factor, epidermal growth factor, and granulocyte-macrophage colony-stimulating factor.[36,40] Although the role of these homologs in natural disease is not clear, experimentally they can modulate immune responses and affect disease severity.[36]

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2.2.1.3.6 Virologic Latency

At the host level, viruses may use several mechanisms to establish persistent infections and avoid immune clearance. Some persistent infections are characterized by continual replication despite the presence of antiviral immune responses. If these types of persistent infections are subclinical, they often are described as clinically latent. However, the viral dynamics at the host level should be differentiated from events at the cellular level. In cells, virologic latency is a specific type of cell interaction and an important mechanism of persistence for some viruses. Virologic latency is defined as the presence of a viral genome that is not producing infectious virus.[41] The genomes of latent viruses also are transcriptionally suppressed and translationally silent so that no viral proteins are expressed that may identify the cell to the immune system as infected. The definition of latency also stipulates that on reactivation, viral gene expression and the production of infectious progeny virions can be resumed, differentiating latently infected cells from cells infected with defective viruses.

The classic latent infection is that of the herpesviruses. For the α herpesviruses, such as EHV1 and EHV4, latent infections are established in the nuclei of sensory neurons and can be maintained indefinitely, and infected animals serve as the reservoir of the virus.[42–44] The only detectable viral gene products in latently infected neurons are a small RNA message called latency-associated transcripts, which are required for maintenance of latency.[41] On reactivation, viral nucleic acids are translocated across synapses to epithelial cells of the nasopharynx, which produce infectious virus. In adult horses the amount of viral replication in the nasopharynx is usually not sufficient to result in clinical disease.[44] The stimuli that induce reactivation are poorly defined, but reactivation can be induced by immunosuppression (e.g., corticosteroids) and presumably by other stressors such as pregnancy, transport, and social stress.[44,45]

2.2.2 Mechanisms of Disease: Virus-Host Interactions

Viral interactions with individual cells are the basis for viral pathogenesis. However, for the whole animal, the severity of disease, or whether infection even results in clinical disease at all, is a much more complex interaction between the classic triad of virus, host, and environment. More specifically, these factors include

viral virulence, viral spread within the animal, the intensity of direct and immune-mediated pathologic response elicited by the virus, and the ability of the virus to avoid clearance by the host. Other than the virulence of the virus, which is strictly a property of the virus, the other virus-host interactions can be influenced by the age and genetics of the host and by environmental factors such as stress and nutrition. These factors account for the observation that considerable variation in disease signs can occur among a group of animals infected with the same viral strain.

2.2.2.1 VIRAL VIRULENCE

Certain strains of a virus are well recognized as causing more severe disease. Although many host factors may influence the severity of clinical disease, virulence per se is strictly a property of the virus. The main properties of a virus that may affect virulence include host cell tropism and replication rate. A tropism change that leads to involvement of additional tissues or facilitates virus spread generally results in more severe disease. The systemic spread of highly pathogenic avian influenza strains described earlier is one example. Outbreaks of EHV1 abortion or neurologic disease strongly suggest that EHV1 strains exist that have a tropism for these tissues compared with EHV1 strains that cause respiratory disease. However, the appropriate studies have not been performed yet to determine the exact basis for this observation. Limited studies of EHV1 genetics and virulence in mouse models have not identified differences between the abortigenic, neurogenic, and respiratory strains.[46] However, the severity of EHV1 respiratory disease in experimentally infected foals can be decreased by deletion of genes that facilitate cell-to-cell spread.[47]

An increase in the viral replication rate usually is associated with an increase in virulence, presumably because of the greater number of infected cells and amount of tissue damage. The virulence of equine infectious anemia virus strains can be correlated directly to plasma virus titers and numbers of infected cells, without any changes in tropism.[48,49] The molecular basis for the increased replication rate is not clear but most likely is caused by variation in viral regulatory sequences and proteins.[50,51]

2.2.2.2 SPREAD OF INFECTION IN THE HOST

Viral infections generally are regarded as localized or systemic. Localized viral infections are those that are restricted to a single organ system, often at the site of entry. Because infection of the tissue is direct, the incubation period for localized viral infections is usually short, often only a few days. Many infections of the skin or mucosal surfaces are localized, and examples in the horse include infections with enteric rotavirus and 81
influenza. For influenza, virus is inhaled into the nasopharynx and replicates in epithelial cells of the upper 82
respiratory tract and trachea. Virus is not present in the blood or tissues outside of the respiratory tract. In general, viruses remain localized because they lack the receptors to infect cells of other tissues or circulating cells such as monocytes or lymphocytes that can disseminate the virus. Some viruses are temperature sensitive and remain localized because they are unable to replicate efficiently at core body temperatures. EHV3, the cause of coital exanthema, is restricted to the surface of the genitalia in horses because of its temperature sensitivity.[52] EHV1 and EHV4 are not temperature sensitive, however, and systemic infection may occur with these viruses. The feline respiratory herpesvirus is also temperature sensitive and normally is restricted to the cooler mucosal surfaces. However, hypothermia may lead to dissemination and multiorgan infections.[53] Temperature sensitivity is also a means by which some viruses, such as equine influenza and infectious bovine rhinotracheitis virus, may be attenuated for use as modified-live intranasal vaccines. Infection by the vaccine strain is limited to the cooler mucosal surfaces; the inability to spread systemically prevents sequelae such as abortion and pneumonia.[54,55]

Systemic infections are those in which virus is disseminated to multiple tissues by blood or lymph. This viremia may exist in the form of cell-free virions in the plasma or lymph or may be cell-associated in circulating blood cells, usually monocytes or lymphocytes. The classic paradigm for a systemic infection is infection of mice with ectromelia virus[56] (Figure 2.2-5). Localized viral replication first occurs at the site of entry and in regional lymph nodes. Depending on the level of replication, clinical disease may be present. The virus then enters the blood or lymphatics and spreads to other tissues such as spleen and liver, in which clinical disease may occur. Virus is amplified and then released again for a second, usually higher-titered, viremia that further disseminates the virus to other organs. Each viremic episode is associated with a febrile response and is the basis for the biphasic fever response associated with some viral infections. Because systemic infections require multiple steps, the incubation periods are longer than for localized infections, typically 1 to several weeks. Infections in the horse by eastern, western, or Venezuelan equine encephalitis virus closely follow this paradigm. Localized viral replication occurs at the site of entry (mosquito bite) followed by viremia and dissemination to the central nervous system.[57] For most horses, even nonvaccinated horses, dissemination is controlled before infection of the brain, and neurologic disease is a rare outcome of infection. A variation on the theme is infection of horses with EHV1. The most common clinical disease associated with EHV1 infection is rhinopneumonitis caused by a localized infection of the nasopharyngeal mucosa.[58] In almost all cases a cell-associated viremia also occurs in lymphocytes, but in most infected horses this does not result in disease. However, in some cases, viremia is associated with infection of endothelial cells, and in the pregnant mare vascular damage to the uterus and placenta may lead to abortion.[58,59] Similarly, infection of the vascular endothelium in the central nervous system results in neurologic disease.[60]

Some viruses also may spread in the host through nerves. In the horse, rabies is the best known infection that relies on neural spread. Following local replication in myocytes at the site of entry, usually a bite wound, rabies virus ascends peripheral nerves into the central nervous system, where it replicates in neurons, and then egresses by way of cranial nerves to the salivary gland.[61] EHV1 and EHV4 establish latency in the nuclei of sensory neurons that innervate the nasopharynx and reach the nucleus by ascending nerve axons. Similarly, on reactivation, these viruses egress back down the axon to infect epithelial cells.[43,62]

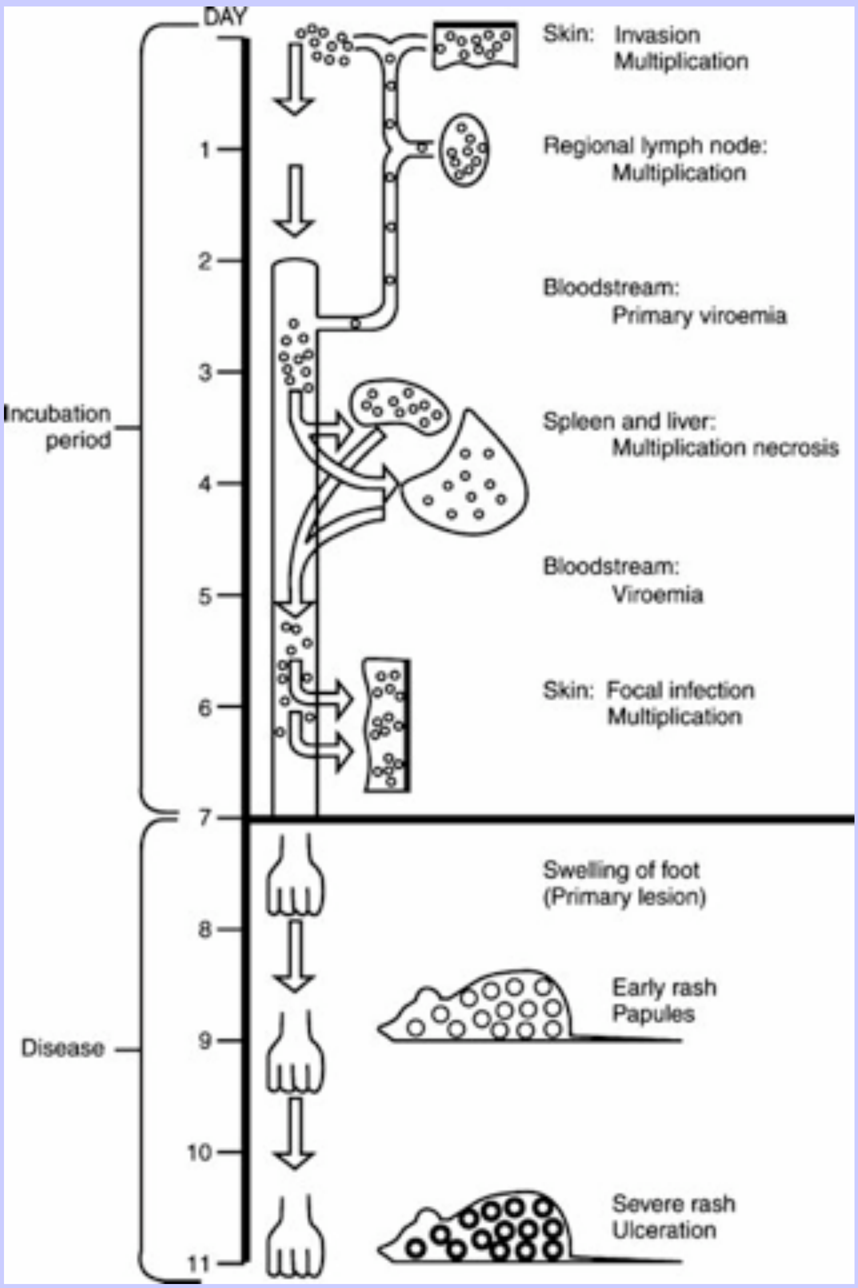
2.2.2.3 VIRAL PATHOLOGY

Once a virus reaches a target organ, virally mediated cell death is the fundamental source of pathologic response, disease, and clinical signs observed by the veterinarian. Despite the great complexity of virus-host interactions and the many factors that influence the expression of clinical disease, in actuality viruses have a limited number of ways by which to cause infection. Cells and tissues may be destroyed directly by cytolytic viral infections or by infections that affect the differentiated function of target cells (e.g., neoplasms and immunodeficiencies). Viral infections of organ systems with bacterial flora (e.g., intestinal and respiratory tracts) can disrupt the normal barrier functions of these organs and result in secondary bacterial infections and toxemia that may contribute significantly to the pathologic response. Cell death and pathologic response also may be caused by host immune responses specifically directed against virally infected cells or by indiscriminate inflammatory responses. Virally induced autoimmune diseases have not been described in horses but are another potential source of pathologic response that may be identified in the future.

For most of the clinically important viral diseases of horses, disease manifestation results from some combination of cytolytic infection and immune-mediated tissue destruction. The relative contribution of these mechanisms is primarily a function of viral virulence and host factors that influence the type and intensity of immune responses. The predominant mechanism of pathologic response also can vary with different stages of

the same disease, as seen in acute versus chronic equine infectious anemia. In acute disease, most of the diseasea manifestation is caused by direct viral damage and cytokines, whereas in chronic disease, immune complex–mediated anemia and glomerulonephritis become more significant. 82 83

Figure 2.2-5 Schematic representation of the pathogenesis of mousepox (ectromelia), illustrating the classic paradigm for the events in a systemic infection. (Fenner F: The pathogenesis of the acute exanthems, *Lancet* 255:915, 1948.)



2.2.2.3.1 Direct Viral Pathology

The most straightforward cause of damage and disease in an organ system is for the virus directly to kill or cause dysfunction in the infected cells. As described previously, cell death may be caused by cytolysis resulting from viral replication and interference with normal cell homeostasis or by initiation of apoptosis. Cell dysfunction usually is observed as neoplastic transformation, although more vague effects on other differentiated cell functions, such as insulin secretion, remain possibilities.

The disease associated with a given viral infection is related to the affected organ system(s), the number of cells destroyed, and the sensitivity of the affected organ system to dysfunction. If the number of infected cells is not sufficient to lead to clinically significant organ dysfunction, then the result is a subclinical infection. Vaccination and naturally acquired immunity are important factors that limit the number of 83
infected cells. However, epidemiologically these infections may still be important because these animals 84
may still be infectious. When enough cells are infected to lead to overt organ dysfunction, then clinical disease becomes apparent. The threshold for clinically significant damage varies considerably between organs and the types of cells. EHV1 infections of the respiratory epithelium, which has a large number of cells with a high turnover rate, produce mild clinical disease even with a high rate of infection. On the other hand, much more significant clinical manifestations of EHV1 infection such as abortion and neurologic disease are caused by infection of a few endothelial cells because minimal vascular damage can lead to thrombosis, ischemic necrosis, and damage to large amounts of tissue. If viral infection results in neoplastic transformation of a cell type, then disease may progress according to the characteristics of the neoplasm, whether or not the virus remains associated with the tumor.

Of particular importance are infections of the respiratory and intestinal tracts that contain normal bacterial flora and have important barrier functions to prevent access of bacteria and toxins to deeper tissues. Consequently, complications of bacterial infection and toxemia are important secondary problems of viral infections.[63,64] For example, upper respiratory viral infections damage the ciliated epithelial cells that function to move mucus and other respiratory secretions outward and provide a barrier between the mucosal bacterial flora and the underlying subepithelial tissues. Damage to this barrier may lead to opportunistic bacterial pneumonias by normal bacterial flora such as *Streptococcus equi zooepidemicus* or *Mannheimia haemolytica* in horses or cattle, respectively.

2.2.2.3.2 Immune-Mediated Viral Pathology

In most viral infections, immune-mediated pathologic response contributes significantly to disease and in some cases may be the predominant cause of disease manifestation. This paradigm has been documented elegantly in classic experiments with lymphocytic choriomeningitis virus infections of mice in which immunodeficient mice develop persistent infections but do not develop disease and do develop disease if their immune systems are reconstituted.[65] Details of equine immune and inflammatory responses are covered in detail in Chapter 1 and are described in this section only in general terms and as they relate to viral infections.

The primary antiviral immune responses initiated in viral infections include natural killer cells, virus-specific cytotoxic ($CD8^+$) T lymphocytes, and to a lesser extent antibody-mediated lysis of infected cells (antibody-directed cellular cytotoxicity, complement fixation, and phagocytosis by macrophages and neutrophils). These immune responses detect and selectively kill virally infected cells and are a significant

cause of cell death. Indiscriminate inflammatory responses also may kill uninfected cells in the vicinity of infected cells, primarily from activation of monocytes and granulocytes with release of a wide array of cytotoxic molecules (e.g., lysozyme, proteases, lipases, and oxygen radicals). Secondary bacterial infections greatly exacerbate indiscriminate tissue damage, not only from the direct effect of bacterial toxins but also because of extensive recruitment of neutrophils and complement, which are highly nonselective components of the antibacterial immune response.

Antibody-mediated immune complex hypersensitivity reactions also may play a major role in the genesis of viral disease. Antibodies bound to soluble viral antigens fix complement and opsonize neutrophils, again leading to indiscriminate cell and tissue destruction. Viral antigens may be on the surface of cells, such as erythrocytes or platelets, resulting in immune-mediated anemia and thrombocytopenia, respectively.[66] When viral antigen is in excess relative to antibody, circulating immune complexes are formed that can be deposited in the capillary beds of tissues such as joints and renal glomeruli. In chronic equine infectious anemia virus, with persistent viral replication and antigenemia in the presence of antiviral antibodies, these mechanisms are primarily responsible for the characteristic lesions of anemia, thrombocytopenia, and glomerulonephritis.[66,67] Nonneutralizing antiviral antibodies actually may serve to increase the number of infected cells and the severity of disease through the mechanism of antibody-dependent enhancement. This enhancement occurs when antibodies bind to the virus and facilitate attachment and entry into Fc receptor–bearing cells such as macrophages by serving as a form of alternative receptor.[68] Antibody-dependent enhancement has been shown to be involved in the pathogenesis of a number of diseases, including feline infectious peritonitis,[69] human respiratory syncytial virus,[70] and dengue hemorrhagic fever.[71]

Soluble inflammatory mediators released by infected cells and inflammatory cells in response to viral infections are also significant contributors to clinical signs and disease manifestation. These mediators include a wide array of cytokines, interleukins, and other proinflammatory molecules with potent systemic and local effects. The most evident of these is the febrile response caused by interleukin-1 released by macrophages in response to virally infected cells. Increased levels of other soluble factors, including interferons, interleukins, transforming growth factor, and tumor necrosis factor are also present in many viral infections.[72,73] These cytokines are primarily proinflammatory and immunoregulatory and are generally important for the control of viral infections but also can be responsible for many of the nonspecific clinical signs of viral infection such as depression, malaise, and in chronic infections, cachexia. 84
Soluble mediators also may have important local effects. Vasoactive factors such as prostaglandins and 85
leukotrienes cause edema and swelling. In equine infectious anemia virus, tumor necrosis factor α, transforming growth factor β, and interferon-α have been shown to suppress hematopoiesis and contribute to the development of anemia and thrombocytopenia.[74]

2.2.2.3.3 Autoimmunity

Although not described in horses, viral infections in other species can induce immune-mediated responses to host cell antigens and autoimmune diseases. The best documented human autoimmune disease suspected to be initiated by viral infections is the Guillain-Barrè syndrome, in which infection with cytomegalovirus or Epstein-Barr herpesvirus elicits antinerve ganglioside immune responses and demyelinating disease.[75] Postinfluenza myocarditis is an occasional sequela in horses and human beings and is a potential autoimmune disease. Although no direct evidence supports this theory, influenza virus is not identified consistently in affected heart muscle and the pathogenesis is not known.[76]

2.2.2.4 IMMUNE AVOIDANCE

A key requirement for viruses to be maintained in nature is to persist successfully in a reservoir host (if the reservoir is an infected animal) and to be transmitted to another susceptible host. One of the most important obstacles to persistence and transmission is detection and elimination by the host immune system. Transmission to a new host also may require that the virus avoid preexisting immunity from prior natural exposures or vaccination. Within the host, rapidly replicating viruses such as influenza may shed and transmit virus before the host can mount specific antiviral immune responses. Herpesviruses avoid detection during latency by not expressing any viral proteins. For persistent viral infections that continually replicate within a host, such as retroviruses, evasion of developing immune responses is necessary. Immunodeficiency viruses may cripple antiviral immune responses by directly infecting immunoregulatory $CD4^{+}$ T lymphocytes. As described previously, many other viruses can dysregulate host immune responses by expressing cytokine mimics.

One of the most important mechanisms of immunologic avoidance is antigenic variation in which neutralizing viral antigens are altered so that they are no longer recognized or accessible by host immune responses. The most important of these antigens includes viral proteins bound by neutralizing antibodies (e.g., virus receptors) and any peptides presented in the context of MHC I or II cell surface molecules for recognition by cytotoxic ($CD8^{+}$) T lymphocytes or helper ($CD4^{+}$) T lymphocytes, respectively. Antigenic variation is generated by nucleotide errors during transcription or replication, which result in amino acid substitutions in the relevant epitopes. This process is facilitated by viral polymerases that are inherently error-prone and lack proofreading functions and by placing the sequences for the relevant epitopes adjacent to other genomic sequences or structures that further predispose to transcriptional errors.[19] Other mechanisms by which some viruses may modify their antigenicity is through intramolecular recombination/duplication or reassortment of segmented genomes (e.g., influenza and African horse sickness).[19,21] For reassortment, co-infection of a single cell with genetically different virions may result in a progeny virion with segments derived from both virions and a major change in antigenicity. In influenza these are called antigenic shifts, and the radical change in the antigenicity of the virus may render preexisting immunity in the host population ineffective at preventing outbreaks of disease with high morbidity and mortality.[21] Viruses also may facilitate the production of nonneutralizing antibodies that sterically interfere with the ability of neutralizing antibodies to bind. Although the mechanism of antigenic variation is primarily random, immunologic selection determines which variants successfully emerge and are capable of replicating within the host or the host population.

2.2.2.5 HOST GENETICS

Genetic differences in susceptibility to disease have been well documented. In an outbred population of animals, the considerable variation in the type or severity of clinical disease is well recognized, even when animals are infected with the same virus strain and have no recognizable differences in other factors such as age, challenge dose, nutrition, and general health status. Conversely, highly inbred populations may be more uniformly susceptible to a viral disease.[77] Thus inbreeding can pose problems for endangered species, such as Przewalski's horse, or other populations with limited genetic variability, which may incur high rates of morbidity or mortality if the animals are infected with a virulent virus.

Although in many virus-host interactions the basis for genetic resistance to disease is not well defined, host genetics have been shown to affect the tropism of the virus and influence the type and intensity of immune

responses to a viral infection. In human immunodeficiency virus infections, genetically determined absence or presence of certain co-receptors has a significant effect on susceptibility to disease.[78,79] Immunologically, host genetics defines the repertoire of antigen-specific recognition molecules (antibodies and T cell receptors) and antigen-presenting molecules (MHC I and II). Consequently, genetically different animals respond to different subsets of viral antigens. The relative inability to react to a neutralizing epitope prevents or delays effective 85
control of viral replication.[80] Different subsets of viral antigens may favor the development of a type 1 T 86
helper cell (cell-mediated) or type 2 T helper cell (antibody-mediated) immune response, which has implications for the ability to control infection and for immune-mediated pathologic response.[81–83]

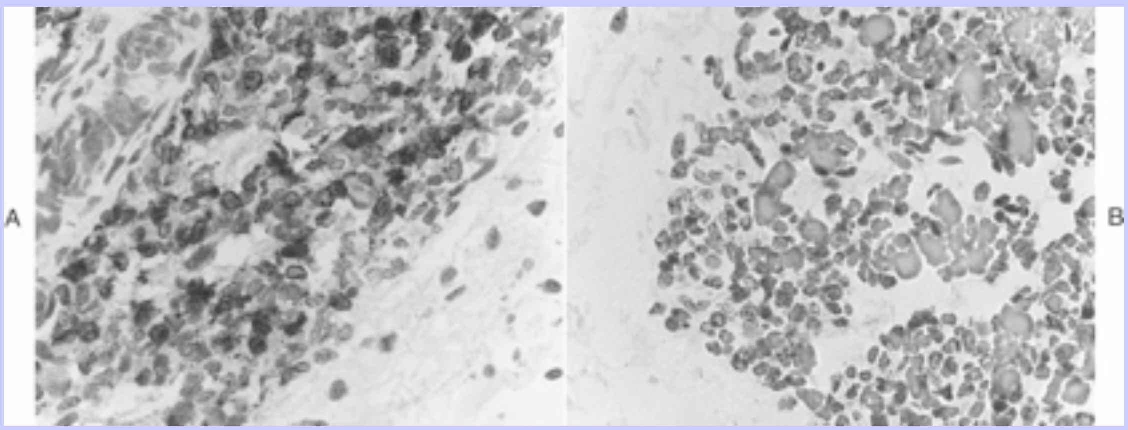
2.2.3 Advances in the Diagnosis of Viral Diseases

The diagnosis of viral infections traditionally has relied on the detection of antiviral antibodies by serologic tests and the direct demonstration of virus by isolation in cultures of living cells or in laboratory animals. Although these principles are still the foundation for the diagnosis of viral diseases, recent molecular biology advances have improved the clinical use of viral diagnostics greatly.

The sensitivity and specificity of antibody testing has been enhanced by the recombinant viral proteins or peptides for use as antigens in serologic tests. One example is the use of recombinant antigens in antibody tests for equine infectious anemia virus. The replacement of whole-virus antigen preparations with recombinant viral proteins has improved the sensitivity and specificity of the agar gel immunodiffusion (Coggins) and enzyme-linked immunosorbent assay tests.[84,85] The improvement in specificity is especially important when screening for a low-prevalence disease in which the predictive value of a positive result is low, as is the case for equine infectious anemia in the United States.

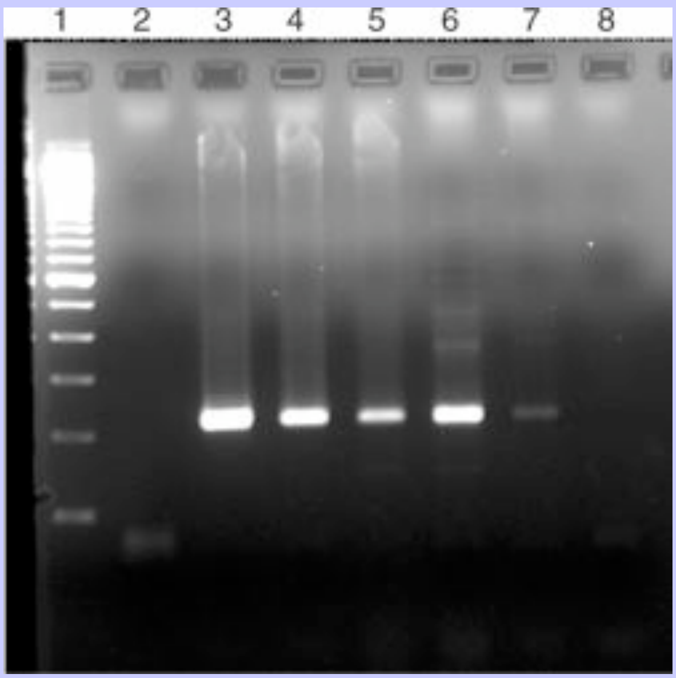
However, the greatest advances have been in the area of detection of virus in clinical specimens. The clinical utility of traditional methods of virus isolation in cell culture often is limited by the sensitivity, specificity, predictive value, or the length of time needed to perform this type of assay. Another significant limitation of virus isolation, particularly for the more labile enveloped viruses, is the requirement for viable virus, which may be compromised by autolysis or transport to the laboratory. In many diagnostic laboratories, virus isolation in cell culture has been replaced by immunohistochemistry and the polymerase chain reaction (PCR), both of which are sensitive and specific tests that can be completed within a day or two. Immunohistochemistry is a method that detects viral antigens in formalin-fixed tissue samples. Although immunohistochemistry technology has been available for many years, this assay has become more widely used because of improvements in the methods for antigen retrieval and the greater availability of antiviral monoclonal or polyclonal antibodies. The aldehydes in formalin cross-link proteins during the fixation process and prevent the recognition of epitopes by antibodies. For many viruses, reliable protocols now have been established that use carefully controlled protease digestion of the tissue sections so that the availability of epitopes is restored.[86] The tissue sections then can be incubated with antiviral antibodies, and bound antibodies can be visualized with a variety of colorimetric systems (Figure 2.2-6). The specificity of immunohistochemistry depends highly on the antiviral antiserum and has been improved greatly by the availability of antiviral monoclonal antibodies and recombinant viral proteins that can be used to generate monospecific antisera.[87–89]

Figure 2.2-6 Immunohistochemistry for equine herpesvirus in tissues of an aborted equine fetus. Panel A is a section of lung stained with anti-EHV antibodies. Bound antibodies are visualized with a secondary antibody conjugated to horseradish peroxidase that when reacted with the chromogen develops a visible precipitate in the cytoplasm of infected cells. Panel B is a negative control, which is a section of lung from the same fetus stained with an isotype-matched antiserum that does not contain anti-EHV antidodies; no precipitate is visible.



The polymerase chain reaction (PCR) assay is a nucleic acid amplification method that detects viral DNA or 86
RNA extracted from fresh or formalin-fixed tissue samples. Only a few years ago the techniques used to purify 87
nucleic acids and perform the PCR reaction were limited by expense and practicality to the research laboratory. However, the simplification and commercialization of these procedures has made this type of test economical and clinically useful for diagnostic laboratories.[90,91] The PCR test uses oligonucleotide primers and a thermostable DNA polymerase (Taq polymerase) to amplify exponentially the viral nucleic acids. The basis for specific amplification in the PCR assay is the sequence of the oligonucleotide primers, which are produced synthetically to be complementary and thus bind to the viral nucleic acid sequences. To produce complementary primers, the sequence of the viral DNA or RNA must be known. Consequently, this assay is only useful for testing for viruses that have been sequenced, and in the past, sequence data limited the ability to design PCR primers. However, because of advances in the technology for sequencing and database management, virtually all of the significant equine pathogens now have complete sequence data available. At the end of the PCR procedure, the amplified nucleic acids, that is, a positive result, are visualized most commonly with agarose gel electrophoresis in which the amplicons in the gel are viewed with a fluorescent dye, and the product presumptively is confirmed as the correct one based on its molecular size (Figure 2.2-7).

Figure 2.2-7 Analysis of PCR reactions for equine infectious anemia virus. PCR products are electrophoresed in an agarose gel. DNA in the gel is stained with ethidium bromide and visualized with ultraviolet light that causes the DNA to fluoresce and appear as white bands in the gel. Lane *1* is a size standard to determine the size of the DNA amplicons. Lane *2* is a negative control, and the lane *3* is a positive control. The samples in lanes *4, 5, 6*, and 7 are positive; the sample in lane *8* is negative.



Immunohistochemistry and PCR are now routine tests that generally are sensitive, specific, and rapid. However, care must be taken not to make assumptions about the performance characteristics of new tests. For example, although PCR assays are often more sensitive than virus isolation, this is not automatically true. The sensitivity and specificity of any new test must be evaluated individually and with respect to the performance of the previous test. Also, the significance of any virus identified in a specimen still must be interpreted in the context of the pathogenicity of the virus and clinical disease, regardless of the method used to detect the virus.

2.2.4

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2.3

2.3—Internal Parasite Infections*

Maureen T. Long

Horses serve as hosts for numerous parasites that induce a wide range of pathologic and immunologic responses,[1] including hypersensitivities and other forms of immunopathology. Immune responses leading to protective resistance against reinfection occur, but the level of this resistance is most often incomplete. Mechanisms associated with these responses have not been investigated extensively in the horse, but information is available from other host-parasite systems that is relevant to the horse. The purpose of this section is to acquaint the reader with contemporary thoughts on host-parasite interactions. Because of their prevalence, major importance to equidae, and information available, coverage is limited to helminth parasites that occur in most developed and nontropical countries.

2.3.1

Parasite-Induced Lesions

Infection with most metazoan parasites results in inflammation and structural and functional changes of the organs invaded, the outcome being an alteration of the host's physiologic state. The degree of alteration depends on the existing physiologic condition of the animal, which is dictated to a great degree by its age, nutritional status, and previous immunologic experience with the parasite. The numbers of parasites introduced and the specific parasite also affect the degree of physiologic change that occurs. When these factors favor major alterations, the results are readily identifiable clinical signs of infection. Subclinical infections, although less apparent, are potentially important to the general health of the animal and continued transmission of the agent. The pathophysiologic effects of infection by ectoparasites, helminths, and microorganisms are in many cases similar. Abnormalities in weight gain, skeletal growth, reproduction, and lactation may result from infections with any of these agents. These changes often are related directly to parasite-induced anorexia, disruption of metabolic processes, and anemia. An understanding of the morphologic and biochemical lesions produced by specific parasites clarifies the role of these agents in clinical and subclinical conditions associated with the infections. A majority of detailed studies on the pathophysiology of parasitic infections have been conducted in 89
laboratory animal models and domestic animal species other than the horse.[2] However, the classical pathologic 90
effect of parasitic infections of the horse has been reviewed.[3,4] The following discussion outlines some recent observations on host-parasite interactions that may be significant to equine medicine. Examples of host-parasite interactions responsible for alterations in host homeostasis are presented as they relate to the gastrointestinal tract, lungs, and skin.

2.3.1.1 GASTROINTESTINAL TRACT

Internal parasites are most important to equine health as mediators of gastrointestinal symptoms such as colic and diarrhea. Although almost all internal parasites have been implicated inferentially as causative agents of colic at some time, large strongyles, principally *Strongylus vulgaris* and to a lesser extent *Parascaris equorum*, classically have been considered major pathogens. Details of the pathogenesis of colic associated with migration of *S. vulgaris* through the mesenteric arteries and the resultant thrombosis, infarctions, and necrosis of the intestine have been described in detail elsewhere.[3,4] Although this condition has been well described, some points are particularly noteworthy. Histologic studies of experimentally infected parasite-free pony foals during the initial stages of the infection indicate that the severity of the lesions produced in the intestine cannot be attributed solely to mechanical disruption caused by larval migrations and that these larval stages induce some biologic amplification system within the mucosa, which results in the degree of inflammation observed.[5] Although the mechanisms involved in this response have not been investigated, the histologic nature of the lesion is characteristic of an Arthus reaction, suggesting an involvement of the immune response. Other experimental studies using the parasite-free pony–*S. vulgaris* system have implicated the immune response in the mediation and regulation of the arterial lesions produced by this parasite. Passive transfer of immune serum, but not normal serum, reduced the severity of arteritis and clinical signs associated with experimental infections without reducing the numbers of parasites that developed in these ponies. However, treatment with immune serum also induced an anamnestic eosinophilia and significant perivascular infiltration of eosinophils in the cecum. The reduction in intravascular lesions may have been associated with an inactivation of parasite-secreted inflammatory factors by antibody or serum enzymes. This serum also may have contained nonspecific host-derived antiinflammatory substances. The exacerbation of the eosinophil response may have been associated with the formation of immune complexes. Although the mechanisms are unknown, the results suggest that the immune response may simultaneously modulate and potentiate inflammation. Larvicidal treatment of *S. vulgaris*–infected horses and killing of intravascular larvae has been postulated to release a bolus of antigenic factors from these larvae within the mesenteric vasculature, resulting in an exacerbation of arterial and intestinal lesions and colic. Experimental testing of this hypothesis indicates that this phenomenon does not occur and further that viable larvae are necessary to maintain the arteritis and eosinophilia seen.[6,7]

P. equorum–associated colic in foals has been related to intestinal impaction and rupture and is not considered to be of major significance in adult horses.[8] However, ascarid nematodes are particularly potent sources of allergens, and conceivably the hypersensitized mature horse may respond to low-level infections by this parasite. Observations made in the author's laboratory are noteworthy in this regard. Two mature *Parascaris*-free adult horses were inoculated intradermally with less than 90 μg of saline-soluble somatic extract of adult *P. equorum* to test for immediate hypersensitivity to this antigen. Both horses experienced an immediate systemic response and colic. One of the horses died within 3 hours of intradermal inoculation. Necropsy results were consistent with the diagnosis of colitis X. Because of the allergic potential of ascarid nematodes and the sensitivity of the equine gut to immediate hypersensitivity reactions, this potential is worthy of further characterization and consideration.

Recent clinical observations have piqued an interest and concern over the pathogenic potential of infections by *Anoplocephala perfoliata*. Case reports have described cecal ruptures and intussusceptions of the cecum and colon associated with these infections.[9,10] However, detailed retrospective analysis of the concomitant occurrence of intussusception and colic and *A. perfoliata* infections has failed to demonstrate any causal relationship between these conditions and tapeworm infections.[11] These parasites inhabit the region of the ileocecal junction and produce ulcerated lesions of the mucosa and submucosal inflammation. However, the

parasites are common, and possibly the association of tapeworm infections and clinical signs attributed to them are caused by chance alone. Detailed experimental investigations of these infections have not been conducted, and thus specific details on the pathogenesis and relevance of these lesions are lacking. However, an association between tapeworm infections and colic of ileocecal origin has been suggested in a case-control study of 231 horses.[12]

Until recently, cyathostomes (small strongyles) have not been considered of major importance, particularly as causative agents of colic. In this regard the field studies of Uhlinger are of particular importance.[13] In these controlled experiments, different anthelmintic treatment regimens were used to test their efficacy in reducing
the incidence of colic. The more efficacious treatment programs significantly reduced the incidence of colic by 90
2 to 13 times that seen in the same herds before implementation of the more efficacious treatment. Because of 91
the management programs used before the initiation of this study and the results of fecal cultures, one can assume that the primary parasites present in these horses were cyathostomes. These data strongly implicate a role for cyathostomes in a substantial proportion of colics observed under field conditions. The parasite or host factors involved in these colic cases are unknown.

Cyathostomes have been implicated in numerous case reports with seasonal diarrhea in adult horses, which is a condition called larval cyathostomiasis. These cases are characterized by a sudden onset of diarrhea during the late winter or spring. Mature horses usually are affected, and infections are often fatal. The condition is difficult to diagnose, and the only consistent signs are weight loss and diarrhea.[14] Large numbers of larval cyathostomes are found in the feces or in intestinal contents and within the mucosa of these horses. These symptoms are related to the synchronous emergence of fourth-stage larvae of these parasites from the mucosa. These larvae build to potentially large numbers within the mucosa because of the arrested development of infective larvae. The seasonality of the occurrence of this condition at present does not appear to vary in different climatic regions as does the analogous bovine condition of type II ostertagiasis. Specific parasite or host factors associated with the regulation of the hypobiotic state of the larvae or the inflammatory response initiated at parasite emergence have not been described.

In view of the paucity of specific mechanistic information on the pathophysiologic effects of equine gastrointestinal parasites, a synopsis of relevant information gathered from other model systems is warranted.[2,15] Parasitic organisms may induce changes in gastrointestinal function directly by mechanical disruption of tissues and cells or by the release of factors that directly alter cell function. Induction of the immune response serves as an anamnestic amplification system. The result of these changes is an alteration in function of the smooth muscle and epithelium of the bowel. A number of helminth parasites, including *P. equorum*, have been demonstrated to produce intestinal smooth muscle hyperplasia. Evidence suggests that this response may be induced by intestinal inflammation or stenosis associated with parasitism. Contractility of these muscles also has been demonstrated to be induced in a Schultz-Dale reaction by stimulation with parasite antigens. This response is mediated in rats by mast-cell–derived 5-hydroxytryptamine and in guinea pigs by histamine. A regulatory relationship of myenteric neurons to these antigen-induced changes also has been demonstrated in this model system. These latter experiments suggest that antigen-induced stimulation of smooth muscle contractility may be blocked correspondingly by γ-aminobutyric acid similarly stimulated by mast cell products. This complex system may be an adaptation by the host to maintain homeostasis in the face of continued antigenic stimulus. What is noteworthy is that strongyle-induced alterations in myoelectric activity of the equine small intestine and colon have been demonstrated in vivo.[16,17] In some of these experiments, dead *S. vulgaris* larvae evoked an alteration of the smooth muscle response in previously exposed ponies, suggesting a role for the immune response in the stimulation of the hyperactivity.[18]

A recent series of in vitro and in vivo experiments on the direct interaction of factors produced by the adult heartworm *Dirofilaria immitis* and arterial endothelium are exciting and noteworthy.[19] The results of these studies indicate that endothelial cell–mediated vasodilation is depressed in the presence of small (100 to 1000 molecular weight) parasite-derived pharmacologically active substances. Seasonal pathologic changes in infected arteries in dogs have been associated with the release of these substances by adult parasites. The nature of the factor or factors involved is unknown at this time. Production of similar factors in the mucosa or mucosal vasculature by nematodes could induce focal alteration in blood flow and other physiologic effects on smooth muscle. In general, these studies demonstrate the potential variety and complexity of metazoan-host interactions that have yet to be defined.

Many parasites and other infectious agents alter the structure of the intestinal epithelium, causing villous atrophy and crypt hyperplasia. Hypersensitivity responses are linked to functional changes in epithelium, such as decreases in epithelial brush border digested enzyme activity, decreased absorption, reduction in fluid absorption, and an increase in fluid secretion.[2] In vitro studies on the Cl^- secretion of isolated intestines from guinea pigs and rats infected with the nematode *Trichinella spiralis* have proved to correlate with in vivo fluid secretion.[15] In this system, antigenic stimulation induces an increase in Cl^- secretion similar to that induced by cholera toxin. The mechanism of this phenomenon in rats is mediated by antiparasite immunoglobulin E–mast cell release of at least two pharmacologically active substances that function in two phases. The immediate response depends on T cell–mediated mast cell hyperplasia, which is important in the release of 5-hydroxytryptamine and histamine. These factors act on enteric neurons to induce the immediate increases in Cl^- secretion from epithelial cells. The second phase is independent of mast cell hyperplasia and is mediated through 5-hydroxytryptamine and histamine–initiated increase in de novo mucosal synthesis of prostaglandin, which mediates a second phase of secretion. These in vitro phenomena can be blocked at various stages by 91
extrinsically supplied pharmacologic agents. The suggestion is that similar levels of control may be active in 92
vivo, mediating these effects and maintaining homeostasis in most cases. Although studies of this nature have not been conducted with equine tissues or parasites, similar mechanisms and activities likely occur.

* The authors acknowledge and appreciate the original contribution of Thomas R. Klei, whose work has been incorporated into this section.

2.3.1.2 RESPIRATORY SYSTEM

Several nematode parasites infect the equine lung. These include migrating stages of *P. equorum* and *Strongyloides* en route to the small intestine. Migrating stages of aberrant parasites, such as *Habronema* sp., *Draschia megastoma*, and *Strongylus* spp., which induce granulomatous foci in the lung parenchyma, and adults and larvae of the lungworm *Dictyocaulus arnfieldi*, which inhabit the bronchi, also occur. Host responses to two of these are noted.

P. equorum larval migrations in the lungs of yearling horses produce more severe clinical signs and inflammatory responses than in foals reared parasite-free. These infections in yearlings are accompanied by focal accumulations of lymphoid tissue, indicating an induction of an active local immune response. The reaction suggests that this is an age-related phenomenon.[8] However, more severe reactions likely could result from previous sensitization to *P. equorum* antigens. Increased responses of this nature have been described in the livers of pigs immunized with *Ascaris suum* antigens following challenge infections.

Dictyocaulus arnfieldi infections of donkeys rarely produce clinical signs, and these equidae have been suggested to be the natural host for this parasite. Infections of horses produce more severe and prolonged

bronchial inflammatory responses similar to *Dictyocaulus* sp. infection in other hosts. The mechanisms associated with this differential response have not been defined but are common in unadapted host-parasite associations. Possibly the more significant inflammatory reaction of the horse to these parasites is caused by the absence of down-regulatory mechanisms that are established in the more adapted natural host, the donkey.

2.3.1.3 SKIN

Reactions to the filarial nematode *Onchocerca cervicalis* illustrate variations seen in responses to chronic parasite infection. Focal, alopecic, depigmented, pruritic lesions often are seen in infected horses. Not all infected horses react to this infection, and the appearance of clinical signs is often seasonal. Detailed studies have not been conducted on the pathogenesis of these lesions in horses. However, similar conditions occur in human onchocerciasis,[20] and the host-parasite responses active in human beings likely are also present in the horse. Lesion development is associated with immune-mediated killing of microfilariae in the skin. Parasites appear to be killed in an antibody-dependent cell-mediated reaction. In this response, antimicrofilarial surface immunoglobulin G and E antibodies mediate adherence and degranulation of granulocytes, which are predominantly eosinophils. The major basic protein of eosinophils has been demonstrated in the tissues of patients with dermal lesions, and eosinophil toxic enzymes and proteins are responsible for many of the changes seen. The reason for the absence of these lesions in most horses is unclear. Human onchocerciasis and filariasis are spectral diseases in which regulation of immune responses has been associated with the lack of pathologic responses to the parasites.[21] Immune regulatory mechanisms associated with these infections include immune tolerance, anergy, induction of immune regulatory circuits involving suppressor T cells or macrophages, and most recently the potential shift in T helper cell subsets, which potentially alters the production of specific cytokines during different phases of the infections. The parasites themselves also may play a role in these regulatory responses. High-molecular-weight proteins and phosphorylcholine-containing proteins isolated from filariae have been demonstrated to suppress lymphoproliferation in vitro.[22] Although the activity of these proteins is yet to be demonstrated in vivo, chemotherapeutic elimination of circulating microfilaria from infected individuals restores previously suppressed parasite-specific lymphocyte responses. The presence of these types of parasite-associated immune regulatory events has yet to be studied critically in the horse. However, the seasonal variability in skin responses to the *Onchocerca* microfilariae by horses in some regions has been investigated. In this instance, the onset of ventral-midline dermatitis during the summer may be related to a seasonal fluctuation in microfilarial burdens of the skin, which also peak at this time.[23] Not only do total numbers increase, but also the microfilariae are found more commonly in the surface layers of the skin. Interestingly, this period of abundant microfilariae corresponds with the seasonal peak in numbers of the vector *Culicoides varripennis*. Although speculative, correlations in the peak availability of microfilariae and vectors may be an evolutionary adaptation by these parasites to maximize transmission and survival of this parasite species.

2.3.2 Protective Resistance

Resistance to infection may be innate or acquired. In some instances, innate resistance to equine parasites has been attributed to age, with older individuals being resistant. Most equine helminth parasites only develop in the horse, and conversely the horse exhibits an innate resistance to most nonequine parasites. Exceptions to this rule are parasites with a broader host range that occasionally infect horses, such as larvae of the tapeworm 92
Echinococcus granulosus and the liver fluke *Fasciola hepatica*. *Trichostrongylus axei*, a parasite of ruminants, 93
establishes readily in the equine stomach and only produces significant lesions when present in large numbers. In

some cases, parasites that develop in the horse induce more severe lesions and clinical signs than in their apparent normal host, as has been described for *D. arnfieldi*.

Age resistance to *P. equorum* and *S. vulgaris* has been described to occur in horses by comparing susceptibility of young and old ponies reared under parasite-free conditions. Apparently the reaction of the lung to migrating *P. equorum* larvae is more significant in mature horses and suggests that an immune response occurs in this site.[9,10] Initial reports on age-acquired resistance to *S. vulgaris* infection have not been substantiated by further experimentation, and these results remain equivocal.[24]

The occurrence of acquired resistance to equine parasites can be inferred from the observation that older, chronically exposed horses generally have lower burdens of parasites than do similarly exposed young horses. With these criteria, acquired resistance is apparent to infections with *Strongyloides, P. equorum, Strongylus* spp., and cyathostome species. Extensive experiments are limited, however, to those on *S. vulgaris*.

The level of resistance acquired in most cases is partial and of a concomitant type; that is, some stages of the parasite, such as arterial larvae of *S. vulgaris*, may reside within the horse in the face of an active acquired resistance against newly acquired infective stages. Resistance to infection with *S. westeri* adult parasites is inferred by the short duration of their life cycle within the small intestine and the failure of subsequent exposures to establish patent infections. Mares, however, remain infected with arrested third-stage larvae, which subsequent to foaling are transmitted to the foals in milk four days postpartum. Although not studied in horses, similar phenomena occur in swine strongyloidosis. In these infections, an apparent protective resistance against the migrating stage L_3 parasite occurs that is effective in preventing reestablishment of the intestinal infection but is ineffective against L_3 parasites, which are sequestered in the abdominal fat of the sow.[25] Similar epidemiologic phenomena occur in *S. westeri* infections of horses implying that similar immunologic mechanisms are also active.

Immunologic mechanisms associated with protective resistance are presented primarily as they relate to parasites that inhabit the lumen of the gastrointestinal tract and secondly as those that undergo extensive extraintestinal tissue migration.

2.3.2.1 GASTROINTESTINAL PARASITES

Immune responses directed toward gastrointestinal nematodes vary significantly among hosts and against different parasite species within a given host.[22] However, some generalities may be stated that may serve as a background for understanding these responses in the horse. A phenomenon termed *self-cure* has been described in sheep, in which the ingestion of significant numbers of infective larvae induces the expulsion of existing adult parasites. This expulsion is initiated by a species-specific immediate-type hypersensitivity response that may cause the nonspecific expulsion of other nematode species. Although this phenomenon has not been examined in the equine, experimental infections of naturally parasitized ponies with large numbers of *S. vulgaris* L_3 induced a dramatic decrease in preexisting strongyle fecal egg counts, suggesting that a self-cure–like reaction may occur under some conditions.

More typically, establishment of primary infections results at some time in spontaneous expulsions of these worms because of senility or, as demonstrated in laboratory animal model systems, active acquired immune responses. This phenomenon occurs experimentally in the absence of reinfection and is thus separate from the self-cure phenomenon. A confusing number of immune effectors have been identified with this phenomenon in various model systems, and likely some if not all are at some time active in the equine intestine. The

mechanisms involved are T cell dependent. Antibodies may be involved but are not sufficient in themselves to induce expulsion. T cell–mediated mastocytosis, eosinophilia, and goblet cell hyperplasia have been demonstrated to be related to expression of expulsion in some systems. These accessory cells are involved in the nonspecific efferent arm of this response. Mediators of inflammation, such as vasoactive amines, prostaglandins, and increased mucus production, have been linked to immune elimination of primary infections in some but not all model systems. A number of specific immunologic events likely initiate several nonspecific effector mechanisms, resulting in this expulsion. These mechanisms vary with the species of parasite involved. The elimination of adult *S. westeri* and *P. equorum* from maturing horses and the hypothetical seasonal turnover in *Strongylus* spp. and cyathostome species may be mediated by such responses.

In addition to immune responses that occur during tissue migrations, protective resistance to reinfection by gastrointestinal nematodes occurs at the surface of the epithelium. This reaction, termed *rapid expulsion* or *immune exclusion*, is separate from self-cure or immune expulsion of primary infection. Infective larvae are expelled from the intestine in a matter of hours. Again mechanisms of expulsion described vary between parasite and host species. However, anaphylactic reactions and mucus entrapment have been observed. Some experiments using the *Trichinella spiralis*–rat system suggest that alterations in the epithelial cells in immune animals are involved directly in the exclusion of these parasites. Although immune-mediated damage of intestinal helminths such as decreased fecundity, reduced size, and morphologic alterations have been noted, infective larvae expelled by rapid expulsion mechanisms remain viable and undamaged. One may speculate that reactions of this nature are responsible in part for resistance to reinfection of equines with cyathostomes. 93 94

2.3.2.2

TISSUE-MIGRATING PARASITES

A number of intestinal helminths migrate through extraintestinal tissues as part of their life cycle. These include parasites such as *P. equorum, Strongyloides westeri*, and *Strongylus* spp., all of which stimulate an acquired immune response in the horse. During this migration, larvae are vulnerable to attack by immune effectors that may encapsulate them in an immune-mediated inflammatory response, disrupt their migrations by interfering with important metabolic or invasive processes, or inhibit molting from the L_3 to L_4 stages. The most studied phenomenon in this regard is antibody-mediated adherence of inflammatory cells, which may result in killing of the larvae. This phenomenon has been demonstrated to involve many cell types and immunoglobulin isotypes in different host-parasite systems. In vitro studies of this nature have been conducted using *S. vulgaris* third-stage larvae and equine immune effectors in the author's laboratory. In these experiments, an antibody-dependent adherence of cells was demonstrated and shown to be parasite species-specific. In vitro killing was mediated by eosinophils and not by neutrophils or monocytes. Activated eosinophils were necessary to mediate this response, and *S. vulgaris* infections have been demonstrated to activate eosinophils and neutrophils in vivo.[26] Although eosinophils are not known with certainty to be essential in this protective immune response, an anamnestic eosinophilia is characteristic in immune ponies but not nonimmune ponies following experimental *S. vulgaris* challenge. Because of its prominence and compelling in vitro and correlative in vivo data, the eosinophil has been considered to be a major effector in immune-mediated helminth killing. However, recent studies in murine parasite model systems in which eosinophilia was blocked by anti–interleukin-5 treatment suggest that this type of cell is not essential for protective resistance in some systems.[27] Possibly, in vivo a number of cells function as effectors and may overcome the absence of sufficient eosinophils under some circumstances. Antibody reactivity with parasite-secreted enzymes and molting fluids, factors important in parasite homeostasis, have been demonstrated in vitro; and similar reactions may be important in vivo.

T cell responses are essential for the induction of protective resistance to tissue-migrating helminths in most systems studied, including the experimental *S. vulgaris* pony model. This dependency likely is caused by the T cell dependency of the antibody response and by the mediation of secondary effector cell responses. Antigenic substances secreted or excreted by migrating nematodes likely are important in the induction of these responses. A combination of immune responses elicited by a combination of specific parasite antigens, including surface antigens and secreted or excreted products, probably is necessary to induce an immune response sufficient to provide protective resistance.

2.3.3 Parasite Evasion of Immune Effectors

Some parasites that live for long periods within a host modulate the host response in a specific and a nonspecific fashion, as described previously for filarial infections. What is inferred is that this modulation inhibits immune responses associated with protective resistance. In addition, blocking antibodies that inhibit antibody-dependent eosinophil killing of schistosome larvae have been described in rodent models and in sera from infected patients. The role of these types of antibodies in other infections is unclear.

A number of parasite-driven mechanisms have been described that promote parasite survival within hosts possessing strong immune responses directed toward their antigenic components.[28] Others mask themselves to avoid recognition or directly inactivate immune effectors. Host molecules shown to be attached to the surface of parasites include glycolipids of blood group antigens, glycoproteins, serum proteins, and class I and II major histocompatibility complex molecules. In some instances, hostlike molecules have been demonstrated to be encoded in parasitic helminth genomes and expressed on their surfaces. This phenomenon, termed *molecular mimicry*, has been postulated to be a mechanism evolved by the parasite to mask itself from the host immune surveillance systems.

Some parasites, notably tapeworm larvae, produce factors that activate complement and may produce a state of localized complement depletion in vivo. Other factors from tapeworm larvae disrupt the coagulation cascade and others have antiinflammatory activity.[29] Filarial nematodes synthesize prostaglandins from host arachidonic acid and release these in vitro and potentially in vivo.[30] These molecules may be responsible for local modulation of inflammatory cell function and may play a role in the survival of parasites.

These types of mechanisms have been identified in most host-parasite systems examined and are likely active in the horse. These intriguing phenomena, however, have yet to be investigated in equine parasite infections. 94

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2.3.4 Mechanisms of Anthelminthic Resistance

Three main target sites for anthelmintic activity against helminth infections are ion channels, microtubules, and energy-requiring transporters.[31] The tetrahydropyrimidines (pyrantal), imidazathiazoles (levamisole), macrocytic lactones (ivermectin and moxidectin), and piperazines target some type of ion channel. Microtubules are the main targets of benzimidazoles.[32] Salicylanilides and chlorinated sulfonamides target energy-requiring processes of the helminths.

Like bacterial resistance to antimicrobials, resistance to anthelmintics is associated with a genetic modification that transforms a susceptible population of parasites into a resistant one.[31] Intensive use and inappropriate or ineffective dosing of therapeutics contributes to development of resistance. Presumably, treatment eventually eliminates susceptible individuals, allowing for accumulation of resistance genes within the remaining

population. Rather than passing genetically encoded material by transmissible elements, resistance is passed through successive generations of allelic inheritance.

Benzimidazoles are a useful class of anthelmintic because of their specificity for nonmammalian microtubules.[31] Interference in microtubule function disrupts the transport of vesicles that contain gut secretions to the outer surface of the parasite.[33] Resistance is thought to be due to selection of certain β-tubule allelic isotypes that are associated with the loss of high-affinity binding sites on the β–tubulin protein.[34] Specifically, the loss of the β-tubulin type 2 isotype allele from the population and the addition of a point mutation of the type 1 isotype results in attainment of high-level resistance in that population.[31,34]

Attainment of resistance to avermectins is complex and a major problem. Eventual resistance to milbemycins does occur. This phenomenon has been studied most extensively in *Haemonchus contortus* infections and *Caenorhabditis elegans*.[35] Macrocyclic lactones induce a flaccid paralysis that is mediated by interfering with the action of the glutamate-gated chloride channel. Acting as an agonist of glutamate, these anthelmintics prolong the opening of the channel. Three mutations of the genes encoding the glutamate-gated chloride channel are associated with high-evel resistance, and one to two changes result in some resistance.[36,37] Genetic changes in another set of nematode molecules, the P-glycoproteins also may be important in attainment of resistance.[31]

Pyrantel compounds are essentially acetylcholine agonists.[31] Spastic paralysis results from binding of the compound to the nicotinic acetylcholine receptor. Populations of worms appear to be heterogeneous in development of a spastic response to acetylcholine agonists. Resistance appears to occur from a shift to a homogenous population that is insensitive to the effect on the acetylcholine receptor.[38] Most of the testing has been performed with levamisole, but it appears that less sensitive receptor subtypes are also responsible for resistance to other nicotinic agents including pyrantel.[31]

Concern for the development of resistance in equine gastrointestinal parasites primarily centers on the development of resistance in cyathostomes.[32,39–45] Cyathostome resistance is a common finding for fenbendazole.[46] Fenbendazole resistance is apparent on 79% to 90% of farms tested in studies that use fecal egg counts as an indication of effectiveness of anthelmintic therapy. Even when sensitive, fecal egg counts are suppressed for less than 2 to 3 weeks. This resistance usually involves multiple species of small strongyles. Resistance to pyrantel is increasing. Efficacy has been reduced to as low as 60% effectiveness. Up to 30% of farms tested have demonstrated resistance to pyrantel. Dual resistance to fenbendazole and pyrantel has been demonstrated. Ivermectin resistance has not been reported for cyathostomes. Ivermectin, although still efficacious, suppresses egg counts for considerably less time than moxidectin.

2.3.5

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2.4 2.4—Rickettsial Diseases

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Potomac horse fever (PHF) and equine ehrlichiosis are two rickettsial diseases known to affect horses. Diagnosis of these diseases requires an awareness of the typical pattern of clinical signs, hematologic values, seasonal pattern, and prevalence of the disease in the area. Because of the frequent international and domestic transport of horses, knowledge of the previous location of the horse also may aid in diagnosis. PHF is transmitted by oral ingestion of trematodes present in aquatic insects. Equine ehrlichiosis is transmitted by ticks. For both diseases, serologic testing by indirect fluorescent antibody (IFA) test is the primary method of laboratory diagnosis. Polymerase chain reaction (PCR) methods are also useful for detection of both infections. When the diseases are diagnosed correctly at acute stages of infection and are treated properly, the prognosis for horses with equine ehrlichiosis is excellent and that for horses with PHF is excellent to good in uncomplicated cases.

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2.4.1 Potomac Horse Fever

2.4.1.1 GEOGRAPHIC DISTRIBUTION

PHF (equine monocytic ehrlichiosis, equine ehrlichial colitis, or acute equine diarrhea syndrome), a rickettsial disease of equidae, was recognized originally in 1979 along the Potomac River in Montgomery County, Maryland.[1] The disease also may have existed in northern California since the 1970s.[2] PHF is now known to occur serologically in 43 of the United States, two provinces (Ontario and Saskatchewan) in Canada, France, Italy, Venezuela, India, and Australia. However, confirmation by isolation of the causative agent has been made only in the United States. The disease is caused by a rickettsial organism currently designated *Neorickettsia risticii* (formerly *Ehrlichia risticii*[3,4]), which is serologically distinguishable from other pathogenic species infecting horses.

2.4.1.2 CAUSE

In 1983, PHF was shown to be transmitted between horses by whole blood transfusion.[1] This finding made laboratory experiments under controlled conditions possible. In 1984, serologic cross-reactivity of sera of infected horses with *Neorickettsia sennetsu* (formerly *Ehrlichia sennetsu*), a human Sennetsu fever agent in Japan, was noted.[5] In the same year, the presence of ehrlichial organisms in macrophages and glandular epithelial cells of the intestinal wall of affected horses was demonstrated by transmission electron microscopy.[6] The causative agent was isolated independently in different laboratories using human histiocytic lymphoma

U-937 cells,[7,8] canine primary monocytes,[5] and murine P388D$_1$ cells.[9,10] The agent was confirmed to reproduce disease in horses and was reisolated from horses with experimentally induced disease.[5,8,9]

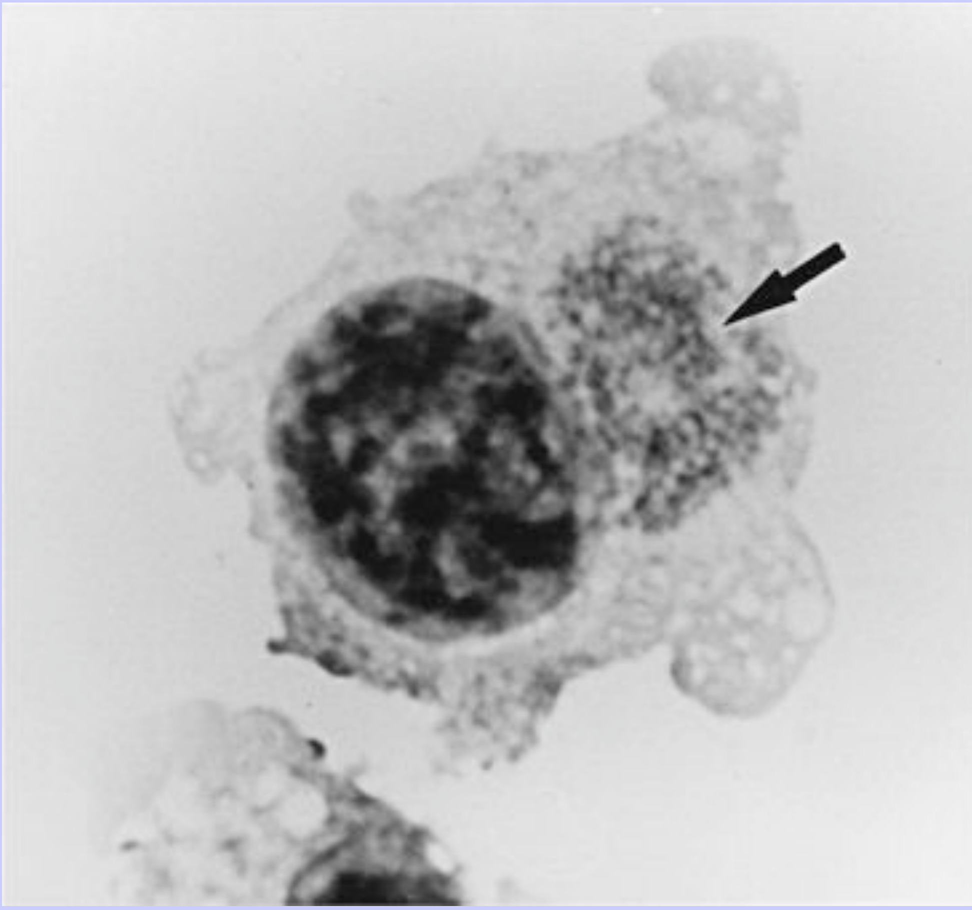
N. risticii currently is classified among three *Neorickettsia* species.[4] The genus *Neorickettsia* belongs to the family Anaplasmataceae along with the genera *Ehrlichia* and *Anaplasma*, which are obligatory intracellular bacteria with a tropism for hematopoietic cells, and the genus *Wolbachia*, which consists of intracellular symbionts of invertebrates. All these microorganisms belong to the α subdivision of the proteobacteria. None of the family Anaplasmataceae has been cultured outside of eukaryotic cells or in yolk sacs.[11] Success in culturing *N. risticii* in macrophage cell lines made possible the obtaining of the quantities of organisms needed for basic research at the molecular and cellular levels and for development of serologic tests and vaccines.

The family Anaplasmataceae is included in the order Rickettsiales along with the family Rickettsiaceae.[4] 16S ribosomal RNA gene sequence comparison currently is considered to be the most reliable method to determine phylogenetic relatedness among bacteria. Studies have shown relatedness (81.7%) in the sequences of the 16S rRNA gene in *N. risticii* and *Rickettsia prowazekii*, which is the causative agent of epidemic typhus, a well-known rickettsial disease responsible for the deaths of millions of persons during wartime and natural disasters. The percentage of 16S rRNA gene sequence homology between *N. risticii* and *N. sennetsu* is 98.9%, and the next related bacterium is *N. helminthoeca* (94.8% 16S rRNA gene sequence homology).[12] *N. helminthoeca* is the causative agent of salmon poisoning disease of the dog. *N. helminthoeca* is transmitted by trematodes, is antigenically cross-reactive with *N. risticii*, and biologically and morphologically resembles *N. risticii*.[13] Furthermore, the 16S rRNA gene of the trematode *Stellantchasmus falcatus* (SF agent) is 99.1% homologous to that of *N. risticii*.[14] Within 11 *N. risticii* strains, a maximum of 10 nucleotides are different (0.7% divergence).[15]

N. risticii is genetically divergent from *Anaplasma phagocytophilum* (formerly *Ehrlichia equi*)—which causes equine ehrlichiosis, tick-borne fever, and human granulocytic ehrlichiosis (HGE)—and *E. canis*, which is the type species of the genus *Ehrlichia*, by 16S rRNA gene sequence comparison, homology being 83.3% and 82.4%, respectively.[16]

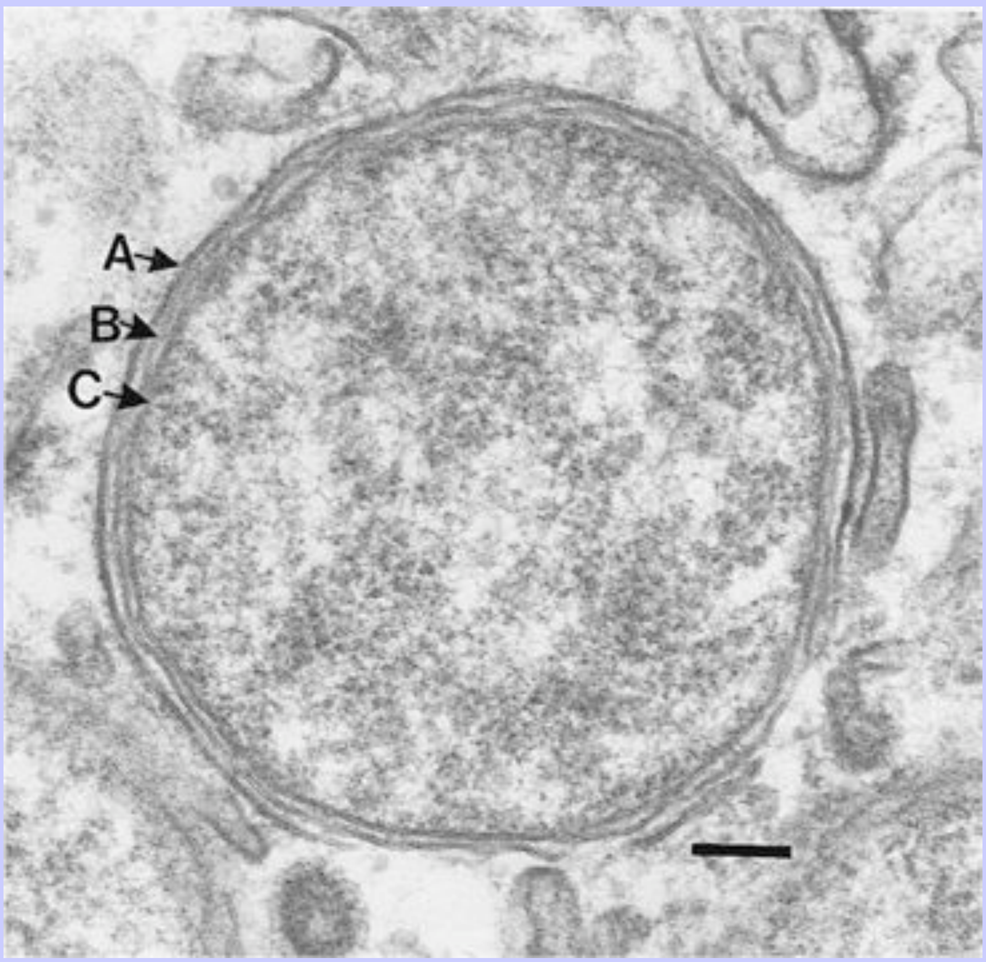
N. risticii is a tiny gram-negative coccus and stains dark blue to purple with Romanowsky's stain (Figure 2.4-1). *N. risticii* tends to occupy one side of the cytoplasm rather than being symmetrically or evenly distributed. *N. risticii* generally is round but sometimes is pleomorphic and may be elongated, especially in tissue culture. The organism divides by binary fission and is found in membrane-lined vacuoles within the cytoplasm of infected eukaryotic host cells, primarily macrophages and glandular epithelial cells in the intestine of the horse. *N. risticii* occurs in at least two different forms: multiple dark, small organisms (0.2 to 0.4 μm) enveloped by the host membrane (called morulae) and relatively light, large forms (0.8 to 1.5 μm) individually tightly wrapped with host membrane.[17,18] Morulae appear to interchange with individually enveloped forms, because an intermediate stage appearing as moderately dense ehrlichial cells tightly 97
enveloped with the host membrane that is continuous with the membrane surrounding a morula, has been seen. 98
[19] *N. risticii* in T-84, P388D$_1$, and U-937 cells, in primary equine monocyte culture, and in infected equine tissues is seen primarily in individual forms, especially in intestinal epithelial cells.[19,20] However, several recent *N. risticii* isolates make inclusions as large and as tightly packed as *E. canis* inclusions.[21,22] Vacuoles containing *N. risticii* do not fuse with lysosomes. The inhibition of lysosomal fusion is not a generalized process but rather is restricted to vesicles that contain *N. risticii*.[23]

Figure 2.4-1 *Neorickettsia risticii* in the cytoplasm of P388D$_1$ cells. Organisms are stained dark purple of Diff-Quik staining *(arrow)*. (×4000.)



Transmission electron microscopy reveals that *N. risticii* has distinct ribosomes and DNA strands.[17–19] Clumps of ribosomes are distributed homogenously in the cytoplasm rather than being marginated beneath the cytoplasmic membrane. Compared with those of the rickettsiae or ordinary bacteria, the DNA and ribosomes in *N. risticii* are packed more loosely in the cytoplasm except for the small, dense form. Thus the electron density of ehrlichial organisms is similar to that of the host cell cytoplasmic background, and this low contrast makes finding *N. risticii* in infected tissue difficult at low magnification under the electron microscope. *N. risticii* is surrounded by thin, bileaflet outer and inner membranes (Figure 2.4-2). Unlike the rickettsiae, ehrlichial organisms show no thickening of either leaflet of the outer membrane.[18,19] Morphologically, *N. risticii* does not appear to contain significant amounts of peptidoglycan.[19] Peptidoglycan is counterproductive when considering the intracellular life of *N. risticii* because the organism requires the provision of an effective diffusion rather than mechanical protection by peptidoglycan.

Figure 2.4-2 Transmission electron micrograph of *Neorickettsia risticii* in the cytoplasm of a macrophage in the large colon of a horse. *N. risticii* is enveloped tightly by the host membrane *(A)* and has its own outer *(B)* and inner *(C)* membranes. (×105,000.) Bar = 0.1 μm. (Reproduced with permission of Yasuko R: Ultrastructure of rickettsiae with special emphasis on ehrlichiae. In Williams JE, Kakoma I, editors: *Ehrlichiosis*, Dordrecht, The Netherlands, 1990, Kluwer Academic Press. Reprinted by permission of Kluwer Academic Publishers.)



No plasmid or phage has been detected by agarose gel electrophoresis of DNA fractions from *N. risticii* cultured in vitro. Lipopolysaccharide is not readily detectable by the conventional extraction method or silver staining.[11] Recent analysis of *N. sennetsu* and *A. phagocytophilum Ehrlichia chaffeensis* genome sequencing data confirms the absence of genes for lipid A biosynthesis and most of genes for peptidoglycan biosynthesis.

[23a] No endospore-like structure has been reported, although minicell-like structures resulting from uneven binary fission and apparent outer membrane vesicles of various sizes have been seen in *N. risticii*.[18]

N. risticii appears to perform aerobic and asaccharolytic catabolism. The metabolic activities of *N. risticii* have been investigated. *N. risticii* can use glutamine and glutamate and generate adenosine triphosphate as rickettsiae do.[10,24] The preference of glutamine over glutamate is likely because glutamine penetrates phagosomes better than glutamate. *N. risticii* cannot use glucose-6-phosphate or glucose[10] and is similar to the genus *Rickettsia*, but the rate of adenosine triphosphate synthesis is much lower than in *Rickettsia typhi*.[24] The greatest metabolic activity of *N. risticii* is observed at a pH of 7.2 to 8.0 and declines drastically at a pH below 98
7.[10] This finding suggests that *N. risticii* can prevent acidification and lysosomal fusion of the vacuole where 99
it resides.

2.4.1.3 EPIZOOTIOLOGY

No sex, age, or breed predilection exists for equine infections based on serologic testing of horses in Ohio.[25] The disease occurs along big rivers and their tributaries mainly during the summer and is most common from June through September. DNA from *N. risticii* has been detected in virgulate cercariae in freshwater operculate snails (Pleuroceridae: *Juga yrekaensis*) from northern California,[26,27] in virgulate xiphidiocercariae isolated from snails (Pleuroceridae: *Elimia livescens*) in central Ohio,[28] and from snails (Pleuroceridae: *Elimia virginica*) in central Pennsylvania.[29] This type of trematode is known to become metacercaria in aquatic insects, and *N. risticii* DNA has been detected in mesocercaria and metacercaria in various aquatic larval and adult insects such as mayflies and caddisflies in northern California[30] and in central Pennsylvania.[29] One horse fed adult caddisflies in northern California[31] and two horses fed adult caddisflies or a mixture of adult caddisflies and mayflies in central Pennsylvania developed PHF.[29] 16S rRNA gene sequences and 51-kd antigen amino acid sequences obtained from culture isolates from horses, horse blood, and caddisflies were identical, proving oral transmission and establishment of stable infection of *N. risticii* in the horse as the cause of PHF in Pennsylvania.[29] Ingestion of the larval stage of aquatic insects or cercariae in the water did not transmit *N. risticii*.[31] Blood-sucking arthropods such as *Dermacentor variabilis*, black flies, eye gnats, biting midges, and mosquitoes have been found to be negative for *N. risticii* transmission.[32]

Experimental injection of cell-cultured *N. risticii* or infected horse blood and reisolation of the organism have revealed that dogs, cats, mice, and nonhuman primates can be infected, although only mice and cats developed significant clinical signs.[33–36] PHF may have zoonotic potential, because *N. risticii* can cause mild clinical signs in primates.[36] Seroepizootiologic studies showed that cats, dogs, foxes, pigs, and goats in an endemic area of Maryland develop antibodies to *N. risticii*.[37] The reservoir potential of these animals is currently unknown. Dogs demonstrating symptoms of canine ehrlichiosis were seropositive to *N. risticii* (but not *E. canis*), and an agent identical to the 16S rRNA *N. risticii* was isolated from the dogs in this report.[38] However, the potential for this canine isolate to infect horses and cause PHF is undetermined.

Owners and veterinarians should be aware of the sporadic nature of PHF. On some farms and racetracks, the disease recurs several summers in a row. Alternatively, the disease may appear suddenly in isolated areas where no previous PHF cases have been reported. Usually, if clinical cases are confirmed serologically on a farm, racetrack, or horseshow, several additional seropositive horses show no clinical signs. Of horses at two racetracks in Ohio, 13% (138 of 840) to 20% (116 of 574) were exposed to *N. risticii*, according to the results

of a serosurvey conducted in the summer of 1986.[25] Of seropositive horses, 80% to 90% did not show clinical signs of infection.

2.4.1.4 PATHOGENESIS AND IMMUNE RESPONSES

2.4.1.4.1 Establishment of Infection

N. risticii can establish infection in mice and horses with or without causing apparent clinical illness. In a murine model of PHF, disease is apparently dose dependent.[39] Only at higher dosages can the organism cause disease and pathologic changes. In mice, susceptibility varies among strains.[40]

N. risticii can be reisolated from the peripheral blood monocytes of horses starting from day 6 to day 11 after ingestion of adult aquatic insects harboring *N. risticii*,[29] and ehrlichemia persists 1 to 2 weeks after spontaneous resolution of clinical signs.[29,41,42] *N. risticii* may persist much longer in the intestinal walls of clinically recovered horses, for the homogenate of intestinal tissues recovered from two ponies at 2 months after infection contained *N. risticii* antigens.[43] Furthermore, in a pregnant mare-fetus system, *N. risticii* infection persists up to 4 months, for *N. risticii* was isolated from the spleen, bone marrow, and mesenteric and colonic lymph nodes of a 7-month-old fetus of a mare that was infected experimentally during the third month of gestation.[44]

2.4.1.4.2 Mechanism of Diarrhea

N. risticii infects blood monocytes and has a predilection for the intestinal wall, especially that of the large colon where it infects tissue macrophages,[6,17] intestinal glandular (crypt) epithelial cells, and mast cells.[16,17] *N. risticii*– infected cells are shed into the intestinal lumen as detected by electron microscopy[18] and by PCR of fecal specimens.[31,42] Watery diarrhea is caused by a reduction in electrolyte transport (Na^+, Cl^-); thus a lack of water resorption occurs, mainly in the large and small colons.[45] Infected intestinal epithelial cells lose microvilli,[20,45] which may contribute to the reduced electrolyte transport and water resorption. An increase in intracellular cyclic adenosine monophosphate is found in infected mouse macrophages and infected mouse and horse intestinal tissues.[45–47] This change in cyclic adenosine monophosphate content also may contribute to the reduced lumenal absorption of Na^+ and Cl^- in the colon and thus to the lack of water absorption and diarrhea.

2.4.1.4.3 Cellular Damage and Cytokines

In contrast to cells infected with virulent strains of the genus *Rickettsia*, host cells infected with *N. risticii*
show little cytolysis in vivo or in vitro until the cytoplasm is filled completely with infecting organisms and 99
cells burst. Ehrlichial release appears to occur not only by cell lysis but also through exocytosis by fusion of 100
the inclusion membrane with the plasma membrane. In the case of intestinal epithelial cells, which make a monolayer tightly connected by circumferential zones of intercellular junctions, ehrlichial organisms appear to be transmitted between adjacent cells in vitro by a coupled exocytosis in one cell and endocytosis in an adjacent cell.[20]

Unlike ordinary gram-negative bacteria, *N. risticii* does not activate macrophages fully in vitro. *N. risticii* induces only low levels of tumor necrosis factor α and prostaglandin E_2 production in macrophages. In contrast, infected macrophages produce large amounts of interleukin-1, and this may be related to the pathogenesis of the disease.[46] This difference may be caused by a lack of lipopolysaccharides in *N. risticii*. *N. risticii* in general does not induce a severe inflammatory reaction in equine tissues.[18,45]

2.4.1.4.4 Mechanism of Infection of Macrophages

Because *N. risticii* is an obligatory intracellular bacterium, the organism must bind to the proper receptor to induce internalization and to maintain an intracellular environment adequate for survival and proliferation. In vitro, professional phagocytes (monocytes and macrophages) of host animal species have no intrinsic resistance to infection with *N. risticii*. In addition, intestinal epithelial cells and mast cells, which are normally not phagocytic, are induced to take up *N. risticii. N. risticii* binds to equine macrophages but not polymorphonuclear leukocytes. *N. risticii* entry into $P388D_1$ cells is blocked by monodansylcadaverine, an inhibitor of transglutaminase that inhibits receptor-mediated endocytosis, suggesting entry in this fashion.[48] Ehrlichial internalization, proliferation, and intercellular spreading, but not binding, depended highly on the host transglutaminase and microtubule system,[48,49] as well as on calcium-calmodulin[50] and protein tyrosine kinase.[51]

N. risticii can be taken up readily by equine polymorphonuclear leukocytes but is destroyed rapidly in those cells.[48] Polyclonal equine antiserum to *N. risticii* does not inhibit binding or internalization of *N. risticii* to $P388D_1$ macrophages, but internalized antibody-coated *N. risticii* fails to survive.[52] Because the antigen-binding fragment of the equine anti–*N. risticii* immunoglobulin G (IgG) blocks the binding of *N. risticii* to $P388D_1$ macrophages, antibody-coated *N. risticii* most likely enters macrophages via the Fc receptor. The mechanisms of destruction of antibody-coated *N. risticii* in macrophages are presently unknown.

2.4.1.4.5 Immune Response

Recovered horses have been reported to be resistant to development of clinical disease on rechallenge for at least 20 months.[53] Humoral and cell-mediated immune responses appear to have significant roles in this protection. *N. risticii* induces a specific antibody response in the natural host or in experimental animals, regardless of the presence of clinical signs. The presence of the antibody, however, does not always correlate with clearance of ehrlichial organisms and presence of protective immunity. An IgM response occurs a few days after the initial infection and lasts for less than 2 months. A challenge injection did not elicit an IgM response. Several days after *N. risticii* infection of the horse, tests for IgG antibody became significantly positive.[54]

Development of neutralizing antibodies in infected horses also was demonstrated using a cell culture system and a murine model for PHF.[41] Three mechanisms of antibody neutralization have been shown. In the first mechanism, antibody blocks ehrlichial binding to its specific receptor, instead making it bind through the Fc receptor. In the second mechanism, the antibody directly inhibits ehrlichial metabolism as demonstrated by reduced $^{14}C\text{-}CO_2$ production from ^{14}C-L-glutamine in host cell–free *N. risticii* in the presence of the antibody.[52] The third mechanism is antibody-dependent cell-mediated cytotoxicity. Macrophages infected with *N. risticii* present ehrlichial antigens on their surface similar to virus-infected cells and are lysed when

incubated with antiehrlichial antibody and normal equine peripheral blood monocytes.[55] Five to seven major antigens were exposed on the surface.[56] These antigens are considered to be the target in antibody-mediated neutralization.

Although mouse resident peritoneal macrophages fail to generate superoxide on interaction with *N. risticii*, peritoneal macrophages from mice previously inoculated with *N. risticii* and further sensitized with *N. risticii* antigen inhibit *N. risticii* in vitro by generating superoxide.[57,58] Interferon-γ has the ability to activate uninfected and infected macrophages and transform them into effector cells. The ehrlichiacidal mechanism exhibited by interferon-γ was investigated using murine peritoneal macrophages. *N. risticii* was highly sensitive to nitric oxide, which was generated by macrophage cytoplasmic nitric oxide synthase induced by interferon-γ.[59] Spleen cells from recovered mice at 28 days after inoculation with *N. risticii* proliferated in response to *N. risticii* antigen.[60] Activities of T cells that generate interferon-γ are, however, severely depressed in infected mice in a dose-related manner.[39] One cause for depressed T cell response was found in macrophages. Class II histocompatibility antigen induction on the surface of *N. risticii*–infected macrophages (antigen-presenting cells) is suppressed in vitro,[61] suggesting inhibition of antigen-specific T cell activation in *N. risticii* infection. Thus an understanding of the immunodepression 100
mechanism and identification of T cell stimulating and ehrlichial antigens that induce neutralization are 101
keys toward developing an improved, more effective vaccine for PHF.

2.4.1.5 CLINICAL FINDINGS

The incubation period for *N. risticii* infection is approximately 1 to 3 weeks. Clinical signs are an acute onset of fever up to 107° F, depression, anorexia, decreased borborygmi in all abdominal quadrants, subcutaneous edema of the legs and ventral abdomen, dehydration, and diarrhea. Laminitis and severe abdominal pain occur in 15% to 25% and 5% to 10% of cases, respectively, which are the major reasons for euthanasia.[62] Laminitis may progress, despite resolution of other clinical signs. Diarrhea may be mild to severe "pipestream" and occurs in 10% to 30% of cases. In some horses diarrhea may be transient; in others cases diarrhea persists for several days; and still other horses may have no diarrhea. Owners and veterinarians should be aware of the variable nature of clinical signs. Case fatality rates vary from 5% to 30%. Transplacental transmission of *N. risticii* is reported, and the organism may induce abortion or resorption of the fetus or produce maladjusted foals, which require extensive neonatal care.[44,63–65] Recurrence of diarrhea and on and off prolonged illness in antibiotic-treated horses has been observed.[66] Leukopenia (white blood cell count <5000/μL) with a left shift and rebound leukocytosis (white blood cell count >14,000/μL) are prominent hematologic changes. Anemia, changes in plasma protein concentration, increased packed cell volume, and thrombocytopenia also may be observed.[67]

2.4.1.6 DIAGNOSIS

In contrast with *A. phagocytophilum* infection, visual observation of blood smears of suspected horses after Romanowsky staining is useless for diagnosis of *N. risticii* infection, because only a few blood monocytes are infected with a few organisms even at the acute stage of infection.

2.4.1.6.1

Serologic Diagnosis

All ehrlichial agents induce specific humoral immune responses that are the basis for serologic diagnosis of ehrlichial diseases. However, because serologic response occurs in every animal exposed to *N. risticii*, regardless of whether infection is established or disease is present, serologic testing alone, with no supporting information, provides limited information for the diagnosis of disease. Serologic diagnosis of ehrlichial infections is performed primarily by IFA.[54,68] The cutoff titer for positive serologic result may vary with the laboratory. In the author's laboratory, IgG IFA titers of 20 or higher represent a positive serologic result. For serologic testing for *N. risticii* infection, the higher the titer is, the greater the correlation is with PHF clinical disease. The chance of healthy horses having a titer of 40 or less is similar to that of ill horses (early and late stages, and low levels of infection with *N. risticii* or possible cross-reaction with other microorganisms). Thus at a titer of 40 or less, although the horse is seropositive, the current disease is not likely to be caused by *N. risticii*. The chance of having a titer of 80 or more and 640 or less is approximately 4 times higher in ill horses than in healthy horses, and the chance of having titers higher than 1280 is 12 to 26 times higher in ill horses.[25] In addition, all experimentally infected horses had an IFA titer of 80 or higher at the onset of clinical disease.

N. risticii has been adapted to grow in a continuous murine monocyte-macrophage cell line and other cell lines; thus antigen slides are easy to prepare.[54,69] IFA testing may produce false-positive results with equine sera when they bind nonspecifically to infected cells. Several means of solving this problem are these: First, *N. risticii* organisms should be seen clearly in the cytoplasm of the host cells by IFA staining at magnification of 1000 times. If staining is blurred, morphologically different from positive control staining or Giemsa-stained infected cells or if extracellular objects are stained, the sample should not be judged positive. Second, positive and negative control equine sera must be tested to ensure the quality of the entire IFA procedure each time, including the quality of the antigen slides used and fluorescein isothiocyanate anti-horse IgG. Third, to reduce nonspecific binding, the author's laboratory uses 0.02% Tween-20 in the phosphate buffered saline washes. Usually serial dilution of the serum takes care of the problem of nonspecific binding if the titer of the sera is sufficiently high. If none of these procedures work, the author preabsorbs the equine sera with uninfected macrophages cultured and prepared under the same condition as the *N. risticii*–infected macrophage antigen. Western immunoblot analysis has been developed and is useful in distinguishing sera that have a nonspecific IFA reactivity or react by IFA to cross-reacting antigens.[70,71]

An enzyme-linked immunosorbent assay (ELISA) has been developed using purified *N. risticii* as the antigen.[54] The IgM titer rises earlier during the course of infection than the IgG titer. A rise in IgM titer occurs only at the time of initial infection, and the titer becomes negative in 1 to 2 months.[55] Thus an IgM ELISA is not useful for detecting chronic or multiple infections but is useful for early diagnosis of a primary infection of nonvaccinated horses. Several other serologic tests such as latex agglutination[72] and monoclonal antibody–based competitive ELISA have been reported.[73] However, to date a rapid and reliable field serodiagnostic test is not yet available.

No significant serologic cross-reactivity occurs between the family Anaplasmataceae and the families 101
Rickettsiaceae and Chlamydiaceae by the IFA test.[3,5,11] *N. risticii* has only minor heat-sensitive antigenic 102
determinants, unlike the genus *Rickettsia*. By Western blot (immunoblot) analysis, however, cross-reactivity of 55-kd heat-shock protein 60 homolog with those of other *Neorickettsia, Anaplasma*, and *Ehrlichia* spp. and *Rickettsia* spp. is detected.[74,75]

Several ehrlichial species antigenically cross-react within the genus when tested by IFA or Western blot analysis.[13] Within the genus *Neoricketsia, N. risticii* and *N. sennetsu* share the strongest common antigens. *N. sennetsu* is nonpathogenic to horses but it protects them from *N. risticii* infection.[71]

Immunologic cross-reactivity among members of the genus *Neorickettsia* does not create a serious problem in serologic diagnosis because of the host animal specificity of *Neorickettsia* spp. in nature and different clinical signs. However, because homologous species or strain antigen provide the most sensitive serodiagnosis, identifying at least the species is still important. Even if infection is misdiagnosed with other species, tetracycline series of antibiotics are effective for all of the ehrlichial diseases, especially at early stages of infection.

Recently the author's laboratory found that one of six *N. risticii* Ohio isolates and three Kentucky isolates were divergent from *N. risticii*, type strain (ATCC VR-986), in their antigen profiles by Western immunoblot analysis and by IFA using two panels of monoclonal antibodies.[21] The isolates also are different in the base sequence of 16S rRNA[15] and translated amino acid sequences of 51-kd antigen genes[28] and in growth morphology in the cell.[22] The 51-kd protein is a unique antigenic protein cloned from the *N. risticii* genome that shows no homology to any other known protein.[76] Amino acid sequences of 51-kd protein derived from *Juga* sp. snails in California differ at 17 amino acid positions over the 179 amino acids that can be compared (90.5% identity) with those of caddisflies and horses infected by ingestion of the caddisflies in Pennsylvania. However, the 51-kd protein amino acid sequence from caddisflies in Pennsylvania differs only at 7 amino acid positions (98.6% identity) from *N. risticii* Maryland strain over the 483 amino acids that can be compared.[29] An association of heterogeneity of *N. risticii* strains and PHF vaccine failure in Virginia and Maryland was reported.[77] Thus multiple strains or subspecies of *N. risticii*, or *Neorickettsia* spp. in the field, are likely to be distinguishable by Western immunoblotting or monoclonal antibody labeling of antigens but not by IFA or ELISA serologic testing of infected horse sera. Sequencing the antigenic protein genes further clarifies these strains. The existence of divergent antigenic variants should be taken into consideration in improving the serodiagnosis and efficacy of vaccines for PHF in the field.

In summary, recommendations for the serologic confirmation of PHF include demonstration of seroconversion, a fourfold rise or fall in titer, or the presence of IgM antibody specific to *N. risticii*. One should emphasize, however, that the initial diagnosis and treatment of PHF should be made on clinical signs, negative test results for other pathogens such as salmonella, for which the use of tetracyclines may be contraindicated, and initial IFA test results if available within 1 to 2 days. Treatment should not be delayed until laboratory confirmation is obtained from a second IFA test. It is important to point out that in the field PHF and salmonellosis can occur concurrently.[70] Weak seropositive or seronegative results of vaccinated horses when they develop clinical signs compatible with PHF are useful for ruling out the possibility of this infection. Seropositive results in vaccinated horses are useless unless confirmed by a fourfold rise in titer or by a positive PCR test.

2.4.1.6.2

Isolation of *Neorickettsia risticii* and Polymerase Chain Reaction

The isolation of *N. risticii* is accomplished by inoculating buffy coat or mononuclear cell fractions of peripheral blood of affected animals into cell culture.[21,22,42] Murine monocyte-macrophage P388D$_1$ cells, human histiocytic lymphoma U-937 cells, or canine primary monocytes are used for isolating *N. risticii*.

The procedure is more sensitive than is direct observation. Even if a single organism cannot be seen in the original buffy coat smear, the organisms can be isolated by this procedure.

Isolation procedures for diagnosing infection, however, are impractical for clinical laboratories because they take too long (3 days to 1 month) relative to the rapid course of PHF and require a reasonable tissue culture technique and facility. Although positive isolation provides a definitive diagnosis, negative results do not necessarily indicate the absence of infection.

A PCR that detects 16S rRNA, *groel*, and 51-kd protein genes of *N. risticii* in peripheral blood monocytes and feces of experimentally infected animals has been developed.[26,42,78] Applicability of this test in field cases has been tested.[42] Although cell culture isolation gives superior sensitivity to PCR procedure for field specimens, PCR procedure will be used more often in the future because of its convenience.

2.4.1.6.3 Postmortem Identification of Ehrlichial Organisms

N. risticii can be demonstrated in intestinal epithelial cells and macrophages in paraffin-embedded tissue specimens with a silver stain or an immunoperoxidase procedure using a specific antibody to *N. risticii*. 102
Immunoperoxide staining detects fewer *N. risticii* than does silver staining, presumably because of partial 103
inactivation of antigens owing to fixation and paraffin embedding. However, silver staining produces background reactions in some specimens and thus is less specific than immunoperoxidase staining.[79] These procedures have not been adapted for routine diagnosis.

2.4.1.7 PATHOLOGIC FINDINGS

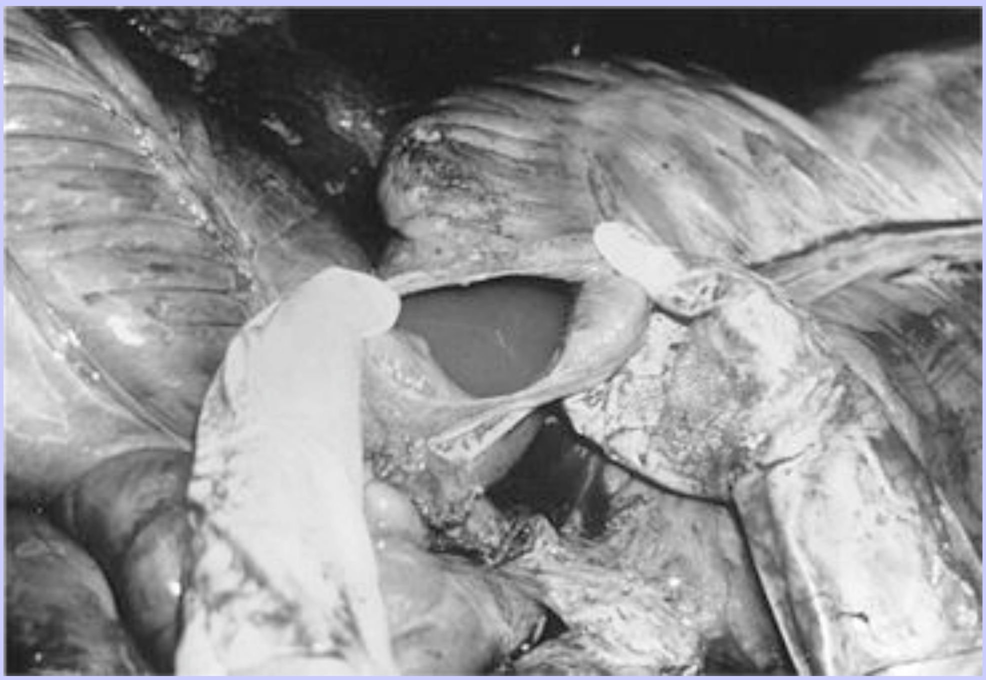
The most obvious postmortem findings in horses with PHF are the grossly distended large colon and cecum filled with watery contents (Figure 2.4-3). Few other consistent gross pathologic changes in horses with PHF occur except for patchy hyperemia along the wall of the large intestine. No destruction, foul odor, or significant inflammatory infiltration occurs such as in enterocolitis caused by salmonella, which may cause similar clinical signs or may co-infect with *N. risticii* in nature.[45] However, remarkable goblet cell mucus depletion and reduced height and increased basophilia of mucosal epithelial cells, dilation of intestinal glands, and entrapment of cellular debris in the glandular lumens are seen in experimental *N. risticii* infection.[45,71,80] Mesenteric lymph nodes are small and consist of prominent sheets of histiocytes, macrophages, occasional giant cells, and severely depleted inactive lymphoid cells.[71] Lack of severe lesions and absence of neutrophil infiltration are thus important in the differential diagnosis of PHF. The combination of lymphohistiocytic enterocolitis, hepatitis, and myocarditis in the aborted fetus at approximately 7 months gestation because of *N. risticii* infection is reported.[65,66]

2.4.1.8 THERAPY

In vitro, *N. risticii* is susceptible to doxycycline, demeclocycline, and oxytetracycline but is resistant to erythromycin and nalidixic acid.[81] As with other rickettsiae, *N. risticii* is resistant to aminoglycosides because they penetrate host cells poorly. Although oxytetracycline alone is bacteriostatic, it was found to induce lysosomal fusion with ehrlichia-containing vacuoles in P388D_1 cells,[23] thus becoming bactericidal. Oxytetracycline and doxycycline frequently correct the pyrexia and other clinical signs of PHF within 24 to 48 hours, which by itself is diagnostic.[67] Although doxycycline is more effective than is oxytetracycline in vitro

and is highly effective in mice inoculated with *N. risticii*,[81,82] oxytetracycline is preferred for the equine species because intravenous administration of doxycycline has an acute toxic effect.[83] Intravenous oxytetracycline at 6.6 mg/kg of body mass twice a day for 7 days was effective in 71% (five of seven) of experimentally infected ponies when ponies were treated after development of severe clinical signs.[67] Oxytetracycline is effective when given immediately after the development of fever but before development of diarrhea.[84] In the murine model of PHF, doxycycline, given on the fifth day, has the best effect on the immune response. When doxycycline is given on the third or seventh day after infection, immunodepression caused by *N. risticii* infection is more severe.[82] Although erythromycin or rifampin alone has a poor effect in controlling *N. risticii* infection in vitro or in infected mice,[80,81] the orally administered combination of erythromycin and rifampin has been efficacious in treating experimentally infected horses.[85]

Figure 2.4-3 Fluid-filled large colon of a horse with Potomac horse fever.



Other supportive therapy frequently is required for acutely ill horses if they are to show clinical improvement. Dehydration is corrected by the oral or intravenous administration of polyionic isotonic fluids. Brief therapy (2 to 7 days) with nonsteroidal antiinflammatory agents (e.g., phenylbutazone, flunixin, meglumine, or aspirin) for horses may be valuable early in the course of treatment to help in the management of clinical signs of PHF.[84]

2.4.1.9

PREVENTION

Ingestion of infected trematodes is probably the only means of transmission under natural circumstances, because susceptible animals housed for several months adjacent to infected animals do not have a higher incidence of the disease than those kept far from infected animals.[86] Because infection-induced immunity is

excellent[53] and a vaccine made of β-propiolactone–inactivated host cell–free *N. risticii* protects mice from homologous challenge based on clinical, pathologic, and immunologic criteria,[87] vaccination is expected to be effective in controlling PHF. Vaccines prepared from inactivated cell-cultured *N. risticii* are available for PHF from three commercial sources. Formalin-inactivated whole organism vaccine with aluminum hydroxide adjuvant has been reviewed. The vaccine is reported to cause no ill effects in horses except for swelling at the injection site in 10% of the animals.[88] Studies published in 1987 reported 78% prevention of all clinical signs except fever.[89] Protection conferred by this vaccine appears to be much shorter in duration compared with infection-induced protection. IFA titers measured after vaccination are also lower and drop rapidly in contrast to what occurs in natural infection.[88] Vaccination is not necessary for up to 2 years in the horse that has recovered from illness or has a high IFA titer from natural exposure or subclinical infection, because infection-induced immunity provides better protection than do vaccines. For nonexposed animals, considering the time required to develop immunity after vaccination, the short-lasting immunity to even the homologous strain of *N. risticii*, and the existence of antigenic variants in the field, how much benefit the vaccine will provide in the field challenge is unknown. Vaccine failures have been reported.[21,42,66,77] Therefore improvement of vaccine for PHF is desired.

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Recombinant clones that produce several *N. risticii* antigenic proteins have been produced in the author's and other laboratories. Using the murine model for PHF, a combination of two recombinant clones that express 44-kd and 70-kd antigens was shown to confer protection against *N. risticii* challenge.[90] The protection was slightly less effective than the protection given by whole *N. risticii* antigen. The efficacy of recombinant antigens for protecting horses from PHF has not been reported.

2.4.2 Equine Ehrlichiosis

2.4.2.1 GEOGRAPHIC DISTRIBUTION

Equine ehrlichiosis and *Anaplasma phagocytophilum* in the cytoplasm of blood neutrophils and eosinophils in affected horses were reported initially in 1969.[91] The disease has been observed chiefly in California. In addition, sporadic cases have been reported in Colorado, Illinois, Florida, Washington, and New Jersey and also in Germany,[92] Switzerland,[93] Sweden,[94] and Israel.[95] In the United States the disease occurs much less frequently than does PHF.

2.4.2.2 CAUSE

A. phagocytophilum is classified along with *A. marginale*, which causes infectious anemia in cattle by infecting erythrocytes, and *A. platys*, which causes canine cyclic thrombocytopenia by infecting platelets, in the family Anaplasmataceae. Because 16S rRNA gene sequences differ only up to three bases among former *Ehrlichia equi; E. phagocytophila*, which infects ruminants (primarily sheep and goats in Europe); and recently discovered human granulocytic ehrlichiosis (HGE) agent, these organisms are now considered strains of *A. phagocytophilum*.[4] By 16S rRNA gene sequence comparison, relatedness between *N. risticii* and *A. phagocytophilum* is low, approximately 83.3%. *A. phagocytophilum* has been cultured in vitro using the tick-embryo cell line IDE8[96] and a human promyelocytic leukemia cell line HL60.[97] Contrary to previous speculation by some researchers, *A. phagocytophilum* is a distinct species different from *E. ewingii* (another granulocytic ehrlichia that infects dogs in the United States) and has a 16S rRNA gene sequence homology of 92.4%.

A. phagocytophilum appears as round, dark purple, small dots or loose aggregates that look like mulberries (morulae) in the cytoplasm of blood granulocytes, primarily in neutrophils and eosinophils by Romanowsky staining.[91] By electron microscopy, several loosely packed ovoid, round, or rod-shaped *A. phagocytophilum* organisms are seen in several membrane-lined vacuoles of equine neutrophils and eosinophils.[98] The size of vacuoles ranges from 1.5 to 5 μm in diameter.[99]

2.4.2.3 EPIZOOTIOLOGY

In contrast to PHF, equine ehrlichiosis occurs during late fall, winter, and spring. Like PHF, in some areas equine ehrlichiosis is endemic and in others the disease is absent. In one seroepidemiologic survey, horses residing in the foothills of northern California had greater exposure to *A. phagocytophilum* than did those residing in the Sacramento Valley.[100] Because most seropositive horses in this study were healthy, subclinical infection with *A. phagocytophilum* seems to occur as PHF. Recent findings indicate that the western black-legged tick, *Ixodes pacificus*, is able to transmit *A. phagocytophilum* and may act as a competent vector in the field.[101,102] *A. phagocytophilum* can cause parasitemia in dogs, cats, goats, sheep, and nonhuman primates with mild to no clinical signs.[91,103,104] Cattle, rats, mice, guinea pigs, hamsters, or rabbits are not susceptible to infection.[103,104] Gribble suggested that the horse may be an accidental host, based on the low incidence of the disease.[91] Whether other species of animals can serve as a reservoir for equine ehrlichiosis is unknown, although white-footed mice recently were shown to serve as an enzootic reservoir of the HGE agent.[105]

A structural protein of *A. phagocytophilum* called *ank* has repeats in amino acids that are similar to the repeats within the human erythrocyte ankyrin protein. Comparison of these amino acid sequences in *A. phagocytophilum* isolated from horses, a dog, cattle, ticks, and human patients from several geographic regions in the United States and Europe revealed that the isolates are heterogeneous and not segregated by host animal species, but rather segregated by geographic locations.[106,107] Intravenous inoculation of horses with infected human blood produces a disease indistinguishable from that caused by *A. phagocytophilum*,[108] 104
and like *N. risticii*, diverse gene sequences detected among horse isolates indicate equine ehrlichiosis is caused 105
by multiple strains of organisms.

2.4.2.4 PATHOGENESIS AND IMMUNE RESPONSE

Infected animals develop antibody detectable by IFA, and leukocytes from infected animals show inhibition of migration when mixed with *A. phagocytophilum* antigen. Because *A. phagocytophilum* from the horse has not been propagated in vitro in sufficient quantity, an antigen prepared from the buffy coat cells of an experimentally infected horse is used as the antigen for immunologic studies.[109] Spontaneously recovered animals are immune to reinfection from 2.5 to 20 months. Maternal antibody protects foals from the disease for up to 2 months, but not from establishment of infection. When an immune mare was challenged, small numbers of infected neutrophils were seen in a 15-day-old foal born to the mare.[91] Oxytetracycline treatment eliminates *A. phagocytophilum* from horses, but rechallenge induces minimal clinical signs, suggesting the development of protective immunity in the recovered horse. Although the blood of recovered horses is reported not to be infectious, blood collected from ponies at 81 or 114 days after primary infection with *A. phagocytophilum* induced mild clinical signs (fever and mild thrombocytopenia) in susceptible recipient ponies but did not protect the recipient animals against a second challenge 100 days later. Thus *A.*

phagocytophilum appears to persist in small numbers despite the concomitant presence of antibodies and the demonstrable inhibition of leukocyte migration.[109]

2.4.2.5 CLINICAL FINDINGS

With experimental transmission using fresh blood from an infected horse, the incubation period for equine ehrlichiosis is 1 to 9 days.[91] However, when naturally infected *I. pacificus* ticks are attached, clinical signs appeared at 18 or 25 days after exposure in two horses and one horse remained normal. All three horses were infected as determined by PCR, but sequences of 16S rRNA gene of *A. phagocytophilum* in three horses were different from that of the type strain, indicating strain and disease variations.[102] Clinical signs of disease include fever lasting 1 to 9 days, depression, partial anorexia, limb edema, petechiae, icterus, ataxia, and reluctance to move.[105] In contrast to *N. risticii* infection, laminitis does not develop in equine ehrlichiosis.[110] Experimental inoculation of seven pregnant mares caused clinical signs of various severity, but none of the mares aborted. Hematologic changes observed included thrombocytopenia, decreased packed cell volume, and significant leukopenia, first involving lymphocytes and then granulocytes. The disease is inapparent to mild and is usually not fatal except for injury resulting from ataxia or secondary infection. *A. phagocytophilum* morulae are found in the cytoplasm of neutrophils and eosinophils only during the acute phase of the disease. The infection rate of peripheral blood neutrophils varies from 0.5% to 73%.[91] Chronic cases have not been reported.

2.4.2.6 DIAGNOSIS

Direct microscopic examination of Romanowsky-stained peripheral blood buffy coat smears (the author prefers Giemsa or Diff-Quik) is simple and inexpensive and provides a permanent record. *A. phagocytophilum* can be seen in granulocytes of buffy coat smears during the acute phase of the disease at 1000 times magnification. When more than three ehrlichial inclusion bodies (morulae) are seen, the diagnosis is considered definitive.[110] Culture isolation of *A. phagocytophilum* from the blood of horses rarely is performed, although *A. phagocytophilum* is isolated routinely from human patients with HGE. An IFA test using the buffy coat cells of the infected horse as the antigen has been developed and has been useful in detecting and titrating antibody in recovered horses.[109] Infected ponies become seropositive by IFA test at 21 days after inoculation, and antibody titers as high as 1:1280 can be detected at day 75 after inoculation. Major surface protein antigens of approximately 44 kd of *A. phagocytophilum* from an HGE patient were cloned in the author's laboratory[111] and others and have been used for serodiagnosis by dot blot Western immunoblot and ELISA of human patients. Because human and horse isolates are highly antigenically cross-reactive, these serologic assays are applicable for diagnosis of equine ehrlichiosis. Nested PCR has been developed for detection of *A. phagocytophilum* 16S rRNA gene in horse blood and ticks.[112] The genes *ank* and *p44* also are used for PCR diagnosis of human HGE patients. None of these methods distinguishes among *A. phagocytophilum* strains (former *E. equi*, the HGE agent, and *E. phagocytophila*) infection.

2.4.2.7 PATHOLOGIC FINDINGS

The characteristic gross lesions are petechial hemorrhages and edema accompanied by proliferative and necrotizing vasculitis of small arteries and veins in the legs. In mature males, orchitis also may be seen.[91]

2.4.2.8

THERAPY

Intravenous administration of oxytetracycline at a dosage of 7 mg/kg of body mass is reported to be effective in treating *A. phagocytophilum* infection.[110] After the initial 48 hours of treatment with oxytetracycline, defervescence is seen in all horses treated.

2.4.2.9

PREVENTION

Because *A. phagocytophilum* most likely is transmitted by *Ixodes* sp. ticks, tick repellent and insecticide are expected to be effective for prevention. A vaccine has not been developed for this disease.

TABLE 2.4-1 Comparison of Biologic Features of *Neorickettsia risticii* and *Anaplasma phagocytophilum*

FEATURE	*N. RISTICII*	*A. PHAGOCYTOPHILUM*
Distribution	United States, Canada, Europe	United States, Europe
Natural host	Horse	Horse, human, sheep, goat, dog, mouse
Experimental host	Mouse, nonhuman primate, cat, dog	Cat, dog, sheep, goat, nonhuman primate but not rat, guinea pig, rabbit, cow
Host cell	Monocyte/macrophage, intestinal epithelial cells, mast cells	Neutrophils, eosinophils
Appearance of inclusion	Individually tightly enveloped by host membrane or densely packed morulae (some of recent Ohio and Kentucky isolates)	Loosely packed small morulae

TABLE 2.4-2 Comparison of Clinical Features of Potomac Horse Fever and Equine Ehrlichiosis

CHARACTERISTIC	POTOMAC HORSE FEVER	EQUINE EHRLICHIOSIS
Mortality	Low to high	None
Acute disease	Yes	Yes
Chronic disease	No	No
Severity	Mild to severe	Mild to moderate
Leukopenia	Yes	Yes
Thrombocytopenia	Yes/no	Yes
Anemia	Yes/no	Yes
Pyrexia; anorexia; depression	Yes	Yes
Laminitis	Yes/no	No
Diarrhea	Yes/no	No
Abortion	Yes/no	No
Ehrlichia	Rarely seen in the blood	Blood granulocytes
Differential diagnosis	Colitis X	Encephalitis
	Salmonellosis	Liver disease
	Endotoxic shock	Purpura hemorrhagica
	Antibiotic-associated diarrhea	Equine infectious anemia
	Dietary changes	Equine viral arteritis
	Intoxications	

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2.4.3 Summary

Tables 2.4-1 and 2.4-2 summarize the biologic and clinical features of PHF and equine ehrlichiosis.

In recent years, because of outbreaks of PHF in the United States and discovery of the HGE agent, a strain of *A. phagocytophilum*, awareness has increased of the importance of rickettsial diseases in horses. With rickettsial diseases, wild animals and vector arthropods or helminths are usually reservoirs of rickettsiae, and domestic animals and human beings are accidental dead-end hosts. Because environmental exposure of horses to vectors and reservoirs is high, improving diagnostic, therapeutic, and vaccination procedures is important.

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3 CHAPTER 3 CLINICAL APPROACH TO COMMONLY ENCOUNTERED PROBLEMS

Melissa T. Hines

3.1 3.1—Syncope and Weakness

Mark V. Crisman

Syncope is a clinical syndrome consisting of a generalized weakness, sudden collapse, and a transient cessation of consciousness. Syncopal episodes are uncommon in horses, and generally few or no premonitory warning or presyncopal (faintness) signs are evident to the rider or handler. The subsequent loss of consciousness and collapse may be potentially harmful or dangerous to the horse and the rider. Despite the infrequent reports of true syncopal episodes in horses, the clinical signs are sufficiently dramatic to cause great concern on the part of the owner. Syncope in horses has been virtually unstudied. Consequently, most of the following information has been drawn from studies of persons and other animal species.

Although presyncopal signs have been well described in human beings (i.e., dizziness, yawning, confusion, and spots before the eyes), these signs are generally not evident in horses. Horses may stumble initially and go down or collapse completely. The depth and duration of unconsciousness may vary, but generally unconsciousness lasts for a few minutes. Horses may be slightly unsteady or struggle during recovery. After a syncopal attack, the horse will completely recover and appear normal.

3.1.1 Pathophysiology

Syncope results from a sudden reduction in cerebral blood flow and subsequent cerebral ischemia. Cerebral blood flow is maintained primarily by arterial blood pressure and cerebrovascular resistance. In response to falling or rising systemic blood pressure, the cerebral blood flow autoregulatory mechanism automatically regulates cerebral vessels to constrict or dilate. This control phenomenon maintains a constant cerebral blood flow despite fluctuations in arterial blood pressure, whether or not these fluctuations are physiologic or pathologic. If perfusion pressure in human beings falls below 60 mm Hg, the cerebral blood flow autoregulatory mechanism may fail. Mean resting arterial pressure measured at the carotid artery in horses has been reported to be 97 ± 12 mm Hg at a heart rate of 42 ± 10 beats/min.[1] Systolic pressure in horses experiencing syncope has not been determined.

Disturbances in oxygen supply to the brain generally result from three primary causes: anoxia, anemia, and ischemia. Although a variety of conditions or diseases may cause these disturbances, all three potentially deprive the brain of its critical oxygen supply.[2] Anoxia generally is described as insufficient oxygen reaching the blood so that arterial oxygen content and tension are low. This insufficiency results from an inability of oxygen to cross the alveolar membrane (e.g., pulmonary disease) or low oxygen tension in the environment (e.g., high altitude). In situations of mild hypoxia, the cerebral blood flow autoregulatory mechanism maintains oxygen delivery to the brain. When the hypoxia is severe or the compensatory mechanism fails, cerebral hypoxia occurs and syncope may result.

Anemia is defined functionally as a decreased oxygen-carrying capacity of the blood. This may be characterized 111
by several mechanisms, including a reduction in the amount of hemoglobin available to bind and transport 112
oxygen or changes in hemoglobin that interfere with oxygen binding (e.g., methemoglobin). If the anemia is

severe, the oxygen concentration drops below the metabolic requirements of the brain despite increased cerebral blood flow.

Finally, cerebral ischemia results when cerebral blood flow is insufficient to supply cerebral tissue. Any disease that greatly reduces cardiac output, such as myocardial infarction or an arrhythmia, ultimately may result in cerebral ischemia. If any of these aforementioned conditions occurs and cerebral blood flow is interrupted or stops with resultant cerebral underperfusion, consciousness is lost. If tissue oxygenation is restored immediately, consciousness generally returns quickly without sequelae.

Areas of the brain that maintain or control consciousness have been the subject of much debate and research. Generally, the level of activity of the brain (alertness) is maintained through sensory input to the ascending reticular activating system in the rostral brainstem, thalamus, and cerebral cortex. More specifically, the bulboreticular facilitatory area within the reticular substance of the middle and lateral pons and mesencephalon is considered to be the central driving component of the excitatory area of the brain. Recent studies have identified the role of the midbrain reticular formation and the thalamic intralaminar nuclei in maintaining consciousness and arousal in animals and human beings.[3] Syncope may result if regional cerebral blood flow to this area is disrupted for any reason.

In horses, syncope may be cardiogenic or extracardiac (neurocardiogenic) in origin. The primary cause of syncope in horses is generally cardiovascular disease. Cardiogenic syncope may result from (1) myocardial disease, (2) cardiac dysrhythmias (i.e., atrial fibrillation and third-degree heart block), (3) congenital heart disease, (4) pulmonary hypertension or stenosis, and (5) pericardial disease. Although many of these conditions are uncommon in horses, atrial fibrillation has been associated with several reports of syncope.[4]

Cardiovascular disease, resulting in an inability to regulate heart rate or in stroke volume, ultimately decreases cardiac output. Atrial fibrillation can lead to heart rates greater than 240 beats/min with submaximal exercise. The lack of effective atrial contraction prevents complete ventricular filling at the end of diastole, thus causing a great reduction in effective cardiac output. Complete heart block may be persistent or intermittent and also has been associated with syncopal episodes in horses. When the block is complete and the pacemaker below the block fails to function, syncope occurs. This situation has been reported in human beings and horses as Morgagni-Adams-Stokes syndrome. This syndrome is the most frequent arrhythmic cause of syncope in human beings.[5] Morgagni-Stokes-Adams attacks result from an advanced atrioventricular block and usually involve a momentary sense of weakness followed by an abrupt loss of consciousness. After cardiac standstill or prolonged periods of asystole, unconsciousness results from cerebral ischemia. These “cardiac faints” have been reported to occur several times a day in human beings. Additional, less common causes of cardiogenic syncope usually involve the distal conduction system (His-Purkinje system) and may be persistent or episodic. Heart block involving the atrioventricular node or proximal conduction system may be congenital or drug induced (e.g., digitalis). Sick sinus syndrome, a condition described in elderly human beings, involves impaired sinoatrial impulse formation or conduction and has been associated with cerebral anoxia. With any of these conditions, cardiac output does not increase sufficiently during skeletal muscle exercise to meet peripheral oxygen demands. Blood preferentially flows to exercising muscle, resulting in systemic arterial hypotension, which results in cerebral ischemia leading to weakness or syncope.

Extracardiac causes of syncope indirectly may involve the cardiovascular system and were referred to previously as vasovagal or vasodepressor syncope. The term *neurocardiogenic syncope* more accurately describes this phenomenon. Neurocardiogenic syncope is the most common type of syncope reported in human beings and often is precipitated by stress or pain.[6] Although not specifically described in horses, a similar mechanism of collapse likely may exist. The critical cardiovascular features include hypotension and paradoxical sinus

bradycardia, heart block, or sinus arrest after sympathetic excitation. Additionally, cardiac asystole may occur as an extreme manifestation of neurocardiogenic syncope. The mediating mechanisms of neurocardiogenic syncope are not well understood; however, several theories have been proposed. Hypercontractile states may cause excessive stimulation of the myocardial mechanoreceptors (C fibers) located in the left ventricle. The result is an exaggerated parasympathetic afferent signal carried by the vagus and glossopharyngeal nerves with a subsequent decrease in sympathetic tone. Inhibition of sympathetic vasoco strictor activity results in vasodilation, which may be especially evident during periods of vigorous activity and increased heart rates and blood pressure. The excess vagal activity produces bradycardia and a decrease in cardiac output. This combination, along with a decrease in peripheral vascular resistance, ultimately leads to syncope.

Regardless of the specific cause, syncope results from a sudden fall in cerebral blood flow. The loss of consciousness is caused by a reduction of oxygenation to the parts of the brain that maintain consciousness. In horses, syncope usually is caused by a fall in systemic blood pressure resulting from a decrease in cardiac output. 112

Additional, less common causes of syncope in horses may include neurologic disease from space-occupying 113
lesions or increased intracranial pressure. Syncopal episodes have been reported in foals with severe respiratory or congenital heart disease.[7] After minimal exercise or restraint in these foals, hypoxia and subsequently reduced cerebral blood flow may result in syncope. Certain drugs, specifically phenothiazine tranquilizers (acepromazine), have been reported to cause syncope in horses. These tranquilizers produce antiadrenergic effects primarily through α_1-blockade with resultant vasodilation and hypotension. If phenothiazine tranquilizers are administered to severely hypovolemic horses or to horses that have hemorrhaged, severe hypotension and syncope may result.

Several disorders often are confused with syncope and should be differentiated carefully by an accurate history and thorough physical examination. These disorders include (1) epilepsy, (2) hypoglycemia, (3) narcolepsy and cataplexy, (4) cerebrovascular disease, and (5) hyperkalemic periodic paralysis.

Epileptic seizures generally differ from syncope in that they have immediate onset and involve loss of consciousness, tonic and clonic convulsive activity with opisthotonos, and changes in visceral function (urination and defecation). Seizures commonly last for several minutes and often are followed by a postictal phase in which the horse may pace, appear blind, and not recognize its surroundings.

Metabolic disturbances such as hypoglycemia frequently are observed in neonatal foals and may be associated with weakness or syncopal-like episodes. Typically, foals are premature or are subject to perinatal stress with subsequent increased glucose use following hypoxia or sepsis. Serum glucose determination is necessary to evaluate hypoglycemia.

Narcolepsy, an abnormal sleep tendency, and cataplexy occasionally may be difficult to distinguish from syncope as a cause of unconsciousness. Attacks of narcolepsy or cataplexy may be preceded by signs of weakness (buckling at the knees) followed by total collapse and areflexia. Rapid eye movements may occur with an absence of spinal reflexes. No other neurologic abnormalities are observed between attacks, although animals may appear sleepy between episodes. Provocative testing with physostigmine (0.05 mg/kg) may induce narcoleptic attacks and might be helpful in differentiating syncope from narcolepsy or cataplexy.

Cerebrovascular disease associated with head trauma and subarachnoid hemorrhage may cause temporary unconsciousness in horses. Clinical signs resulting from brain trauma generally are associated with focal cerebral dysfunction and therefore are readily distinguishable from syncope.

Hyperkalemic periodic paralysis causes weakness and collapse without alterations in consciousness. This autosomal dominant disorder has been reported in certain lines of registered Quarter Horses, Paints, and Appaloosas. A reliable DNA-based test is available to diagnose hyperkalemic periodic paralysis in horses.

3.1.2 Evaluation of Syncope

A thorough evaluation of syncope in the horse consists of the following:

1. *History:* Emphasis should be placed on obtaining a detailed history. The onset and the duration of the problem along with performance history should be determined.
2. *Physical examination:* After a thorough physical examination and determination of vital signs, a detailed cardiovascular and neurologic examination should be performed. In addition to heart rate at rest and pulse characteristics, a thorough cardiac auscultation should be performed in a quiet room to identify any murmurs or cardiac dysrhythmias. An electrocardiogram and echocardiogram also provide valuable information. A neurologic examination should evaluate reflexes and sensory and motor function carefully to identify any central or peripheral neuropathies.
3. *Complete blood count and biochemical profile:* To rule out other potential causes of syncopelike episodes (e.g., hypoglycemia and sepsis), a complete blood count and biochemical profile should be performed. Additionally, serum lactate dehydrogenase (isoenzymes 1 and 2) and creatine kinase (CK-2) concentration determinations may be helpful in identifying cardiac dysfunction.
4. *Exercise/stress test:* A thorough cardiac evaluation should be performed following strenuous exercise, including auscultation and an electrocardiogram. If available, a high-speed treadmill may be helpful in this phase of the evaluation. If any cardiac abnormalities are detected on physical examination, exercise testing on a treadmill will allow a more thorough evaluation of the cardiovascular system, although care must be taken to ensure that such testing does not exacerbate the condition of the horse.

Diagnosis of the cause of syncope in horses is not always easy, because the cause should be considered a symptom complex rather than a primary disease. In addition to the infrequent reports of syncope, the history is often vague and the neurologic and cardiovascular examinations may not lead to a specific cause. Even in the absence of apparently overt cardiovascular disease (e.g., atrial fibrillation), cardiac dysrhythmias cannot be excluded as the possible cause of syncope.

3.1.3 Treatment of Syncope

Options for treating syncope in horses are limited. The frequency of the syncopal attacks and the underlying 113
cause (i.e., cardiogenic or neurocardiogenic) may determine if a course of treatment should be undertaken. 114
Generally, treatment of syncope should be directed toward preventing or correcting the cause of the decreased cerebral perfusion. An accurate pathophysiologic diagnosis is essential for treating cardiogenic syncope. A few reports in the literature indicate successful treatment of syncope in horses associated with atrial fibrillation.[4] A horse with a complete heart block returned to work after implantation of a transvenous cardiac pacing system.[8]

3.1.4

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3.2

3.2—Polyuria and Polydipsia

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Bernard Hansen

The complaint of excessive urination and drinking may be encountered with some frequency in equine practice. Before pursuing a lengthy diagnostic workup, verifying that 24-hour urine production and voluntary water consumption exceed reference ranges is important. Urine production in adult horses may range from 15 to 30 ml/kg/day, and values as high as 48 ml/kg/day have been reported.[1–4] Daily urine volume is affected by diet; more water is lost in the urine in horses fed pelleted diets and legume hays than in horses fed grass hay. The latter excrete more water in feces.[5,6] Generally, any component of the diet that increases renal solute load increases urine volume (e.g., high salt content in the diet). Voluntary water intake also is affected by the ambient temperature (Table 3.2-1). When temperatures are high and evaporative water losses increase to cool the horse, voluntary water intake also increases. Diet and climatic conditions therefore must be considered when interpreting water consumption and urine production data. Water requirements are proportional to metabolic body size rather than to body mass. Thus larger horses, particularly draft breeds, require less water per kilogram than do smaller horses, ponies, or miniature horses. In addition, fat is low in water content compared with lean body tissue, and fat animals require proportionately less water than do lean animals.[7]

Some owners may misinterpret polyalkiuria (frequent urination usually of small volume) as polyuria. Quantitative collection of urine for a 24-hour period may be required to verify excessive urine production. Several simple collection apparatuses have been described.[8,9]

3.2.1 Maintenance of Water Balance in Health

Maintenance of water homeostasis depends on establishing a balance between intake and excretion such that plasma osmolality remains constant (within approximately 2% of normal).[10] The primary determinant of renal water excretion is antidiuretic hormone (ADH).[11] ADH is a polypeptide synthesized in three nuclei in the hypothalamus (suprachiasmatic, paraventricular, and supraoptic nuclei)[12] and transported from the latter two 114
nuclei in secretory granules down axons of the supraopticohypophyseal tract into the posterior lobe of the 115
pituitary where ADH is stored. Some ADH enters the cerebrospinal fluid or portal capillaries of the median eminence from the paraventricular nucleus.[11] In addition, neurons from the suprachiasmatic nucleus deposit ADH in other areas in the central nervous system.[12] In human beings, lesions of the posterior pituitary or supraopticohypophyseal tract below the median eminence usually do not lead to permanent central diabetes insipidus because ADH still has access to systemic circulation in these cases.[11] The clinical importance of these anatomic relationships in horses is not known.

TABLE 3.2-1 Voluntary Water Consumption in Healthy Horses

	WATER CONSUMPTION	
AMBIENT TEMPERATURE	**ml/kg/day**	**L/450 kg**
5°–16°C (41°–61°F)	44–61	19.8–27.5
25°C (77°F)	70	31.5

Data from Tasker JB: Fluid and electrolyte studies in the horse. III. Intake and output of water, sodium and potassium in normal horses, *Cornell Vet* 57:649–657, 1967; Rose BD: *Clinical physiology acid-base and electrolyte disorders,* New York, 1989, McGraw-Hill Information Services; and Groenedyk S, English PB, Abetz I: External balance of water and electrolytes in the horse, *Equine Vet J* 20:189–193, 1988.

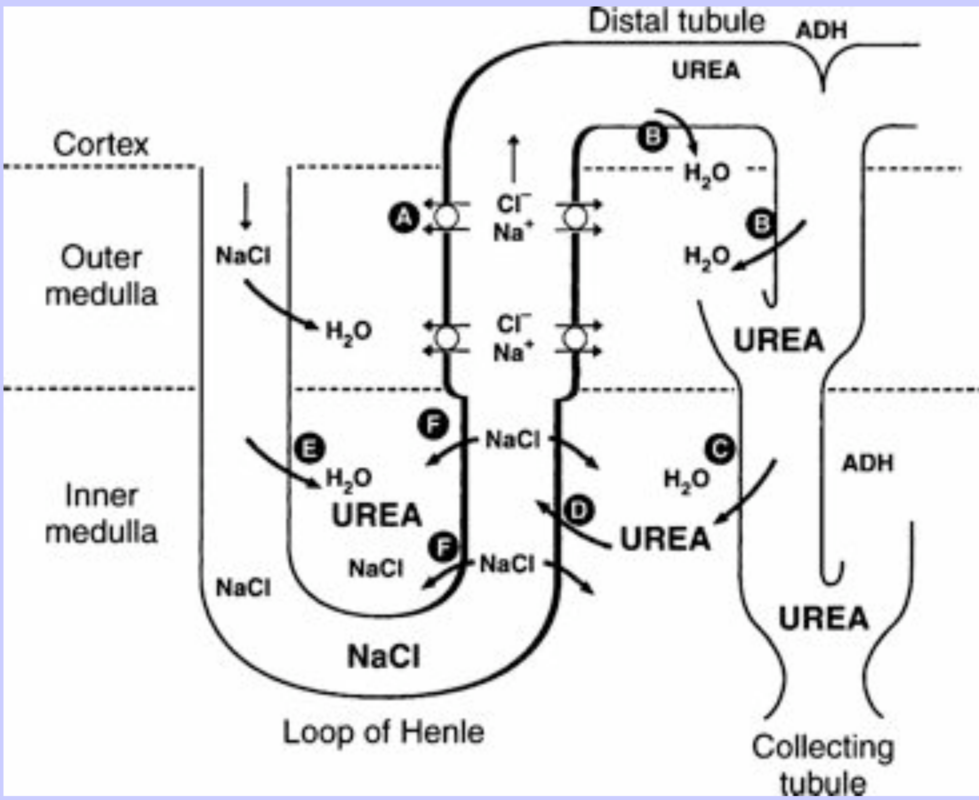
ADH increases renal water reabsorption and urine osmolality by augmenting water permeability of luminal membranes of cortical and medullary collecting tubules. ADH augments urea, and in some species NaCl, accumulation in the interstitium, therefore promoting medullary hypertonicity. The primary stimuli for ADH release are plasma hyperosmolality and depletion of the effective circulating blood volume. Osmoreceptors in the hypothalamus detect changes in plasma osmolality of as little as 1%.[11] Although the threshold for ADH release in the horse is not known, 24-hour water deprivation in healthy ponies resulted in approximately an 8 mOsm/kg increase in plasma osmolality (about 3%), from 287 ±3 mOsm/kg to 295 ± 4 mOsm/kg, which was associated with an increase in plasma ADH concentration from 1.53 ± 0.36 pg/ml to 4.32 ± 1.12 pg/ml.[13] In another study of ponies, water deprivation for 19 hours resulted in an increase in plasma osmolality from 297 ± 1 mOsm/L to 306 ± 2 mOsm/L.[14] In human beings, plasma osmolalities of 280 to 290 mOsm/L stimulate ADH release. The organs that sense changes in effective circulating blood volume include arterial and left atrial baroreceptors. These stretch receptors function indirectly as volume sensors by responding to the reductions in intraluminal pressure that typically accompany loss of plasma volume. Reduced activation of these receptors by hypovolemia or heart failure is a potent cause of ADH release, even in the absence of increased plasma osmolality. ADH secretion also may be stimulated by stress (pain), nausea, hyp glycemia, and certain drugs including morphine and lithium.[11]

When the need for water in body fluids cannot be met by conservation via the renal/ADH axis, thirst is stimulated. Thirst is regulated primarily by plasma tonicity; however, in human beings the threshold for stimulation of thirst is approximately 2 to 5 mOsm/kg greater than that for stimulation of ADH release.[11] Thirst is controlled by osmosensitive neurons in close proximity in the hypothalamus to osmoreceptors that mediate ADH secretion.[12] Thirst is sensed peripherally by oropharyngeal mechanoreceptors as dryness of the mouth. Thirst also may be stimulated by volume depletion through an incompletely understood mechanism. Experimental ponies drank when their plasma osmolalities increased by 3% after water deprivation, when plasma Na concentrations increased by approximately 5%, and after induction of a plasma volume deficit of 6%.[14]

3.2.2 Mechanism of Urine Concentration

For the kidney to make concentrated urine, ADH must be produced, the renal collecting tubules must respond to ADH, and the renal medullary interstitium must be hypertonic. Generation of medullary hypertonicity is initiated in the thick ascending limb of the loop of Henle by active transport of NaCl out of the lumen. Because the thick ascending limb is impermeable to water, active resorption of NaCl results in hypotonicity of the fluid entering the distal tubule in the renal cortex (Figure 3.2-1, *A*). The distal tubules and cortical portions of the collecting ducts are permeable to water (Figure 3.2-1, *B*), which is reabsorbed down its concentration gradient into the interstitium. Reabsorbed water is transported rapidly out of the interstitium by the extensive cortical capillary network, and interstitial hypertonicity is preserved. Urea remains in the lumen of the distal tubule and cortical collecting duct and is concentrated further. Luminal fluid flows into the medullary collecting duct, which is permeable to water and urea when under the influence of ADH (Figure 3.2-1, *C*). Water is reabsorbed down its progressively steeper concentration gradient as luminal fluid moves through the medullary collecting ducts. Some urea also is reabsorbed into the interstitium. Reabsorbed water is removed efficiently by the vasa recta in the renal medulla. Because these blood vessels also are arranged in a hairpin loop, minimal loss of medullary interstitial solute occurs with water removal. Some reabsorbed urea enters the loop of Henle (Figure 3.2-1, *D*) and thus is recycled, helping to maintain medullary hypertonicity. In the absence of ADH, the collecting ducts are relatively impermeable to water and urea, resulting in water and urea loss in urine and reduction of medullary solute. Prolonged diuresis of any cause may result in the loss of medullary hypertonicity (medullary washout) with subsequent impairment of renal concentrating ability. Water is reabsorbed down its concentration gradient from the thin descending limb of the loop of Henle (Figure 3.2-1, *E*) as a consequence of medullary hypertonicity. This segment of the nephron is impermeable to NaCl and urea, thus the osmolality of luminal fluid in the most distal portion of the loop approaches that of the interstitium. The thin ascending limb of the loop of Henle is permeable to NaCl, which diffuses down its concentration gradient into the interstitium (Figure 3.2-1, *F*). As previously mentioned, this segment is also permeable to urea, and some interstitial urea enters the tubule 115
lumen by diffusion down its concentration gradient. Luminal fluid entering the thick ascending limb of the loop 116
of Henle is thus hypotonic to the interstitium.

Figure 3.2-1 The countercurrent hypothesis identifies the roles of sodium chloride and urea transport in the generation of concentrated urine. From Hansen B: Polyuria and polydipsia. In Fenner WR: *Quick reference to veterinary medicine,* ed 2, Philadelphia, 1991, JB Lippincott. Adapted from Jamison RL, Maffly RH: The urinary concentration mechanism, *N Engl J Med* 295:1059-1067, 1976.)



When luminal fluid reaches the thick ascending limb of the loop of Henle, approximately 80% of the glomerular filtrate has been reabsorbed. Therefore only 20% of the glomerular filtrate is available for reabsorption via the action of ADH.[15,16]

3.2.3 Primary Polydipsia

Excessive water intake may result in water diuresis. Primary polydipsia has been described in horses residing in the southern United States during months when ambient temperature and humidity are high. Apparent psychogenic polydipsia may result from boredom, especially in stalled, young horses.[8] Psychogenic polydipsia also has been reported anecdotally in horses with chronic liver disease and central nervous system signs that had been treated with intravenous fluids.[17] Primary disorders of thirst are poorly understood in horses.

3.2.4 Causes of Polyuria With Secondary Polydipsia

Increased urine flow may be induced by solute or water diuresis (Box 3.2-1). Solute diuresis results in increased urine flow because of excessive renal excretion of a nonreabsorbed solute such as glucose or sodium. During solute diuresis, the urine osmolality is equal to or higher than the plasma osmolality. Primary renal insufficiency or failure (33% or fewer intact nephrons) result in solute diuresis, because each functional nephron must filter an increased amount of solute to maintain daily obligatory solute excretion. Fractional clearances of solutes such as Na, K, and Cl therefore appropriately increase. Solute diuresis caused by glucosuria occurs in hyperglycemic horses when the maximal renal reabsorptive capacity for glucose is exceeded (180 to 200 mg/dl).[18] Solute diuresis caused by glucosuria has been reported in horses with pituitary adenoma and in a hyperglycemic horse with bilateral granulosa cell tumors.[19,20] Primary diabetes mellitus, a common cause of hyperglycemia and glucosuria in other species, is uncommon in the horse, although type 2 diabetes mellitus was diagnosed in a 15-year-old Quarter Horse mare.[21] Primary renal tubular glucosuria caused by a defect in proximal tubular glucose reabsorption (as is seen in Basenji dogs with Fanconi-like syndrome)[15] has not been reported in horses. Psychogenic salt consumption also has been reported to cause solute diuresis in a horse.[22] Postobstructive solute diuresis is not diagnosed commonly in horses because nephrolithiasis and ureterolithiasis are uncommon; when they occur, the condition is often bilateral and associated with chronic renal failure, and treatment is usually unsuccessful.[23,24]

Decreased water resorption in the collecting tubules or inappropriately large voluntary water intake causes water diuresis. The osmolality of the urine during water diuresis is less than that of plasma. Water diuresis may be caused by insufficient ADH secretion, insensitivity of the receptors of the distal collecting duct and collecting tubules to the action of ADH, renal medullary solute washout, or apparent psychogenic polydipsia. Insufficient secretion of ADH (central diabetes insipidus) may be associated with adenoma of the pars intermedia of horses but has never been documented[25] and with head trauma and potassium depletion in other species. A case of idiopathic central diabetes insipidus was reported in a Welsh pony.[26] Insensitivity of collecting duct receptors to ADH may occur during endotoxemia and hyperadrenocorticism (glucocorticoid excess associated with tumors of the pars intermedia). In other species, potassium depletion, hypercalcemia, and the administration of certain drugs (including gentamicin) have been reported to cause insensitivity of the collecting duct receptors to ADH.[15] Congenital diabetes insipidus also has been reported in other species.[26] True nephrogenic diabetes insipidus implies isolated dysfunction of response to ADH by collecting tubules that are not associated with other structural or metabolic lesions of the kidney. The occurrence of nephrogenic diabetes insipidus in two sibling Thoroughbred colts has suggested that the condition might be heritable in some horses.[27] Renal medullary 116
washout (loss of medullary Na, Cl, and urea) leading to water diuresis may result from chronic diuresis of any 117
cause. Diuresis is associated with increased tubular flow rates and inability to resorb sodium and urea adequately from the tubular lumen. Enhanced medullary blood flow may deplete medullary solute further. Water diuresis also has been reported in association with pyometra, hypoadrenocorticism (chronic renal sodium loss), chronic liver disease (increased aldosterone concentration promotes sodium retention, smaller daily load of urea for excretion caused by decreased conversion of ammonia to urea), primary polycythemia, hypercalcemia, and potassium depletion in other species.[15]

3.2.4.1 BOX 3.2-1 CAUSES OF POLYURIA AND POLYDIPSIA

3.2.4.1.1 Solute Diuresis

Primary renal insufficiency or failure

Glucosuria (adenoma of the pars intermedia of the pituitary)

Psychogenic salt consumption

Diabetes mellitus

Postobstructive diuresis

3.2.4.1.2 Water Diuresis

Insufficient antidiuretic hormone (central diabetes insipidus)

- Adenoma of the pars intermedia of the pituitary
- Head trauma
- (Potassium depletion)*

Insufficient response of collecting ducts to antidiuretic hormone

Acquired nephrogenic diabetes insipidus

- Hyperadrenocorticism (glucocorticoid excess with adenoma of the pars intermedia of the pituitary)
- Endotoxemia
- (Drugs: gentamicin, lithium, methoxyflurane, amphotericin B, propoxyphene, etc.)

(Congenital nephrogenic diabetes insipidus)

Renal medullary solute washout

- Chronic diuresis of any cause
- Inappropriate renal tubular sodium handling

Apparent psychogenic polydipsia

(Chronic liver disease)

(Polycythemia)

(Pyometra)

(Hypercalcemia)

(Potassium depletion)

3.2.4.1.3

Iatrogenic

Intravenous fluid therapy

Excess dietary salt

Drugs:

- Diuretics
- Glucocorticoids
- (Drugs causing acquired diabetes insipidus)

Modified from Hansen B: Polyuria and polydipsia. In Fenner WR: *Quick reference to veterinary medicine,* ed 2, Philadelphia, 1991, JB Lippincott.

* *(),* Not reported in horses.

3.2.5

Approach to the Horse With Polyuria and Polydipsia

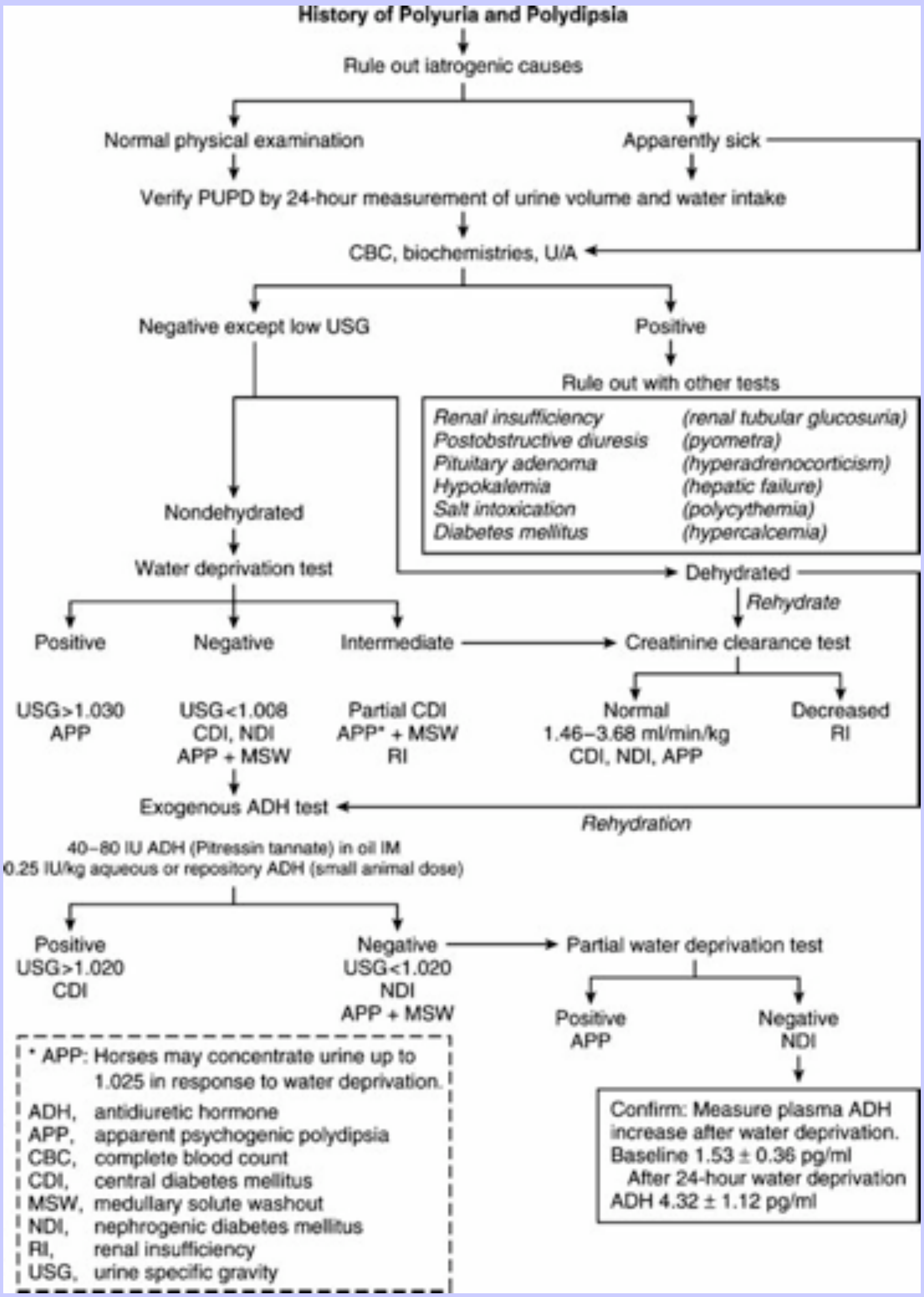
Iatrogenic causes of polyuria and polydipsia (see Box 3.2-1) should be ruled out by careful assessment of the history and by documentation of return to normal urine volume and water intake after withdrawal of intravenous fluids, excess dietary salt, or drugs implicated in causing polyuria and polydipsia (Figure 3.2-2). Verification of 24-hour urine volume and water intake should be undertaken for horses suspected of having polyuria and polydipsia that do not display obvious polyuria (frequent large volume urination and wet stall) and polydipsia (water bucket always empty and overt thirst). Hemogram, serum biochemistries, and urinalysis should be assessed for all horses with polyuria and polydipsia. A hallmark finding in horses with polyuria and polydipsia is a decreased urine specific gravity (USG). Identification of other abnormalities on laboratory tests (e.g., increased blood urea nitrogen or creatinine concentrations, hyperglycemia, and hypokalemia) necessitates ruling out the presence of underlying diseases (such as renal insufficiency and adenoma of the pars intermedia of the pituitary) using specialized laboratory tests.

The hydration status of horses then should be assessed carefully. Those horses that are dehydrated should be rehydrated judiciously with intravenous fluids, taking care not to overhydrate horses with renal insufficiency. After rehydration, when possible, creatinine clearance should be determined by using a urine collection

apparatus to allow 24-hour volumetric urine collection. A creatinine clearance value below the reference range (1.46 to 3.68 ml/min/kg)[28] suggests that renal insufficiency with decreased glomerular filtration rate and solute diuresis are likely present. A creatinine clearance within the reference range indicates that central diabetes insipidus (CDI), nephrogenic diabetes insipidus (NDI), or apparent psychogenic polydypsia (APP) is present. To distinguish among these differential diagnoses, an exogenous ADH challenge test should be performed (see the subsequent discussion).

Horses with polyuria and polydipsia that are well hydrated and healthy based on physical examination and
results of hemogram and serum biochemistry determinations should be subjected to a water deprivation test to 117
assess renal ability to conserve water.[2,29,30] Water deprivation testing is contraindicated in a dehydrated horse 118
with a low USG. Such horses have already undergone an endogenous water deprivation test (clinical dehydration is present) and have responded with an inappropriately low USG. The following guidelines for interpretation of water deprivation test results are based on practical experience and the limited data available. A positive response to water deprivation (USG >1.030) indicates that the horse has APP, whereas a negative response (USG <1.008) after 24 hours of water deprivation or greater than 5% weight loss[31] is consistent with a diagnosis of
CDI, true NDI, insensitivity of collecting duct receptors to ADH, or apparent psychogenic polydipsia and 118
medullary solute washout (APP plus MSW). Horses with a negative response to water deprivation testing should 119
undergo an exogenous ADH challenge. Some horses may have an intermediate response to water deprivation. An intermediate response is consistent with partial CDI or APP plus MSW or renal insufficiency, and assessment of creatinine clearance is indicated. Consult Chapter 18 for a more detailed discussion of water deprivation testing.

Figure 3.2-2 Approach to the polyuric patient. *PUPD,* Polyuria and polydipsia; *U/A,* Urinalysis. Modified from Hansen B: Polyuria and polydipsia. In Fenner WR: *Quick reference to veterinary medicine,* ed 2, Philadelphia, 1991, JB Lippincott.



An evaluation of a response to the administration of exogenous ADH is indicated for horses that do not concentrate their urine adequately during water deprivation testing, for horses that require rehydration and subsequently demonstrate creatinine clearance values within reference ranges, and for rehydrated horses for which creatinine clearance determinations are impractical. Two regimens for exogenous ADH administration have been reported: 40 to 80 IU ADH as Pitressin tannate in oil intramuscularly[32] or 0.25 IU/kg aqueous or repository ADH intramuscularly. A few reports of responses of horses to exogenous ADH administration have been made, and the following recommendations are based on clinical experience. A positive response to exogenous ADH (USG >1.020) confirms the diagnosis of CDI.A negative response (USG <1.020) implies that NDI or APP plus MSW is present.[31]

MSW may result in a decreased USG despite the presence of adequate ADH. A partial water deprivation test should result in an increase in USG in horses with APP plus MSW but should have no effect on horses with true NDI or insensitivity of collecting duct receptors to ADH. The horse is allowed to consume its normal diet and water ad libitum. Voluntary water consumption is monitored closely for 3 to 4 days to establish a baseline. Water available to the horse then is decreased by 5% to 10% of the baseline voluntary intake. Water should be offered in aliquots several times a day to prevent the horse from consuming most of the water in a short time. Water intake should never be restricted below maintenance requirements (about 40 ml/kg/day). During water restriction, the horse is allowed to eat its regular diet. The horse should be weighed daily if possible and should be observed carefully for signs of dehydration (prolonged capillary refill time, increasing heart rate, prolonged skin tenting, and hypernatremia). Moderate water restriction in the face of continued intake of dietary solutes facilitates reestablishment of the corticomedullary osmotic gradient.[15] Results of partial water deprivation tests in horses have been reported infrequently.

The diagnosis of true NDI or insensitivity of collecting duct receptors to ADH may be confirmed by measuring plasma ADH concentrations before and after partial water deprivation. ADH concentrations have been reported to increase from baseline values of 1.53 ± 0.36 pg/ml to 4.32 ± 1.12 pg/ml after 24 hours of water deprivation in ponies.[13]

Because CDI, NDI, and APP are uncommon in horses, the presenting complaint of polyuria and polydipsia usually signifies other underlying disease. The most likely underlying disease is renal insufficiency. Pituitary adenoma should be considered in horses with compatible clinical signs (hirsutism, weight loss, and laminitis) and supporting laboratory data (hyperglycemia and failure of suppression of cortisol production by dexamethasone).[33] Medullary washout may be a more common complication of primary diseases and their therapy in horses than has been reported to date. Potential causes of diuresis compatible with the case history and clinical signs should be investigated, and a partial water deprivation test should be considered when horses exhibit polyuria and polydipsia.

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3.3

3.3—Edema

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Edema is the excessive and abnormal accumulation of fluid in the interstitium. Interstitial fluid accumulates because of imbalances in the rates with which fluid enters and exits the interstitium. Factors that increase the rate of fluid flux from the capillary or impair lymph drainage sufficiently to overwhelm normal compensatory mechanisms result in accumulation of fluid and the development of edema.

3.3.1

Physiology

The volume of interstitial fluid and lymph fluid in the normal horse is 8% to 10% of body mass,[1] or 36 to 45 L in a 450-kg horse. Interstitial fluid consists of water, protein, and electrolytes. Compared with plasma, interstitial fluid has a slightly lower concentration of cationic electrolytes, a slightly higher concentration of chloride, and a much lower concentration of protein (1.2 versus 0.2 mOsm/L of water).[2] The amount and function of plasma proteins within the interstitial space are not inconsequential. A constant circulation of plasma proteins occurs between the vascular and interstitial spaces, with about half of the protein circulating every 24 hours in human beings. More than half of the plasma protein content of the body is contained within the interstitial space at any one time. Plasma proteins within the interstitial space are important in the transport of water-insoluble substances from the vascular space and in resistance to infection.[3]

Interstitial fluid is contained within the interstitium, the intercellular connective tissues that lie between the cellular elements of the vascular and cellular compartments of the body. The extracellular tissue of the interstitium, except in the case of bone, consists of a three-dimensional collagen fiber network embedded in a proteoglycan gel matrix.[4] Interstitial water exists as free water and as water within the proteoglycan gel. Normally, only a small proportion of interstitial fluid exists as free water, most of the water being contained in

the interstitial gel. However, in edematous states, the proportion of fluid as free water within the interstitium increases.[2]

The source of interstitial fluid is the intravascular space. The volume of interstitial fluid is determined by the functional relationships of three major anatomic structures: the capillary, the interstitial space, and the lymphatics.[5] Functionally, the volume of fluid that accumulates in the interstitium is determined by the rate of ingress of fluid from the vascular space, the compliance of the interstitium, and the rate at which fluid is evacuated from the interstitium. The net rate of ingress of fluid from capillaries into the interstitium is determined by a number of factors acting across the capillary membrane, the effects of which are related by Starling's equation:

$$J = Kf[(P_c - P_t) - \sigma(\pi_p - \pi_t)]$$

in which J equals the volume flow across the capillary wall; Kf equals the filtration coefficient of the capillary wall (volume flow per unit time per 100 g of tissue per unit pressure); P_c equals capillary hydrostatic pressure; P_t equals interstitial fluid hydrostatic pressure; σ equals the osmotic reflection coefficient; π_p equals the colloid osmotic (oncotic) pressure of the plasma; and π_t equals the colloid osmotic (oncotic) pressure of the interstitial fluid.[6] Although all these factors act in concert to determine the rate of net fluid efflux from the capillary, considering them individually is conceptually easier.

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3.3.1.1 FILTRATION (Kf) AND REFLECTION (σ) COEFFICIENTS

Together the filtration and reflection coefficients describe the properties of the capillary membrane that determine the ease with which water, protein, and other plasma constituents move from the vascular space to the interstitium. The filtration coefficient, which is the product of the hydraulic permeability and surface area of the capillary, is a measure of the ease with which water crosses the capillary membrane. The reflection coefficient is an indicator of the degree to which the capillary membrane resists the passage of a substance, such as protein. A reflection coefficient can be defined for each substance; a reflection coefficient of 0 indicates that the molecule crosses the membrane as readily as does water, whereas a value of 1 indicates that the membrane is impermeable to the substance. The reflection coefficient for a substance may vary with the anatomic site of the capillary[7,8]: Capillaries in the liver are permeable to albumin, whereas capillaries in muscle are much less permeable and cerebral capillaries are among the least permeable to albumin.

The movement of fluid and protein across the vascular membrane is assumed to be passive, with plasma water and protein exiting the vascular space through pores in the capillary membrane. However, the rate with which various plasma constituents cross the capillary membrane varies considerably depending on the constituent and the tissue. For example, muscle capillary pores are permeable to water molecules (reflection coefficient of 0) but much less permeable to albumin (reflection coefficient of approximately 0.9).[2] Movement of solutes across the endothelium is not understood fully, being complex, but is affected by the concentration of the solutes on either side of the membrane, solute charge and interaction with other solutes, and capillary pore configuration.[9]

Together the filtration and reflection coefficients partially determine the rate of fluid flux across the capillary wall, and the composition of the fluid. For a given hydrostatic and oncotic pressure difference, tissues with higher filtration coefficients (whether because of a larger capillary surface area or more porous capillaries) will have a greater fluid flux. Conversely, under the same circumstance, increases in the reflection coefficient of the capillary wall reduce fluid flux. The differential permeability of the capillary membrane to water and

protein has important consequences in the maintenance of the oncotic pressure difference between plasma and interstitial fluid.

3.3.1.2 HYDROSTATIC AND COLLOID OSMOTIC PRESSURES

Transcapillary fluid flow is results from an imbalance between the hydraulic forces favoring movement of water from the capillary into the interstitium and the forces favoring movement of water in the reverse direction. The forces contributing to fluid movement out of the capillary are the intracapillary hydrostatic pressure and the interstitial colloid osmotic pressure, whereas those forces favoring movement of fluid from the interstitium to the capillary are the interstitial hydrostatic pressure (if it is positive) and the plasma colloid osmotic pressure.[10]

The principal force favoring fluid efflux from the capillary is the hydrostatic pressure within the capillary. Capillary hydrostatic pressure varies among different tissues and decreases along the length of the capillary. Hydrostatic pressure within a capillary is determined by the arterial and venous pressures and by the precapillary and postcapillary resistances.[11] Specifically, capillary pressure is determined by the ratio of the postcapillary resistance (R_a) to the precapillary resistance (R_v), and the arterial (P_a) and venous (P_v) pressures:

$$P_c = \frac{(R_v/R_a)P_a + P_v}{1 + (R_v/R_a)}$$

Thus a small increase in venous pressure has a much greater effect on capillary pressure than does an increase in arterial pressure. For this reason the hydrostatic pressure is greater in capillaries below the heart (e.g., legs) than in those above the heart (e.g., head).

The colloid osmotic pressure of the plasma is the principal force minimizing fluid efflux from the capillary. The colloid osmotic pressure is generated because the plasma and interstitial fluid are separated by a semipermeable membrane—the endothelium—and vary slightly, but significantly, in composition. As noted previously, the interstitial fluid has a lower protein concentration than does plasma but has an essentially identical electrolyte concentration. The difference in protein concentration across the semipermeable endothelium generates an osmotic force that tends to draw water from the interstitium into the plasma.

In addition to the capillary hydrostatic pressure, the colloid osmotic pressure and negative hydrostatic pressure of the interstitial fluid favor fluid movement out of the capillary. Fluid flux across the capillary results from the summation of these forces (Table 3.3-1). These figures should be recognized as representing the forces at the midpoint of an idealized capillary and that the forces are dynamic, changing between tissues and even along the length of the capillary. In fact, a large net flux of fluid from the capillary occurs at its arteriolar end, 121
where capillary hydrostatic forces are greatest and plasma oncotic forces are least, and a net flux of fluid into 122
the capillary toward its venous end, where capillary hydrostatic forces are least and plasma oncotic pressure is greatest.

TABLE 3.3-1 Mean Forces (mm Hg) Influencing Fluid Movement Into or Out of the Capillary

HYDROSTATIC PRESSURES	
Mean capillary pressure	17.0
Interstitial pressure	–5.3
Total hydrostatic pressure favoring filtration	22.3
COLLOID ONCOTIC PRESSURES	
Plasma oncotic pressure	28.0
Interstitial oncotic pressure	6.0
Total oncotic pressure opposing filtration	22.0
TOTAL PRESSURE FAVORING FILTRATION	0.3

Data from Guyton AC: *Textbook of medical physiology,* ed 8, Philadelphia, 1986, WB Saunders.

The small imbalance in filtration forces results in a net efflux of fluid from the capillary into the interstitial tissue. This fluid does not accumulate in the interstitium; it is removed by the lymphatics.

3.3.1.3 LYMPHATICS

The lymphatics drain the interstitium of fluid and substances, notably proteins, that are not absorbed by the capillaries. The lymphatics represent the only means by which interstitial protein is returned to the circulation. Interstitial fluid, and with it protein, moves down a pressure gradient into lymphatic capillaries through clefts between the lymphatic endothelial cells. Lymphatic endothelial cells are supported, and the lymphatic capillaries maintained patent, by anchoring filaments that attach the endothelial cells to surrounding connective tissue. Lymphatic fluid progresses centripetally through progressively larger vessels before draining into the great veins of the chest. Lymphatic valves prevent the retrograde flow of fluid from the lymphatics. Lymph is propelled by factors extrinsic to the lymphatics, including muscle activity, active and passive motion, posture, respiration, and blood vessel pulsation. Exercise causes a significant increase in lymph flow, at least in part because of the increase in tissue pressure that is associated with muscle contraction, although passive motion also increases lymph flow. Standing results in significant diminution or cessation of lymph flow from, and the prompt accumulation of interstitial fluid in, the lower extremities of human beings. In addition to the extrinsic factors affecting lymph flow, coordinated contractions of lymphatic vessels contribute substantially to the centripetal flow of lymph.[12]

3.3.2 Mechanisms of Edema Formation

Simply stated, accumulation of excessive fluid in the interstitial spaces—edema—results from an imbalance of the rates of fluid filtration from the capillaries and drainage by the lymphatics. Perturbations of one or more of the forces that affect filtration across the capillary alter the rate at which fluid enters the interstitium. Increases in capillary hydrostatic pressure, decreases in plasma oncotic pressure, and increases in interstitial oncotic pressure all favor increased fluid filtration. Conversely, increased interstitial hydrostatic pressure and decreased interstitial oncotic pressure act to inhibit fluid filtration.

Box 3.3-1 lists the fundamental mechanisms of accumulation of excessive interstitial fluid. Increases in capillary hydrostatic pressure, which occur with venous obstruction or arteriolar dilation, such as that associated with inflammation, increase net fluid efflux. The edema that occurs with congestive heart failure likely has an increase in capillary hydrostatic pressure as one of its causes, although the mechanism is complex.[13] Posture also affects capillary hydrostatic pressure; capillaries below the level of the heart have higher hydrostatic pressures than do capillaries above the level of the heart.

A decrease in the oncotic gradient across the capillary endothelium, which occurs with a decreased plasma oncotic pressure or an increased interstitial oncotic pressure, results in an increase in efflux of fluid from the capillary. A decrease in plasma oncotic pressure decreases the oncotic gradient that favors movement of fluid into the capillary. Consequently, the capillary hydrostatic pressure, which favors filtration, predominates and fluid accumulates in the interstitium. Plasma oncotic pressure decreases when plasma protein concentration declines. Albumin is the plasma protein that exerts the preponderance of the oncotic force[8]; therefore clinically, edema often is associated with hypoalbuminemia. An increase in the permeability of the capillary membrane greatly increases fluid and protein transport into the interstitium and decreases the ability of the membrane to maintain a difference in oncotic pressure between the plasma and the interstitium.[5] Capillary permeability increases when the endothelium is damaged, such as by vasculitis or inflammatory reactions. 122 123

3.3.2.1 BOX 3.3-1 PATHOGENESIS OF EDEMA

3.3.2.1.1 Increased Capillary Hydrostatic Pressure

Venous obstruction

- Thrombophlebitis
  - Compression (mass, tourniquet)

Venous congestion

- Posture (dependent limbs)
- Congestive heart failure

Arteriolar dilation

- Inflammation
- Increased body water

3.3.2.1.2 Decreased Plasma Oncotic Pressure

Panhypoproteinemia

Hypoalbuminemia

3.3.2.1.3 Increased Interstitial Oncotic Pressure

Increased capillary permeability

3.3.2.1.4 Decreased Lymph Flow

Lymphatic obstruction

Lymphatic obstruction prevents the removal of interstitial fluid and protein. Filtration of fluid and passage of small amounts of protein into the interstitial space continues in the presence of lymphatic obstruction. The interstitial fluid is reabsorbed by the capillaries; however, the protein is not. Consequently, the protein content of the interstitial fluid gradually increases, with a resultant increase in interstitial oncotic pressure that favors filtration of fluid. The increased interstitial oncotic pressure causes fluid to accumulate in the interstitium, thus exacerbating the edema.[2]

Alterations in the magnitude of one or more of Starling's forces may be offset by compensatory changes in lymph flow and other of Starling's forces. In concert, Starling's forces and lymph flow act as “edema safety factors” to prevent the excess accumulation of interstitial fluid and development of frank edema. For example, lymph flow increases with the increased filtration associated with increased capillary hydrostatic pressure. Thus a larger volume of fluid enters and is removed from the interstitial space. The interstitial protein concentration decreases as increased fluid flow washes protein out of the interstitial space. Reduced interstitial space protein concentration increases the oncotic gradient, inhibiting fluid efflux from the capillary, and decreases the rate of movement of fluid from the capillary to the interstitial space.[6]

3.3.3 Diagnostic Approach to the Patient With Edema

Edema is not of itself a disease; rather it is a sign of a disease process. Therefore the diagnostic approach to the patient with edema is based on an understanding of the pathogenesis of edema and a knowledge of the diseases 123
likely to be involved (Box 3.3-2). The diagnostic approach to an animal with edema should not be any different 124
than for any other sign of disease. A clinical examination, including history and physical examination, permit the development of a list of potential diagnoses and dictate the appropriate subsequent steps in confirming the diagnosis. The reader is referred to those sections of the text that deal with specific diseases for a description of the appropriate diagnostic aids.

3.3.3.1 BOX 3.3-2 COMMON CAUSES OF PERIPHERAL OR VENTRAL EDEMA IN HORSES

3.3.3.1.1 Congestive Heart Failure

Valvular disease

Myocarditis

Monensin toxicosis

3.3.3.1.2
Vasculitis

Equine viral arteritis

Equine ehrlichiosis

Purpura hemorrhagica

Equine infectious anemia

3.3.3.1.3
Venous Obstruction and Congestion

Catheter-related thrombophlebitis

Disseminated intravascular coagulation

Tight bandages

Tumors

Immobility

3.3.3.1.4
Cellulitis

Staphylococcal

Clostridial

Counterirritant application

3.3.3.1.5
Lymphatic Obstruction

Ulcerative lymphangitis

Lymphadenitis *(Streptococcus equi, Corynebacterium pseudotuberculosis)*

Lymphosarcoma

Tumors

3.3.3.1.6
Hypoalbuminemia

Parasitism

Pleural and peritoneal effusions

Protein loss (gastrointestinal, renal, or wounds)

Inadequate production (starvation)

Hemodilution (subsequent to hemorrhage)

3.3.3.1.7 Shock

Hemorrhagic

Endotoxic

3.3.3.1.8 Pleuritis

Late-Term Pregnancy

Prepubic Tendon Rupture

Starvation

- Inadequate intake
- Malabsorption

When taking the history of a horse that has edema, one should focus on acquiring those facts that have the greatest diagnostic use in differentiating among those diseases that have edema as a sign. One should consider the following aspects:

- Housing, season, and geographic region
- Vaccine and parasiticide administration
- Exposure to other horses and diseases present within the herd
- The duration of the edema, its distribution, and the presence of any other clinical signs

One should investigate the remainder of the history depending on the responses to initial questions.

The physical examination should begin with a visual evaluation of the attitude and physical condition of the horse. The temperature, pulse, and respiration should be recorded. Although the physical examination should be complete, particular attention should be paid to those body systems that the preliminary examination indicates may be involved in the disease process. The physical examination reveals the distribution and severity of edema. Edema that is localized to one extremity or is not bilaterally symmetric is more likely to be caused by local factors (e.g., lymphangitis or venous obstruction) than by systemic disease. Conversely, edema that involves several areas of the body and has a symmetric distribution is likely to be associated with systemic disease (e.g., the ventral edema of congestive heart failure).

Following the initial clinical examination, the clinician will have developed an ordered list of potential diagnoses. Confirmation, or elimination, of these diagnoses depends on subsequent diagnostic procedures, including the response to therapy. Sections of this text deal with the specific disease processes for appropriate diagnostic procedures.

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3.4 3.4—Changes in Body Weight

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An unwelcome or unexpected change in the body weight of a horse is a commonly encountered problem in equine practice. Although obesity may be a more common problem, weight loss often represents a more serious situation, with potentially severe consequences. Normal or acceptable body weight is also in the eye of the beholder, because a horse with a given body weight might look overweight as an endurance horse, appropriate as a Thoroughbred racehorse, or too thin as a show hunter.

Whether dealing with a problem of weight loss or weight gain, the veterinarian always should investigate the
feeding practices of the horse. Not uncommonly the owner reports that the horse is receiving 3 lb of grain twice 124
daily when the actual measuring device (usually the everyday coffee can) differs in net grain weight once the 125
volume of the measuring device and grain density are taken into account. Observing firsthand the feeding practices of the stable may be necessary to document that the horse actually is getting the reported amount of grain 2 or 3 times daily. Hay should be examined for type, quality (color, texture, leafiness, and steminess), mold, weeds, and potentially toxic plants. The horse in question should be observed eating hay and grain to ensure that it really does consume the amounts the owner or feeder reports.

The veterinarian also should observe nursing foals when they suckle. The udder should be examined before and after nursing to ensure that the mare actually is producing sufficient milk and that the foal actually is nursing the mare completely until her udder is empty. The milk itself should be examined from both halves of the udder to see that it appears grossly normal (no evidence of mastitis). The nostrils of the foal should be examined after nursing to determine the presence of milk reflux caused by dysphagia, esophageal obstruction, or gastric reflux associated with gastrointestinal ulcers.

3.4.1 Decreased Body Weight

Losses in body weight are usually insidious and chronic but may be surprisingly rapid in the face of acute overwhelming systemic infections (Box 3.4-1). Causes have been classified variously as gastrointestinal, nutritional, infectious, or hypoproteinemic.[1,2] Differential mechanisms include decreased feed intake, decreased absorption of nutrients, decreased nutrient utilization, and increased loss of energy or protein leading to a catabolic "sink."[1–3]

Decreased feed intake may be caused by management factors, poor dentition, dysphagia, or esophageal obstruction. Management factors leading to weight loss may be multifactorial and include inadequate amounts of feed, inadequate quality of feed, or inability of the horse to eat the proper amounts of the feed given. A horse with severe lameness (e.g., chronic laminitis) may not be able to ambulate to the feed source. A horse low on the pecking order in a pasture hierarchy may be unable to eat because it cannot approach the feed without the other horses bullying it and fending it away. The feed must be palatable and digestible. Appropriate amounts and types of concentrates must be fed considering the work schedule or pregnancy status of the horse. Proper investigation of stable feeding practices is described earlier.

Poor dentition may cause the horse not to eat some or all of its grain or hay. Parrot-mouthed horses or aged horses with receding incisor teeth (more than 25 years old) may have difficulty in tearing off grass when grazing. A horse with one or more oral sores from a poorly fitting bit or from sharp cheek teeth may exhibit partial or complete inappetence because of pain associated with chewing. Sharp cheek teeth, wave mouth, or step mouth may lead to poor digestion and incomplete absorption of nutrients because of inadequate mastication of hay leading to poor fiber use during the hindgut (cecum) fermentation process.

3.4.1.1 BOX 3.4-1 MECHANISMS AND DIFFERENTIAL DIAGNOSES FOR DECREASED BODY WEIGHT

3.4.1.1.1 Decreased Dietary Intake

Inadequate diet

Lameness

Pecking order

Poor dentition

Dysphagia

Esophageal obstruction

3.4.1.1.2

Maldigestion and Malabsorption

Lactose intolerance

Gastrointestinal ulceration

Parasitism

Diarrhea

Inflammatory intestinal disease

- Granulomatous enterocolitis
- Eosinophilic enterocolitis
- Lymphocytic/plasmacytic enterocolitis

Gastrointestinal neoplasia

3.4.1.1.3

Inappropriate Hepatic Utilization

Inadequate circulation and respiration

- Heart failure
- Chronic obstructive pulmonary disease

3.4.1.1.4

Increased Rate of Protein and Energy Loss

Infection

- Pneumonia
- Pleuritis
- Peritonitis
- Equine infectious anemia

Protein-losing eneropathy

- Diarrhea

- Gastrointestinal ulceration
- Parasitism
- Inflammatory intestinal disease
- Gastrointestinal neoplasia

Renal disease (glomerular)

Increased metabolic energy use

- Chronic pain
- Secondary hyperadrenocorticism

Dysphagia has many causes, including abnormal prehension, chewing, or swallowing.[4] Abnormal prehension 125
can be caused by tongue lacerations; dental, mandibular, or maxillary fractures; damage to nerves supplying the 126
tongue or facial musculature (local trauma, equine protozoal myelitis, or polyneuritis equi); or central neurologic disease (equine protozoal myelitis). Basal ganglia lesions caused by poisoning by ingestion of yellow star thistle or Russian knapweed prevent normal prehension in the pharynx.[5] Swallowing abnormalities may be caused by neurologic (equine protozoal myelitis, viral encephalitis, or guttural pouch infection), muscular, or physical obstructions such as strangles, abscesses, or guttural pouch distention.[4] Muscular causes include hyperkalemic periodic paralysis in Quarter Horse foals, vitamin E or selenium deficiency in neonates, botulism in neonates and adults, and local trauma subsequent to laryngeal surgery (laryngoplasty).

Esophageal obstruction usually presents acutely because an apparently dysphagic horse regurgitates food from its nostrils while attempting to eat or drink. Chronic choke, or anorexia related to painful swallowing caused by partial esophageal obstruction may lead to weight loss without the owner realizing that the horse is not eating adequately. Esophageal endoscopy is usually diagnostic, but positive contrast radiography may be helpful and is sometimes necessary to establish an accurate diagnosis.

If the horse with weight loss has been observed fully to ingest adequate amounts of good-quality hay and grain, then decreased feed absorption must be considered the reason for weight loss. Maldigestion and malabsorption are not easily confirmed diagnoses, but tests based on luminal absorption of simple sugars (xylose or glucose tolerance tests) have been used to document malabsorption syndromes.[3,6,7] These tests are described in greater detail in Chapter 13.4. Malabsorption may be caused by parasitism, diarrhea, and inflammatory or neoplastic intestinal disease.

Gastrointestinal parasitism results in weight loss because of several mechanisms.[2] Parasites may compete directly for nutrients within the lumen of the bowel. Malabsorption may result from a lack of mucosal integrity, a decrease in intestinal villi size and number (and subsequent decrease in mucosal absorptive surface area), and a decrease in digestive enzymes that originate in the mucosa. Competition of parasites for protein sources may

result in decreased availability of amino acids for production of digestive enzymes or mucosal transport proteins. Increased mucosal permeability caused by leakiness in mucosal intercellular bridges may result in mucosal edema and increased transudation of intercellular fluid and its associated electrolytes, amino acids, and sugars into the lumen of the intestine.

Chronic diarrhea results in partial or complete anorexia, which contributes directly to weight loss. More rapid (decreased) gastrointestinal transit time results in increased losses of incompletely digested dietary feedstuffs. Malabsorption may result from decreased transit time and from villus blunting caused by specific pathogens, such as in viral diarrhea (see Chapter 13.4). Bacterial pathogens may compete directly for luminal nutrients. Mucosal invasion by viral and bacterial pathogens may cause mild to severe degrees of mucosal sloughing (ulcers), which result in maldigestion, malabsorption, and increased mucosal losses of intercellular fluid (e.g., in parasitism).

Given that the horse has adequate feed intake and absorption, inappropriate hepatic use of amino acids and sugars must be considered as a differential diagnosis for weight loss. Chronic liver disease may result in weight loss because of inappetance, maldigestion (caused by inadequate bile acid production), and inadequate or improper processing of amino acids into normal plasma proteins in the liver. These abnormalities may result in lowered concentrations of serum albumin, liver-dependent clotting factors (factors II, VII, IX, and X), and total plasma or serum protein. Lowered circulating proteins (especially albumin) may result in decreased plasma colloid osmotic pressure and thus may manifest as peripheral dependent edema in the distal limbs, pectoral region, and ventral midline. This peripheral edema may mask further weight loss by making the torso of the horse appear to be heavier than it actually is. Decreases in clotting factors may result in bleeding diatheses. Hyperlipemia, hyperlipidemia, fatty liver syndrome, and ketosis may be seen in poorly fed ponies and in miniature horses with acute anorexia or overwhelming energy demands, such as pregnancy or lactation.[8]

Increased loss of protein or energy is a common cause of decreased body weight in horses. Luminal losses of fluid, electrolytes, and nutrients were described earlier for intestinal parasitism and diarrhea. Acute inflammatory protein losses may occur into major body cavities in overwhelming infections such as pleuritis or peritonitis. Chronic abscessing pneumonia, pleuritis, and peritonitis often result in increased, rather than acutely decreased, serum total protein because of increased γ-globulin production in response to chronic antigenic stimulation from the chronic infection. These chronic infections also usually have weight loss as an additional clinical sign because of the continuing catabolic processes associated with the infection itself. Equine infectious anemia is a type of persistent systemic infection that in its symptomatic form may result in chronic weight loss and varying levels of anemia.[9] Asymptomatic equine infectious anemia carriers may have no weight loss or other obvious clinical signs but can infect pasture mates via vector transmission.

Protein-losing enteropathy is not a definitive diagnosis but rather is a group of diseases, each of which results in luminal losses of fluid, electrolytes, plasma proteins, and nutrients. Mechanisms of protein and fluid loss were described earlier for intestinal parasitism and diarrhea. Gastrointestinal ulcers have been reported to result in lowered serum total protein and weight loss.[10] One of the early indications of nonsteroidal antiinflammatory drug toxicity is detection of a lowered serum total protein. Horses with such a condition also may manifest varying degrees of inappetence and colic, especially associated with the immediate postprandial period. Intestinal neoplasms (usually lymphosarcoma) often manifest as a protein-losing enteropathy with weight loss.[11]

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Acute or chronic renal diseases, especially involving glomerulonephritis, can result in urinary protein loss and subsequent body weight loss.[12] Horses with this condition may have polyuria and polydipsia as associated clinical signs. Owners or handlers often report polyuria as increased wetness in stall bedding. The veterinarian should question owners thoroughly regarding the water intake of the horse. The veterinarian may need to observe

stable watering habits, often including actually measuring the volume of the water buckets to establish definitively the presence of polydipsia. Turning off automatic waterers in the stall or pasture and offering the horse measured volumes of water from additional buckets may be necessary to establish a diagnosis of polydipsia. Urine puddles in stalls or collected urine samples may foam excessively because of increased protein concentrations. Increased urinary protein concentrations can be diagnosed quickly on the farm with the proper interpretation of urine dipstick protein indicators.

Neoplasms or abscesses within the thorax or abdomen serve as catabolic energy and protein sinks, resulting in chronic weight loss.[11–13] Chronic pain, such as that associated with severe, unresponsive laminitis, results in increased catabolism and weight loss, probably because of chronically elevated systemic catecholamine levels. Increased circulating epinephrine and norepinephrine levels result in a whole-body catabolic state with increased breakdown of stored energy sources and ultimately result in chronic weight loss. Similar weight loss caused by systemic catabolism can result from chronically elevated serum cortisol associated with pituitary adenoma and secondary hyperadrenocorticism.

Heart murmurs and resultant heart failure can cause weight loss because of inefficiency of circulation of nutrients and oxygen to peripheral tissues. Chronic obstructive pulmonary disease or heaves may result in weight loss because of an increase in the work of breathing and poor oxygenation of peripheral tissues. Although ventral abdominal musculature may hypertrophy and result in a heave line, weight loss is manifested by increased depth between the ribs and decreased muscular thickness and definition along the dorsal midline. Suckling foals with severe pneumonia may manifest weight loss if they become inappetent because of decreased suckling related to their severe dyspnea.

An appropriately taken history should document the type, amount, and quality of feed and hay being provided daily. Documentation of deworming products used and intervals of administration is critical. The history also may document the presence of anorexia, depression, polyuria, polydipsia, diarrhea, or other important historical signs that may point more quickly toward a specific cause of the weight loss.

The physical examination should reveal the presence of weight loss, a cardiac murmur, pneumonia or pleuropneumonia (increased lung sounds), chronic obstructive pulmonary disease (increased abnormal expiratory lung sounds), dental abnormalities, peripheral edema, urine staining on the hindlimbs, diarrhea, icterus, nasal discharge (dysphagia, pneumonia), fever, or hirsutism (secondary hyperadrenocorticism). The rectal examination may document the presence of intraabdominal masses (abscesses or neoplasms), enlarged left kidney, thickened intestinal or rectal wall, colonic displacement, gritty peritoneal surfaces (peritonitis), gritty feces (sand impaction), or diarrhea.

Fecal flotation may serve as an adequate screening tool to determine whether any evidence of parasitism exists. In the event of a positive fecal flotation, Baermann sedimentation may be necessary to determine quantitatively the severity of the patent parasitic load in the horse with weight loss. Fecal occult blood may be positive with gastrointestinal ulceration or neoplasms, but parasites or a recent rectal examination also may result in positive results.

Routine hematologic testing (complete blood count and fibrinogen) should assist in diagnosing infectious conditions such as pleuritis or peritonitis. Decreased serum or plasma total protein and albumin concentrations are evidence of hypoproteinemia and make the following conditions more likely: severe malnutrition, protein-losing enteropathy (diarrhea, parasitism, ulceration, intestinal neoplasms, or inflammatory intestinal disease), glomerular disease, acute pleuritis or peritonitis, or chronic liver disease. Increased total protein concentrations, especially γ-globulins, make chronic closed-cavity infections such as abscesses, peritonitis, or pleuritis more likely. Increased β-globulin fractions suggest the presence of parasitism.

Routine serum biochemistries should aid in diagnosing renal (renal azotemia, electrolyte abnormalities) and liver disease (increased γ-glutamyltransferase, aspartate aminotransferase, serum alkaline phosphatase, and lactate dehydrogenase). Urinalysis should reveal increased protein levels on dipstick or quantitative analysis in the event of glomerular protein losses. Metabolic alkalosis may be evident in the aftermath of salivary bicarbonate losses caused by dysphagia or esophageal obstruction. 127

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Endoscopy may aid in diagnosing causes of dysphagia or esophageal obstruction. Lengthy endoscopes are necessary for examination of large adult horses for suspected gastrointestinal ulcers, but shorter endoscopes may suffice for foals or shorter-necked adults (e.g., Arabians and ponies).

Peritoneal fluid analysis documents the presence of a transudate (equivocal infection) or exudate (probable infection).[14,15] Aerobic and anaerobic peritoneal fluid cultures should be performed if intraabdominal infection is suspected. Exfoliative cytologic examination rarely may document the presence of neoplastic cells from intraabdominal neoplasms.[11–16]

Nonroutine tests should be performed only as indicated and should include oral absorption tests (see Chapter 13.4) and biopsies of the liver, kidney, or intestinal wall. Abdominal or thoracic ultrasonography should help to rule out abnormalities of the liver or kidneys and may document the presence of abnormal fluid (peritonitis or pleuritis) or masses (abscesses or neoplasms). Cardiac ultrasound should be definitive in the event of a murmur and suspected heart failure. Radiography also may be helpful to document the presence of thoracic masses or chronic obstructive pulmonary disease, but increased pleural fluid obscures visualization of other intrathoracic structures.

3.4.2 Increased Body Weight

Overfeeding may be the most common cause of obesity in horses and also may be the easiest to correct. The veterinarian should investigate the feeding practices of the stable and feed and hay sources thoroughly. Novice horse owners, single horse owners, and pony owners commonly overfeed their animals.

Ponies seem to be particularly susceptible to obesity, perhaps because their size renders them more easily overfed. However, at least one author has proposed that this tendency toward obesity in ponies receiving modern confinement diets may be because of their having evolved in the inhospitable ice age climates of northern Europe.[17] In that era, the lack of readily available grazing feedstuffs might have placed greater selection pressure on survival of ponies with more efficient dentition and better nutrient and fluid absorption from the gastrointestinal tract. The author argues that those ponies that had greater feed conversion efficiency would have been stronger, had longer lives, and been more available for breeding. Current illustrations of this theory may lie in the Welsh and Connemara pony breeds that still thrive and flourish in the wild in the inhospitable north Atlantic climates of the western coasts of Wales and Ireland, respectively.

Pregnancy in mares is a normal physiologic event that leads to increased body weight. Surprisingly, many new owners of mares may not know that their new purchase is pregnant. For an earlier negative pregnancy diagnosis to have been in error is not uncommon. Any mare that is gaining weight in an unexpected manner should be examined rectally, and by ultrasonography if necessary, for a possible pregnancy.

Hypothyroidism has been reported to be associated with weight gain and failure to become pregnant in broodmares.[18] Evidence for hypothyroid-associated weight gain and infertility was lacking in surgically created hypothyroid pony[19] and Quarter Horse[20] subjects. An abundance of field experience exists, however, from

which to infer a relationship between obesity, hypothyroidism, and infertility in mares.[17] Documentation of hypothyroidism must be by performance of a thyroid-stimulating hormone or thyroid-releasing hormone stimulation test,[21,22] because resting thyroid levels vary diurnally[23] and do not truly reflect thyroid function. One must also remember that only 5 days of normal phenylbutazone therapy results in abnormally low resting serum thyroid levels because of direct competition of phenylbutazone with thyroid hormone for serum protein-binding sites.[21] The diagnosis and treatment of hypothyroidism is described in greater detail elsewhere in this text.

Differential diagnoses for increased body weight include overfeeding, pregnancy, hypothyroidism, and other conditions that result in abdominal distention, such as bloat, ascites, uroperitoneum, fetal hydrops, and rupture of the prepubic tendon or abdominal wall musculature. The latter conditions are described in greater detail in Chapter 16.

Feeding practices should be investigated and observed firsthand if necessary. A positive pregnancy status should be an easy historical and rectal diagnosis. Most hematologic and biochemical tests are normal in the pregnant or simply overweight horse. Thyroid status should be assessed appropriately, not by simple resting thyroid hormone concentrations, but by thyroid-stimulating hormone or thyroid-releasing hormone stimulation tests that have been described previously and that are presented elsewhere in this text.[21,22]

Education of the client is important regarding feeding practices, especially if the overweight horse is determined simply to have been overfed by a novice owner. Dangerous consequences, including colic and laminitis, should be explained to the client.

3.4.3

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3.5

3.5—Abdominal Distention

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Increases in body weight because of overeating or pregnancy must be distinguished from increases in body girth caused by bloat, ascites, uroperitoneum, fetal hydrops, or ruptured prepubic tendon. In each of these conditions, body weight actually may increase because of fetal growth or fluid accumulation. More important, however, a perceptible change in the shape of the abdomen of the horse occurs.

Bloat usually is associated with colic signs in horses and is caused by gaseous intestinal distention resulting from ileus or simple obstruction of the large, or rarely small, intestine. Ileus caused by diarrhea, peritonitis, colic surgery, or parasympatholytic agents (e.g., atropine) can result in sufficient accumulation of intraluminal gas to be manifested as tympany, bloat, and mild to severe abdominal pain.[1] If optic topical atropine application is overly aggressive, secondary ileus and bloat may result. Rapid and severe gas production may follow grain overload; cecal and colonic fermentation of readily available carbohydrate sources results in rapid-onset colonic tympany and abdominal distention.[2] Exhaustion in endurance horses also is associated with intestinal shutdown and subsequent abdominal distention.[3] In any of these bloat conditions, abdominal auscultation in the flank area reveals decreased

or absent intestinal motility sounds (borborygmi) and perhaps increased gaseous distention sounds (pinging). Decreased borborygmi in the right flank are specific for cecal ileus.

Simple colonic obstruction also results in tympany and bloat. Strangulating obstruction results in greater pain than usually is manifested in simple obstruction and bloat. Colonic displacements are more common in older postpartum mares.[4] These horses often initially show mild colic signs and progressively develop more dramatic pain and abdominal distention. Miniature horses with simple obstructions caused by fecoliths often have bloat as the initial clinical sign.[5] Such cases have the additional complication that rectal examination may be impossible for differentiation of the source of the bloat. Even in full-sized horses, rectal examination may reveal that the abdomen is so filled with distended colon that the examiner can push an arm into the rectum no farther than wrist-deep. Colonic or cecal bloat can be relieved by trocarization through the flank, but relief is merely palliative and is usually temporary because the cause of the obstruction still has not been resolved.

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Ascites does not occur commonly in horses and usually is caused by peritonitis or abdominal neoplasms. Peritonitis is caused by septicemia, laparotomy, intestinal leakage, internal abscess, or a penetrating external wound resulting in inflammation and usually infection of the peritoneal lining of the abdomen. Such inflammation results in increased fluid production by the squamous abdominal epithelium. Initially, this increased abdominal fluid may be characterized as a transudate (low cell count and low total protein). If inflammation with infection persists, the character of the fluid may change to that of an exudate (increased cell count >5000 nucleated cells/μl, increased neutrophil count, increased degenerate neutrophils, microscopically visible bacteria, and increased total protein).[6,7] These increases in abdominal fluid volume can be substantive and can result in abdominal distention that eventually becomes clinically apparent. Fluid ballottement in the equine abdomen is not an easily performed diagnostic technique but may be easier in foals, ponies, or miniature horses than in full-sized horses.

Ascites also may result from abdominal neoplasms. Tumors reported to cause ascites and weight loss in horses include lymphosarcoma, squamous cell carcinoma, mammary adenocarcinoma, and mesothelioma.[8,9] Although rare, mesothelioma may cause the most fluid production, because it is a tumor of the fluid-producing cells of the peritoneal lining. Mesothelioma may result in the production of large volumes of fluid (several liters) in a short time (24 hours) after a similarly large volume is drained from the same horse via abdominal catheterization or trocarization.

Ascites also may result from any condition that produces lowered serum total protein and albumin. With lowered intravascular colloid osmotic pressure, fluid diffuses or moves from the vasculature and results in dependent peripheral edema. Fluid also may accumulate within the major body cavities (i.e., the thorax and the abdomen).[7] The mechanisms for such low-protein conditions include poor protein intake, malabsorption, poor hepatic utilization, and increased rate of protein loss such as in glomerular renal disease, peritonitis/pleuritis, or gastrointestinal transudation (diarrhea or ulceration). Causes of peripheral edema are described elsewhere in this text.

Increased preload because of right ventricular heart failure also can result in a transudate fluid accumulation within the abdomen.[7] A horse with right ventricular heart failure usually has tricuspid insufficiency and manifests other signs of right ventricular heart failure, such as a murmur, exercise intolerance, jugular pulse, and edema of the ventral abdomen, pectoral muscles, and distal limbs. Severe mitral insufficiency also can result in right ventricular heart failure, but only after the development of left ventricular heart failure and its associated pulmonary edema, which is manifested by exercise intolerance, coughing, epistaxis, and increased respiratory effort.

Uroperitoneum results from leakage of urine from some part of the urinary tract into the abdomen and most commonly is associated with a ruptured bladder in neonatal foals (usually male). Uroperitoneum also may result

from a necrotic bladder caused by neonatal sepsis and urachal abscesses. Such foals often have pendulous, bloated abdomens that ballotte more easily than do the abdomens of adult horses with accumulation of fluid. Abdominal fluid actually may smell like urine, and peritoneal fluid creatinine concentrations will be high—often more than twice those of peripheral blood.[10–12] Because most classically described neonatal urinary bladder tears are dorsal near the trigone, the foal still may be able to produce a stream of urine despite having a leaking bladder. A ruptured urinary bladder abscess should be suspected in a foal with sepsis that initially responds to therapy for sepsis and then, several days later, has acute-onset depression, anorexia, ileus, and abdominal distention. Adults horses rarely have uroperitoneum; however, uroperitoneum has been associated with ruptured urinary bladders during stressful parturition in mares that manifest mild postpartum abdominal pain and abdominal distention.[12,13]

Fetal hydrops results from an accumulation of excessive amounts of fluid within the amnion (hydrops amnion) or chorioallantois (hydrops allantois).[14] Hydrops results in a bilaterally pendulous abdomen in a late-term pregnant mare. A rapid accumulation of fluid over 10 to 14 days may makes walking or perhaps even breathing difficult for the mare. A diagnosis may be made after taking history and performing a rectal examination, although palpating the fetus is usually difficult because the excess fluid causes the uterus to descend out of reach of the examiner. If necessary, a percutaneous ultrasonographic examination may be used to confirm the diagnosis by documenting the presence of increased intrauterine fluid within the fetal membranes.

A ruptured prepubic tendon results in a unilateral lowering of the abdominal margin and apparent distention of the abdomen only on the affected side. The condition is associated routinely with later-term pregnancy in mares and is thought to occur simply because of the increased weight of the pregnant uterus pressing downward on the abdominal wall. Rupture of the rectus, transverse, or oblique abdominal muscles also can result in ventral dropping or herniation of the abdomen late in gestation.[14] Ruptures may be more common in older or more sedentary mares, probably because of decreased abdominal wall strength and tone. Other than a focal abdominal wall hernia, a unilateral prepubic tendon rupture results in the only form of prominent unilateral abdominal distention in horses. Mares with ruptured prepubic tendons may have elicitable pain in the local abdominal wall and may demonstrate a 130
reluctance to walk. They may need assistance during parturition, because they may have difficulty performing an 131
effective abdominal press to aid in fetal expulsion.

Pregnancy, diarrhea, colic signs, colic surgery, and the use of parasympatholytic agents should be evident from the history. The rate of onset of abdominal distention may help to distinguish more acute conditions (e.g., gastrointestinal bloat from grain overload) from more chronic conditions (e.g., ascites caused by heart or liver failure). Signalment and history may assist in indicating specific conditions. A depressed, 48- to 72-hour-old male foal with fluid abdominal distention may be a likely candidate to have a ruptured urinary bladder and uroperitoneum. Miniature horses with bloat and colic signs frequently have simple obstructions owing to fecoliths or enteroliths.

A complete physical examination reveals the presence of a murmur that may be associated with heart failure and ascites. Other signs of heart failure also may be evident on physical examination. An actual defect in the integrity of the abdominal wall may be palpable on external examination of the abdomen in a mare with a ruptured prepubic tendon or ruptured abdominal wall musculature.[14] The veterinarian should attempt ballottement to discern the presence of increased free abdominal fluid in suspected ascites or uroperitoneum. Fever may indicate the presence of an infectious peritonitis or umbilical abscess.

A rectal examination is a critical part of examining a horse with bloat or colic but may be difficult to accomplish if colonic distention is dramatic or if the patient is small (i.e., a foal, pony, or miniature horse). A rectal examination further may document advanced pregnancy, resulting in mild bilateral abdominal distention (normal pregnancy), abnormal or severe bilateral distention (hydrops or bilateral ruptured prepubic tendon), or unilateral distention

(unilateral ruptured prepubic tendon or focal abdominal wall hernia). A rectal examination also may reveal abnormalities of the urinary tract (enlarged kidney or ureter, abscess, or neoplasm), which may result in uroperitoneum in adults.

An ultrasonographic examination may be helpful and is sometimes necessary to examine the distended abdomen and fetus in a pregnant mare. Such an examination must be performed percutaneously in late gestation. Ultrasonography can determine the location of increased abdominal fluid (intrauterine or extrauterine) and the health status of the fetus. Percutaneous placement of base-apex electrocardiographic leads across the abdomen of the mare may help to document that the fetus is still viable if an ultrasound examination does not produce definitive evidence (heart movement or gross fetal movement).[15]

Cardiac ultrasonography may help to document the presence of a cardiac valvular defect that can be the cause of ascites in a horse with heart failure. Abdominal radiography may assist in the diagnosis of abdominal distention caused by intestinal obstruction in a foal or miniature horse. Percutaneous ultrasound examination also may assist in documenting the source of abdominal distention (e.g., intussusception) in smaller horses or foals[16,17] and in characterizing umbilical and urachal abnormalities.[17]

Complete blood counts and plasma fibrinogen concentrations assist in diagnosing inflammatory conditions such as infectious peritonitis. Urachal or urinary bladder abscesses also may be associated with inflammatory leukograms. Blood or peritoneal fluid cultures may assist in documenting the offending bacterial agent(s). Foals or adults with uroperitoneum have elevated serum urea nitrogen, creatinine, and potassium and decreased serum sodium, chloride, and bicarbonate concentrations.[10,11]

Abdominocentesis should be attempted to distinguish the cause of ascites. Care must be taken, however, in obtaining peritoneal fluid from late-term pregnant mares to avoid penetrating directly into the distended uterus. Analysis of peritoneal fluid reveals abdominal fluid to be a transudate (equivocal infection) or exudate (probable infection).[6,7] Fluid should be cultured aerobically and anaerobically when infectious peritonitis is suspected. Exfoliative cytologic examination rarely may document the presence of neoplastic cells.[8,9] Peritoneal fluid creatinine concentration approaches or often exceeds (more than twice) that of serum if uroperitoneum is present.[10,11] Serum and peritoneal urea nitrogen concentrations are less reliable for such a diagnosis because the peritoneal membrane does not differentially sequester urea nitrogen (but does creatinine) within the abdominal cavity.

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3.6

3.6—Dysphagia

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3.6.1

Normal Eating

Normal eating is complex and requires normal anatomic structures and neurologic function. The process of eating can be divided into prehension (uptake of food into the oral cavity) and deglutition (transport of food from the oral cavity to the stomach). Prehension requires the lips to grasp and the incisors to tear the food.[1] Motor innervation to the tongue, lips, and muscles of mastication is provided by the hypoglossal, facial, and trigeminal nerves. Sensory input is important for successful prehension and requires intact olfactory, optic, and trigeminal nerves, providing smell, sight, and sensation of the rostral oral mucosa and lips. Normal prehension depends on the central nervous system to coordinate movements of the tongue and lips.

Deglutition involves mastication, swallowing, and transport of food through the esophagus to the stomach. Mastication or chewing of food initiates mechanical digestion and insalivation. Mastication is specifically a function of the molars to grind feed and the tongue and buccal muscles to position the food. The facial nerve provides motor and sensory fibers to the tongue and pharynx. The glossopharyngeal nerve provides sensory fibers to the caudal third of the tongue. The trigeminal nerve is sensory to the teeth and provides the important parasympathetic fibers to the parotid salivary gland. Function of this gland is critical to help liquefy food and provides a small amount of digestive enzymes.

Swallowing is complex and is performed in a series of steps. Initially, food must be moved to the base of the tongue and formed into a bolus. This action requires coordinated movements of the tongue and pharynx. Second, the bolus is forced caudally. As this action takes place, the oropharynx relaxes and the soft palate elevates to seal the palatopharyngeal arch and nasopharynx.[1] Next, the bolus enters the oropharynx and the hyoid apparatus swings rostrodorsally, which draws the larynx and the common pharynx forward.[1,2] The epiglottis is tipped caudally and prevents the bolus from entering the larynx. Finally, the bolus is moved into the common pharynx with pharyngeal muscle contractions and enters the open cranial esophageal sphincter. The sphincter closes to prevent esophagopharyngeal reflux and aerophagia. Herbivores are unique, because breathing continues uninterrupted during swallowing, unlike other animals.[1] The glossopharyngeal, vagus, and spinal accessory nerves provide sensory and motor fibers to the pharynx, larynx, and soft palate.

The esophageal phase of eating involves the transport of the food bolus to the stomach, with primary peristaltic waves, which are generated by continuous contraction of the pharyngeal peristalsis. The bolus is transported to the caudal esophageal sphincter, which relaxes to allow the bolus to enter the stomach and then contracts to prevent gastroesophageal reflux. If reflux does occur, esophageal clearance is achieved by secondary peristaltic waves. Antiperistalsis is normal in ruminants during eructation and regurgitation but is not normal in horses.[1]

3.6.2 Dysphagia

Dysphagia is defined as difficulty in swallowing but often is used to describe problems with eating.[2] Problems with eating may include problems with prehension, mastication, swallowing, and esophageal transport. In this section, the term *dysphagia* is used in the broader sense to describe problems with eating. Dysphagia can result from a number of disorders affecting any part of the upper gastrointestinal system (oral cavity, pharynx, and esophagus). Clinical signs of dysphagia vary depending on the cause and the location of the problem but may include ptyalism (excessive salivation), gagging, dropping food, nasal discharge, and coughing. Dysphagia can result from morphologic or functional disorders. The causes of these diseases may be acquired or congenital. Morphologic causes of dysphagia include abnormal anatomy, obstruction of the upper gastrointestinal tract, inflammation, and pain. Examples of anatomic abnormalities include a cleft palate and subepiglottic cysts.[3,4] Obstruction of the upper gastrointestinal tract most commonly includes feed impactions of the esophagus but also can include pharyngeal obstructions secondary to retropharyngeal lymph node masses or severe guttural pouch tympany.[5–10] Inflammatory conditions resulting in pain and dysphagia include periodontal diseases, foreign bodies, pharyngitis, epiglottitis, and mandibular or maxillary fractures.[3,6]

Functional disorders resulting in dysphagia include neurologic, neuromuscular, and muscular diseases. Functional disorders frequently result in problems with swallowing but less commonly involve mastication and prehension and rarely occur with esophageal transport. Neurologic diseases resulting in dysphagia may be peripheral or central. Peripheral neurologic problems frequently result from abnormalities of the guttural pouch but also can include toxic peripheral neuropathies, such as lead toxicity. Problems of the guttural pouch include infection (tympany, empyema, or mycosis), iatrogenic problems (infusion of caustic substances), and trauma (rupture of the longus capitis muscle from the basisphenoid bone and hemorrhage into the guttural pouch).[2,11,12] Central neurologic diseases may result in problems in prehension, mastication, or swallowing. Specific examples include equine protozoal myelitis, viral encephalitis (rabies and eastern and western encephalitis), toxic neuropathies (leukoencephalomalacia and nigropallidal encephalomalacia), and cerebral trauma.[1,2,7,13–15] Neuromuscular problems resulting in dysphagia generally present as a systemic disease and include diseases

such as botulism and organophosphate toxicity.[1,2,16,17] Muscular diseases resulting in dysphagia are rare but include nutritional muscular dystrophy (white muscle disease) in foals.[18]

3.6.2.1 BASIC APPROACH TO DYSPHAGIA

The initial evaluation of dysphagia focuses on determining whether morphologic or functional abnormalities exist. To answer these questions best, a thorough history, physical examination (including observation of the horse eating), and additional tests (e.g., endoscopic examination and radiographs) are required. A history of an acute onset of dysphagia is often consistent with trauma, whereas a slow progressive onset of clinical signs is more consistent with a neurologic problem such as guttural pouch mycosis, equine protozoal myelitis, or toxicities. The clinician should assess exposure of the horse to toxic substances or plants (lead or yellow star thistle). A history of treatment before the onset of dysphagia suggests trauma or injury to the pharynx. Use of a balling gun or flushing of guttural pouches may result in iatrogenic injury to the pharynx, esophagus, and guttural pouches. The clinician should determine concurrent problems in other horses (e.g., strangles or other bacterial infections of the submandibular lymph nodes).

In performing the physical examination, the clinician should pay close attention to the head and neck. Because rabies is a potential cause of dysphagia, protective measures while performing a careful and thorough physical examination are necessary. Ideally, all clinicians working on horses should have an adequate rabies antibody titer. An examination of the oral cavity is best accomplished with a mouth speculum, good light, and if necessary, the administration of sedation. The teeth should be examined carefully for retained deciduous caps, sharp points or hooks, wave mouth or step mouth, dental fractures, or patent infundibula.[3] Foreign bodies may become wedged between the molars or under the tongue. The tongue should be examined for lacerations, foreign bodies, and evidence of neoplasia. The throat latch area and neck should be examined for heat or swelling, which might be caused by a ruptured esophagus. The lungs should be auscultated carefully to determine if the horse shows evidence of aspiration pneumonia resulting from dysphagia.

A valuable activity is to watch the horse eat. The distinction between dysphagia and anorexia is important. Dysphagic horses usually are hungry and will attempt to eat. Problems with prehension generally suggest a primary neurologic problem. Watching the horse try to graze and eat hay or grain may be necessary. Ingestion of yellow star thistle or Russian knapweed results in basal ganglia lesions (nigropallidal encephalomalacia). Horses with these lesions are unable to prehend food (with lack of coordination of the lips and tongue), but they can swallow.[14] Their ability to drink water should be evaluated carefully, because some horses continue to drink despite having difficulty in swallowing. Horses that expel food while chewing may have problems with mastication. Coughing and nasal discharge indicate aspiration of food into the trachea. Problems with swallowing or regurgitation may cause aspiration. Esophageal obstruction results in regurgitation of food through the nares. Regurgitation often is observed during feeding but may occur shortly after or even hours after feeding. Ptyalism, without dysphagia, may result from ingestion of legume plants (especially second-cutting red clover) contaminated with *Rhizoctonia leguminicola*. This fungus produces a mycotoxin called slaframine, which has parasympathomimetic properties.[19] The excess salivation disappears once the animal stops feeding on the plant. 133

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3.6.2.2 MORPHOLOGIC ABNORMALITIES

Morphologic abnormalities that cause dysphagia are easier to diagnose than are functional disorders. Morphologic problems of the oral cavity generally result in problems of prehension or mastication. An oral

examination (as outlined earlier) is particularly useful. The passing of a nasogastric tube, endoscopic examination, and radiographs (if necessary) are other diagnostic tests that may help to identify the anatomic localization and cause of dysphagia. Complete obstruction of the esophagus can be excluded if a nasogastric tube is passed successfully into the stomach. Feed impactions of the esophagus are common in horses. Esophageal impactions of feed may occur because of poor mastication or esophageal strictures or diverticulum.[5–7] The most common sites for obstructions occur in the cranial esophagus, at the thoracic inlet, and at the base of the heart.[7] Other esophageal abnormalities include rupture, fistula, cyst, megaesophagus, and neoplasms.[5,20] An endoscopic examination allows visualization of the nasal passageways, nasopharynx, guttural pouches, pharynx, larynx, and esophagus. Inflammation of the pharynx, larynx, or esophagus is assessed best by endoscopic examination. Partial obstructions of the pharynx often result in dyspnea, especially during exercise, and sometimes can cause dysphagia. Retropharyngeal masses, guttural pouch tympany, and rarely neoplasms may result in pharyngeal obstruction and collapse.[2,9,10] Depending on the length of the endoscope available, the clinician can evaluate all or part of the esophagus for inflammation or obstruction.

Radiographs can provide additional information in horses with morphologic causes of dysphagia; however, they are not required in all situations. Radiographs of the skull can help demonstrate the presence of periodontal disease, fractures of the mandible or maxilla, lesions of the temporomandibular joint, or radioopaque foreign bodies.[3] Radiographs of the larynx or pharynx are indicated in cases of pharyngeal obstruction and are especially useful to evaluate retropharyngeal masses, neoplasms, or trauma.[8–10] Radiographs of esophageal perforations reveal subcutaneous air, which shows up as extraluminal radiolucencies.[6] Contrast studies of the esophagus, with the use of barium sulfate, may help differentiate cases of esophageal strictures, dilation, or diverticulum.[5] Radiographs of the thorax are indicated in horses with nasal discharge and abnormal thoracic auscultation because of the concerns of aspiration pneumonia.

3.6.2.3

FUNCTIONAL ABNORMALITIES

Functional disorders that cause dysphagia are more difficult to diagnose and should be pursued after morphologic causes are not identified. The clinician also should consider a functional abnormality if the initial physical examination provides strong evidence of a neurologic, neuromuscular, or muscular problem. The initial step to evaluate functional causes of dysphagia is to perform a neurologic examination. The neurologic examination helps establish a neuroanatomic localization by (1) assessing brain, brainstem, and spinal cord functions; (2) determining if the problem is focal, multifocal, or diffuse; and (3) determining if the problem is a peripheral or central problem.

Cerebral disease usually manifests as seizures, head pressing, wandering, depression, and changes in mentation. Brainstem function can be assessed by cranial nerve examination. Evaluation of an abnormal response of the cranial nerves should establish the location of the problem within the brainstem. For example, the optic nerve can be assessed by the menace response (requiring the facial nerve) and by the pupillary light reflex (requiring the oculomotor nerve). Abnormalities of the oculomotor, trochlear, and abducens nerves manifest as strabismus or lack of a pupillary light reflex. Facial nerve paralysis (ear, eyelid, and muzzle droop) and vestibular disease (circling, nystagmus, and head tilt) often occur together because of the close proximity of these nerves as they exit the brainstem.[21] Endoscopic examination is a valuable tool to determine if pharyngeal or laryngeal paralysis is present. These problems may be caused by peripheral or central diseases. The dorsolateral wall of the medial compartment of the guttural pouch contains a plexus of nerves, including the glossopharyngeal nerve; branches of the vagus, spinal accessory, and hypoglossal nerves; and the cranial

cervical ganglion. Mycotic plaques, empyema, and trauma (hematoma) of the guttural pouch can result in pharyngeal paralysis, dorsal displacement of the soft palate, laryngeal hemiplegia, and occasionally Horner's syndrome.[1,2,11,12,21] The clinician should obtain skull radiographs in many horses with dysphagia, and they are especially helpful when traumatic injuries are suspected. Rupture of the longus capitis muscle results in ventral deviation of the dorsal pharynx and narrowing of the nasopharynx. Bony fragments may be evident ventral to the basisphenoid bones in these horses.[12] Otitis media and pathologic fracture of the petrous temporal bone frequently result in vestibular disease and facial nerve paralysis and occasionally in glossopharyngeal and vagus nerve involvement. An endoscopic examination of the guttural pouches is helpful with this problem, because the distal stylohyoid bone is thickened and irregular. Ventrodorsal, lateral, and rostrolateral oblique radiographs also may reveal osseous changes of the stylohyoid bone, tympanic bulla, or petrous temporal bone.[21]

The clinical examination should include an evaluation of gait. Signs of ataxia, generalized weakness, and hypermetria along with cranial nerve signs may be observable with brainstem involvement. The clinician 134
should evaluate the horse at the walk, trot, down an incline, over a step, and backing and turning in tight 135
circles. The clinician may wish to place the feet of the horse in abnormal positions and determine if the horse can reposition the leg correctly in a reasonable time. Generalized weakness (without ataxia) may manifest with a decrease in tail, eyelid, and tongue tone and muscle fasciculations. Weakness generally suggests a neuromuscular (botulism, organophosphate poisoning) or muscular problem.[16–18] Equine lower motor neuron disease results in generalized weakness and weight loss; however, horses are not dysphagic and do not exhibit cranial nerve abnormalities.[22] Ataxia or hypermetria along with dysphagia suggests a diffuse or multifocal disease that affects the spinal cord and brainstem. Examples of such diseases include equine protozoal myelitis, rabies, equine herpes myeloencephalopathy, polyneuritis equi, and a migrating parasite.[12,23,24] Further diagnostic tests are indicated in these cases, such as an evaluation of spinal fluid for cytologic abnormalities and chemistry and Western blot analysis for antibodies to *Sarcocystis neurona*.[25] Grass sickness, a disease found in Great Britain and in other northern European countries, results in ileus and colic. Grass sickness can result in dysphagia, with problems in swallowing or esophageal transport.[26] Grass sickness is regarded as a fatal disease, resulting in ileus of the gastrointestinal tract, dysphagia, and weight loss, which most likely is caused by an unidentified neurotoxin. Grass sickness can be defined as a dysautonomia characterized by pathologic lesions in autonomic ganglia, enteric plexi, and specific nuclei in the central nervous system.[27] Additional information about the specific causes of dysphagia are covered elsewhere in this text.

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3.7 3.7—Respiratory Distress

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Respiratory distress is defined as labored breathing and is characterized by an inappropriate degree of effort to breathe based on rate, rhythm, and subjective evaluation of respiratory effort.[1] Dyspnea is the sensation of arduous, uncomfortable, or difficult breathing that occurs when the demand for ventilation exceeds the patient's ability to respond.[2] Dyspnea describes a symptom rather than a clinical sign, and although the term often is used, dyspnea is not technically applicable in veterinary medicine. The clinical signs of respiratory distress vary with the severity and origin of impaired gas exchange. Clinical signs commonly observed in horses with respiratory distress include flared nostrils, exercise intolerance, inactivity, exaggerated abdominal effort, abnormal respiratory noise (stridor), anxious expression, extended head and neck, cyanosis, and synchronous pumping of the anus with the respiratory cycle.[1] Horses with chronic respiratory distress may develop a heave line resulting from hypertrophy of the cutaneous trunci and abdominal muscles, which assist during forced expiration.[3] Respiratory distress usually results from inefficient exchange of oxygen and carbon dioxide caused by primary pulmonary disease, airway obstruction, or impairment of the muscles and supporting structures necessary for ventilation. In some cases, ventilation increases in the absence of impaired gas exchange in response to pain, metabolic acidosis, or high environmental temperature. Familiarity with the mechanics of breathing and control of ventilation in healthy and diseased lungs facilitates the diagnosis and treatment of respiratory distress.[3,4]

3.7.1 Control of Ventilation

The partial pressure of oxygen (Pao_2) and carbon dioxide ($Paco_2$) in arterial blood are maintained within a narrow range through rigid control of gas exchange.[2] The center controller of respiration in the medulla alters the rate and depth of respiration via efferent signals to the muscles of respiration in response to afferent signals from chemoreceptors in the peripheral vasculature and central nervous system and mechanoreceptors in the upper and lower respiratory tract, diaphragm, and thoracic wall. The central controller therefore adjusts alveolar ventilation to the metabolic rate of the individual.[4]

3.7.1.1 SENSORS

The chemoreceptors identify changes in metabolism and oxygen requirements and provide feedback to the central controller, thus allowing for modification of ventilation. Central chemoreceptors respond predominantly to hypercapnia, whereas peripheral chemoreceptors respond to hypoxia and hypercapnia. Central chemoreceptors, located in the ventral medulla, monitor alterations in the pH of intracerebral interstitial fluid and cerebrospinal fluid. The blood-brain barrier is impermeable to bicarbonate and hydrogen ions but is freely permeable to carbon dioxide. Therefore acidification of the intracerebral interstitial fluid and stimulation of the central chemoreceptors occur predominantly in response to hypercapnia. The severity of acidosis in the intracerebral interstitial fluid caused by hypercapnia is amplified by two features of the central nervous system: (1) hypercapnia produces cerebral vasodilation, increasing the delivery of CO_2 to the central

nervous system, and (2) cerebrospinal fluid has poor buffering capacity because of low total protein concentrations.[2]

Peripheral chemoreceptors are located in the arterial circulation and respond to acidemia, hypercapnia, and hypoxemia. The carotid bodies are situated at the bifurcation of the common carotid artery, and the aortic bodies are located near the aortic arch. These receptors relay information to the central controller regarding arterial gas tensions via the glossopharyngeal and vagus nerves. Their responsiveness to alterations in $Paco_2$ is less consequential than the central chemoreceptors; however, the peripheral chemoreceptors are solely responsible for the hypoxic ventilatory drive. The peripheral chemoreceptors demonstrate a nonlinear response to low arterial oxygen tension. They are insensitive to alterations in Pao_2 above 100 mm Hg, exhibit moderate response to arterial O_2 tensions between 50 and 100 mm Hg, and demonstrate a dramatic increase in responsiveness when the partial pressure of oxygen falls below 50 mm Hg in the arterial circulation.[2] The respiratory pattern elicited by hypoxia differs from that stimulated by hypercapnia.[5,6] Hypoxia evokes an increase in respiratory frequency, whereas hypercapnia triggers an elevation in tidal volume. In addition, hypoxia stimulates recruitment of the inspiratory muscles, whereas hypercapnia potentiates the activity of inspiratory and expiratory muscles.

The sensitivity of peripheral chemoreceptors should be considered in the treatment of patients with complex acid-base and blood-gas abnormalities. A patient suffering from impaired gas exchange caused by pulmonary disease and metabolic acidosis resulting from shock manifests respiratory distress in response to hypoxemia, hypercapnia, and acidosis. Oxygen supplementation likely will improve the patient's arterial oxygen tension. Such treatment, however, may abolish the hypoxic ventilatory drive and consequently slow the ventilatory rate. This decreased ventilation could exacerbate respiratory acidosis and may result in decompensation of the patient.[4] To avoid life-threatening acidemia, treatment of metabolic acidosis in addition to oxygen supplementation is indicated.

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Receptors located in the upper and lower respiratory tract respond to mechanical and chemical stimuli and relay afferent information to the central controller of respiration via the vagus nerve.[1,2] Vagal blockade abolishes tachypnea in horses with pulmonary disease; therefore these receptors are likely to play an important role in development of respiratory distress associated with primary pulmonary disease.[7–9] Pulmonary stretch receptors, also called slow-adapting stretch receptors, are located within smooth muscle fibers in the walls of the trachea and bronchi.[1,2,4] These receptors are stimulated by pulmonary inflation and inhibit further inflation of the lung (Hering-Breuer reflex). Conversely, at end expiration these receptors stimulate inspiratory activity. These receptors are considered to be partially responsible for controlling the depth and rate of respiration.

Irritant receptors (rapid-adjusting stretch receptors) are believed to be located between epithelial cells of the conducting airways.[2] They are not likely to function in regulation of breathing in a normal resting horse.[4] Stimulation of these receptors by noxious stimuli triggers bronchoconstriction, cough, tachypnea, mucus production, and release of inflammatory mediators.[1,2] Irritant receptors can be triggered by exogenous stimuli (smoke, irritant gases, dust) or by endogenously produced inflammatory mediators including histamine and prostaglandins. Production of histamine, prostaglandins, and other inflammatory mediators increases in horses with chronic obstructive pulmonary disease (COPD).[10–12] Stimulation of irritant receptors by these inflammatory mediators may be responsible in part for bronchoconstriction, mucus production, and tachypnea observed in horses with allergic airway disease. In addition to their role as chemoreceptors, irritant receptors also function as mechanoreceptors.[1] An abrupt change in end-expiratory lung volume, such as occurs with pneumothorax or pleural effusion, produces a tachypneic breathing pattern attributed to stimulation of irritant

receptors. Juxtacapillary receptors are believed to be located within the wall of the alveolus. Stimulation by increased interstitial fluid volume triggers the sensation of difficult breathing.[2] Nonmyelinated C fibers are located in the pulmonary parenchyma, conducting airways, and blood vessels. These receptors respond to pulmonary edema, congestion, and inflammatory mediators, and stimulation activates a tachypneic breathing pattern. In addition, C fiber receptors may stimulate the release of pulmonary neuropeptides, which produce bronchoconstriction, vasodilation, protein extravasation, and cytokine production.[1] Increased negative pressure (upper airway obstruction) within the airway stimulates mechanoreceptors of the larynx and produces prolongation of inspiratory time and activation of upper airway dilator muscles.[13]

3.7.1.2 CENTRAL CONTROL OF RESPIRATION

The central controller consists of a group of motor neurons in the pons and medulla that receive input from the peripheral and central receptors and initiate phasic activity of diaphragmatic, intercostal, and abdominal respiratory muscles.[2] The medullary respiratory center, which is located in the reticular formation, controls the rhythmic pattern of respiration. The dorsal respiratory group coordinates inspiratory activity by assimilating afferent information from the glossopharyngeal and vagus nerves and transmits efferent signals to the muscles of inspiration and neurons in the ventral respiratory group. The ventral respiratory group consists of inspiratory and expiratory motor neurons. This nucleus is relatively inactive at rest and has a more dominant role during exercise. The apneustic center, located in the pons, provides stimulatory input to inspiratory motor neurons. Damage to the apneustic center, from trauma or neonatal maladjustment syndrome, results in prolonged inspiratory gasps interrupted by transient expiratory efforts.[4] The pneumotaxic center, also located in the pons, inhibits the inspiratory centers and regulates the volume and rate of respiration. The pneumotaxic center is not required to maintain a normal respiratory rhythm; instead, this center functions to fine tune the respiratory rhythm,[2] receiving afferent input from the vagus nerve regarding Pao_2, $Paco_2$, and pulmonary inflation.

3.7.1.3 EFFECTORS OF RESPIRATION

The muscles required for ventilation include the diaphragm, the external and internal intercostal muscles, and the abdominal muscles. The single most important muscle required for the inspiratory phase of the respiratory cycle is the diaphragm. Contraction of the diaphragm forces the abdominal contents back, increasing the length of the thoracic cavity, and pulls the ribs abaxially, increasing the width of the abdominal cavity. In addition, the external intercostal muscles participate in inspiration by pulling the ribs abaxially to increase the width of the thoracic cavity. The net effect is an increase in the size of the thoracic cavity, producing subatmospheric intrathoracic pressure, to drive inspiration and pulmonary inflation. Expiration at rest is a passive process in most species and relies on elastic recoil of the lung to create positive intrathoracic pressure.[14] In horses, the first portion of expiration relies on elastic recoil of the lung to the point of relaxation volume, whereby the tendency for pulmonary collapse equals the tendency for expansion by the thoracic wall. 137
However, horses further decrease lung volume by active compression of the chest wall, through contraction of 138
the internal intercostal muscles and muscles of the abdominal wall.[15] Conversely, the first part of inhalation is passive until the relaxation volume is reached, at which point the diaphragm and external intercostal muscles complete the inspiratory phase. Mechanical (abdominal distention, trauma to the thoracic wall) and neuromuscular (botulism, phrenic nerve damage, nutritional muscular dystrophy) dysfunction of the diaphragm and intercostal muscles prevent expansion of the thoracic wall and produce hypoventilation, hypoxemia, and respiratory distress.[4] Horses with torsion of the large colon develop significant abdominal

distention and respiratory distress. Respiratory failure caused by impaired diaphragmatic function plays an important role in the pathophysiology and mortality associated with this intestinal accident.

The diameter of the conducting airways is an important determinant of the degree of pulmonary resistance and work of breathing and is controlled by the autonomic nervous system. Vagal-mediated parasympathetic stimulation causes airway narrowing and is one mechanism of bronchoconstriction associated with allergic airway disease. Administration of atropine results in rapid relief of bronchoconstriction in some horses with COPD, demonstrating the important role of parasympathetic bronchoconstriction in the pathogenesis of this disease.[16,17] β_2-Receptor stimulation produces smooth muscle relaxation and bronchodilation. β_2-Adrenergic receptors are abundant throughout the lung; however, sympathetic innervation is sparse and β-receptors within the lung must rely on circulating catecholamines for stimulation.[4] Airways must be constricted for β_2-receptor stimulation or atropine blockade to produce increased airway caliber.[18,19] β-Adrenergic receptors are less abundant than β_2-receptors and play no important role in the regulation of airway diameter. However, α-receptors appear to be upregulated in horses with COPD and contribute to bronchoconstriction associated with this disease.[20]

Nonadrenergic-noncholinergic (NANC) innervation also contributes to large airway diameter. Smooth muscles of the trachea and bronchi relax in response to activation of the inhibitory NANC system. In COPD-affected horses with clinical signs of airway obstruction, inhibitory NANC function is absent.[21] Failure of the inhibitory NANC system may result from the inflammatory response during acute COPD or may be an inherent autonomic dysfunction of the conducting airways of COPD-affected horses.

3.7.2 Hypoxemia

Respiratory distress most often originates from inadequate pulmonary gas exchange to meet the metabolic demands of the individual, resulting in hypoxia and hypercapnia. Hypoxia results from one or more of five basic pathophysiologic mechanisms: hypoventilation, ventilation-perfusion mismatch, right to left shunting of blood, diffusion impairment, and reduced inspired oxygen concentration. The degree of hypercapnia and response to oxygen supplementation varies depending on the mechanism of impaired gas exchange. Determination of these two parameters is useful in identifying the pathophysiologic process predominantly responsible for the development of hypoxia.[22]

3.7.2.1 HYPOVENTILATION

The hallmark of hypoventilation is hypercapnia.[22] The elevation in $Paco_2$ is inversely proportional to the reduction in alveolar ventilation; halving alveolar ventilation doubles $Paco_2$.[2] The reduction in arterial oxygen tension is almost directly proportional to the increase in CO_2. For instance, if $Paco_2$ increases from 40 to 80 mm Hg, then the Pao_2 decreases from 100 to 60 mm Hg. Therefore hypoxemia resulting from hypoventilation is rarely life-threatening. In addition, oxygen supplementation easily abolishes hypoxemia caused by pure hypoventilation. Acidosis caused by hypercapnia is the most clinically significant feature of hypoventilation and may threaten the life of the patient.[22] Metabolic alkalosis or central nervous system depression (head trauma, encephalitis, narcotic drugs) can produce hypoventilation; however, horses with these disorders may not demonstrate clinical signs of respiratory distress. The following disorders can cause alveolar hypoventilation, and affected patients usually demonstrate clinical signs of respiratory distress: mechanical (abdominal distention, trauma to the thoracic wall) and neuromuscular (botulism, phrenic nerve damage,

nutritional muscular dystrophy) dysfunction of the diaphragm and intercostal muscles, restrictive pulmonary disease (silicosis, pulmonary fibrosis, pneumothorax, pleural effusion), and upper airway obstruction.[4]

3.7.2.2 VENTILATION-PERFUSION MISMATCH

Ventilation-perfusion (V-Q) mismatch is the most common cause of hypoxemia and is characterized by unequal distribution of alveolar ventilation and blood flow.[4] Pulmonary regions that are overperfused in relation to ventilation (low V-Q ratio) contribute disproportionate amounts of blood with low arterial oxygen content to the systemic circulation.[2,22] Respiratory diseases characterized by low V-Q ratios include COPD, pulmonary atelectasis, and consolidation.[4] If ventilation exceeds perfusion (high V-Q ratio), the ventilated pulmonary units are inefficient for CO_2 elimination and O_2 uptake. Ventilation of poorly or nonperfused units is wasted ventilation, termed *alveolar dead space*.[2,22] Conditions associated with high V-Q ratios include pulmonary thromboembolism and shock (low pulmonary artery pressure). Patients with V-Q mismatch often 138
have a normal arterial Pco_2. The ventilatory drive to maintain normal $Paco_2$ is powerful. Because the CO_2 139
dissociation curve is basically a straight line (direct relationship), increased ventilation efficiently decreases $Paco_2$ at high and low V-Q ratios. Because the nearly flat shape of the O_2 dissociation curve, increasing ventilation is inefficient for proportionally increasing the arterial Po_2. Only pulmonary units with moderate to low V-Q ratios benefit from increased ventilation. Therefore the increased ventilatory effort to maintain normal $Paco_2$ is wasted and unnecessarily increases the work of breathing. Oxygen supplementation increases $Paco_2$ in patients with a V-Q mismatch. However, elevation in arterial O_2 is delayed compared with hypoventilation and in some cases may be incomplete.[22] Compensatory mechanisms are present to minimize unequal distribution of ventilation and perfusion in diseased lungs to prevent the development of hypoxemia until pulmonary pathologic condition is severe.[23] Reflex pulmonary arterial constriction (hypoxic vasoconstriction) prevents perfusion of unventilated alveolar units and attempts to redirect blood flow to alveoli that are ventilated adequately. Airway hypocapnia causes bronchoconstriction of airways that conduct to unperfused alveolar units, redirecting air flow to better perfused alveoli.

3.7.2.3 SHUNT

Shunt is defined as blood that is not exposed to ventilated areas of the lung and is added to the arteries of the systemic circulation.[22] Shunting can occur as an extreme form of V-Q mismatch or with direct addition of unoxygenated blood to the arterial system. *Physiologic shunting* is defined as perfusion of nonventilated or collapsed regions of the lung and occurs with pulmonary consolidation, atelectasis, and edema. Congenital heart disease, such as tetralogy of Fallot and some cardiac septal defects, is an example of a direct right-to-left shunt wherein unoxygenated blood from the right side of the heart is added to oxygenated blood from the left side of the heart. In these conditions, hypoxemia cannot be abolished by increasing the oxygen content of inspired air. The shunted blood is never exposed to the higher concentration of inspired oxygen in the alveolus, and the addition of a small amount of shunted blood with its low O_2 content greatly reduces the Po_2 of arterial blood. Compared with breathing room air, the decrement in Po_2 is much greater at Po_2 levels associated with the inhalation of O_2-enriched air because the O_2 dissociation curve is so flat at high Po_2 levels. Only hypoxemia caused by right-to-left shunting behaves in this manner when the patient is permitted to inspire high percentages of oxygen (70% to 100%). Shunts do not usually cause hypercapnia.[23] Chemoreceptors detect excess arterial CO_2, and ventilation increases to reduce the content of CO_2 in

unshunted blood until arterial Pco_2 reaches the normal range. In some cases of shunt, the arterial Pco_2 is below normal because of hyperventilation stimulated by the hypoxemic ventilatory drive.

3.7.2.4 DIFFUSION IMPAIRMENT

Gas exchange between the alveolus and the capillary occurs by passive diffusion, which is driven by the property of molecules to move randomly from an area of high concentration to one of low concentration.[23] Factors that determine the rate of gas exchange include the concentration gradient between the alveolus and capillary blood, solubility of the gas, surface area available for diffusion, and the width of the air-blood barrier. Diseases characterized by pure diffusion impairment are rare in veterinary medicine.[4] Diffusion impairment can occur with pulmonary fibrosis, interstitial pneumonia, silicosis, or edema caused by increased width of the barrier or decreased surface area available for gas exchange. The clinician should recognize that the major component of hypoxemia for these conditions is a V-Q mismatch; however, diffusion impairment can contribute to the severity of hypoxemia. Supplemental oxygen therapy is effective in treating hypoxemia caused by diffusion impairment because it creates a more favorable concentration gradient and increases the driving pressure of oxygen to move from the alveolus into the blood. Transport of CO_2 is less affected by diseases of diffusion impairment because of its greater solubility compared with O_2.[23]

3.7.2.5 REDUCTION OF INSPIRED OXYGEN

Hypoxemia resulting from decreased inspired oxygen content is uncommon and occurs only under special circumstances. High altitude and iatrogenic ventilation with a low oxygen concentration are the most common circumstances in which hypoxemia is attributed to reduction of inspired oxygen content.[22]

Most pulmonary diseases in horses incorporate more than one of these pathophysiologic mechanisms for the development of hypoxemia. Horses with pleuropneumonia, for example, may develop hypoxemia caused by hypoventilation (extrapulmonary restriction by pleural effusion), V-Q mismatch (accumulation of exudate and edema within alveoli and conducting airways), and diffusion impairment (exudate and edema within the interstitial spaces).

3.7.3 Obstructive Disease

The location (intrathoracic or extrathoracic) and nature (fixed or dynamic) of airway obstruction determines whether impedance to air flow occurs during inspiration, expiration, or both.[3] The phase of the respiration cycle affected by air flow obstruction are prolonged and may be associated with a respiratory noise (stridor or wheeze).[24,25] 139

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The horse is an obligate nasal breather and can only breathe efficiently through the nares.[4] Therefore upper airway obstruction within the nasal passages cannot be bypassed by mouth breathing. In addition, approximately 80% of the total airway resistance to air flow is located in the upper airway.[25] A 50% decrease in the radius of an airway increases its resistance by sixteenfold (Poiseuille's law).[14] Therefore small changes in the upper airway diameter dramatically affect the overall resistance to air flow and work of breathing for the horse. Extrathoracic airway pressures are subatmospheric during inspiration; therefore poorly supported structures in the upper airway narrow or collapse during inspiration (dynamic collapse). The most common cause of non–fixed upper airway obstruction in horses is laryngeal hemiplegia, which produces inspiratory stridor during exercise.

Intraluminal masses and arytenoid chondritis cause fixed upper airway obstruction and produce inspiratory and expiratory respiratory distress.[3]

Twenty percent of the total airway resistance is attributable to the small airways.[25] Although the radius of individual bronchioles is small, many of them exist and the sum or collective radius is large, with the result that their overall contribution to pulmonary resistance is low.[23] Because the resistance of the bronchioles is low, advanced disease must be present for routine measurements of airway resistance to detect an abnormality, and obstruction of these airways must be extensive before a horse would suffer from respiratory distress. During pulmonary inflation, intrathoracic pressures are subatmospheric. Small airways are pulled open by negative intrathoracic pressure and stretched parenchymal attachments at high lung volumes. Thus resistance to air flow in small airways is low during the inspiratory phase of respiration.[24] During exhalation, intrathoracic pressure is positive and the diameter of small airways is decreased, and bronchioles may even close at low lung volumes. Therefore resistance to air flow in small airways is greatest during the expiratory phase. In horses with COPD, the airway diameter is reduced by inflammatory exudate, edema, and bronchoconstriction.[16,17] As lung volume decreases during expiration, the narrowed bronchioles are compressed shut (dynamic airway collapse) and trap air distal to the site of closure.[4] This is an example of severe flow limitation, which may lead ultimately to the development of emphysema. Flow limitation forces horses with COPD to breathe at higher lung volumes and maintain a higher functional residual capacity to reduce or avoid dynamic airway collapse. Affected horses attempt to reduce the end-expiratory lung volume by recruiting abdominal muscles to increase the intrathoracic pressures during expiration. However, the greater the end-expiratory pressure, the greater is the likelihood of small airway compression and collapse. Hypertrophy of the cutaneous trunci and expiratory abdominal muscles, especially the external abdominal oblique, produces the characteristic heave line associated with COPD.[4] Because dynamic airway narrowing and collapse occurs during exhalation, wheezes are loudest at end expiration in horses with COPD.[16,17]

3.7.4 Restrictive Disease

Restrictive disease is less common than is obstructive pulmonary disease in horses.[4] By definition, restrictive disease inhibits pulmonary expansion and leads to inspiratory respiratory distress.[26] The vital capacity and compliance (pulmonary or chest wall) decrease, expiratory flow rates and elastic recoil increase, and airway resistance is normal. The characteristic respiratory pattern in horses with restrictive pulmonary disease is rapid, shallow respiration at low lung volumes.[4] This strategy takes advantage of high pulmonary compliance at low lung volumes and decreases the work of breathing. This respiratory pattern has the disadvantage of increased ventilation of anatomic dead space.[26] Restrictive diseases may be classified as intrapulmonary (pulmonary fibrosis, silicosis,[27] and interstitial pneumonia[28,29]) and extrapulmonary (pleural effusion, pneumothorax, mediastinal mass, botulism, and nutritional muscular dystrophy).[4] Hypoxemia observed in horses with intrapulmonary restrictive disease is attributed to V-Q mismatch and diffusion impairment. Stimulation of juxtacapillary receptors may contribute to respiratory distress observed in these patients.[26] The pathophysiologic mechanism for hypoxemia in horses with extrapulmonary restriction is hypoventilation.[4] In cases of pleural effusion and pneumothorax, respiratory distress is likely to be exacerbated by thoracic pain.

3.7.5 Nonpulmonary Respiratory Distress

Respiratory distress does not always originate from dysfunction of the pulmonary system and its supporting structures. Nonpulmonary respiratory distress can occur because of inadequate oxygen-carrying capacity of the blood, compensation for metabolic acidosis, pain, and hyperthermia.

Impaired oxygen-carrying capacity of the blood may occur because of anemia (blood loss, hemolytic, or aplastic) or dysfunction of red blood cells (methemoglobinemia, carbon monoxide toxicity). In these cases, the arterial Po_2 tension is normal; however, the oxygen content of the blood is reduced greatly.[2] Tachypnea and respiratory distress occur in response to impaired oxygen delivery and tissue hypoxia.[3]

The respiratory system can compensate for metabolic acidosis by increasing ventilation to lower $Paco_2$ and 140
attenuate acidemia.[2] The ventilatory drive increases in response to stimulation by peripheral chemoreceptors by 141
circulating hydrogen ions. Hypocarbic compensation for mild to moderate metabolic acidosis is effective in returning blood pH to normal until renal compensatory mechanisms can be established.[2]

Pain and anxiety are physiologic causes of tachypnea and hyperpnea. Horses with musculoskeletal pain are unlikely to demonstrate significant respiratory distress; however, rhabdomyolysis and laminitis are painful musculoskeletal conditions that may produce tachypnea.[3] Marked respiratory distress is observed frequently in horses with abdominal pain; however, the respiratory distress is not caused solely by pain and is exacerbated by abdominal distention, shock, acidosis, and endotoxemia.

Hyperthermia caused by fever, high environmental temperature, exercise, and heat stress can produce respiratory distress in horses. Tachypnea and elevation in body temperature are the most prominent clinical signs in horses with anhydrosis.[30] Hyperpnea is an effective mechanism for heat dissipation in human beings, dogs, and ruminants.[3] Unfortunately, increased ventilation is an inefficient mechanism for heat dissipation in horses and appears to be wasted effort.[3,4]

3.7.6 Clinical Evaluation of Respiratory Distress

A thorough physical examination is essential to determine the origin of respiratory distress, identify concurrent disease, and direct further diagnostic testing. Prolonged inspiration is consistent with restrictive or extrathoracic, nonfixed, obstructive disease, whereas horses with intrathoracic airway obstruction exhibit expiratory difficulty.[3,24] Respiratory distress associated with inspiration and expiration may indicate an extrathoracic fixed obstruction. Stridor is an abnormal respiratory noise that usually is generated by obstruction of the upper airway and is audible most often during inspiration.[3] Horses with nonpulmonary respiratory distress demonstrate increased rate and depth of respiration, without producing abnormal respiratory noise.

Thoracic auscultation identifies abnormal respiratory sounds (crackles and wheezes) or regions of decreased breath sounds caused by pleural effusion, pneumothorax, or pulmonary consolidation. Percussion of the thoracic wall generates a resonant and hollow sound when performed over regions of normal lung. Pleural effusion and pulmonary consolidation sound dull and flat during thoracic percussion, whereas pneumothorax produces a hyperresonant sound.[31]

Normal air flow occurs in laminar flow; therefore normal horses at rest do not generate easily audible sounds.[4] Respiratory sounds are generated from vibration in tissue and sudden changes in pressure of gas moving within the airway lumen. Airway narrowing and exudate generate audible sounds by creating disturbances in laminar flow, turbulence, and sudden changes in pressure of moving gas.[14] Crackles are intermittent or explosive sounds, generated by bubbling of air through secretions or by equilibration of airway pressures after sudden opening of collapsed small airways. The generation of crackles requires an air-fluid interface, and these abnormal lung sounds occur in horses with pneumonia, interstitial fibrosis, COPD, pulmonary edema, and atelectasis.[4] Wheezes are continuous, musical sounds that originate from oscillation of small airway walls before complete closing (expiratory wheeze) or opening (inspiratory wheeze).[14] Expiratory wheezes are the hallmark of obstructive pulmonary disease.[24]

Arterial blood gas determination provides a quantitative evaluation of pulmonary function, alveolar ventilation, and acid-base status and may identify the origin of respiratory distress (hypercapnia, hypoxemia, or acidemia).[22] The clinician may determine the pathophysiologic mechanism of hypoxemia by examining the $Paco_2$ level and by investigating the response of Pao_2 to supplemental oxygen therapy. In addition, serial blood gas monitoring can determine response to bronchodilator, parasympathomimetic, or antiinflammatory therapy.

Additional diagnostic tests that may be indicated in horses with respiratory distress include thoracic radiography, thoracic ultrasonography, endoscopic examination of the upper airway, and atropine challenge. The findings during thoracic auscultation and percussion are valuable in determining indication for ultrasonography versus radiography. Pulmonary consolidation, abscessation, fibrosis, interstitial pneumonia, peribronchial infiltration, and mediastinal mass are differentiated and diagnosed readily via thoracic radiography. Thoracic ultrasonography is superior to radiography in detecting and characterizing pleural fluid and peripheral pulmonary abscessation and consolidation in horses. Air reflects the ultrasound beam; therefore ultrasonography does not image deep pulmonary lesions if the overlying lung is aerated.[32] An endoscopic examination of the upper airway is indicated in horses with inspiratory stridor and suspected upper airway obstruction.[33] Horses with extreme respiratory distress may resent endoscopic examination, and forced examination may precipitate a respiratory crisis. Atropine administration in horses with COPD may provide rapid relief of respiratory distress, if the major component of airway obstruction is reversible bronchoconstriction. Horses that respond to an atropine challenge likely will respond favorably to bronchodilator therapy. Incomplete response to atropine in horses with COPD indicates that exudate or fibrosis is contributing to airway obstruction, and limited response to bronchodilator therapy is anticipated.[16,17] 141

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3.8

3.8—Cough

Catherine W. Kohn

Cough, a sudden explosive expulsion of air through the glottis, is a common sign of respiratory disease and a reflex pulmonary defense mechanism. Coughing facilitates the removal of noxious substances and excessive secretions from the airways by creating maximum expiratory airflow. A high-velocity airflow generates the shear forces required to separate mucus from the airway walls, enabling expulsion of exudate and debris from the airway.[1] An understanding of the cough reflex provides insight into the pathophysiology of diseases characterized by cough.

The cough reflex has been studied infrequently in horses. Descriptions of the cough cycle and the neural basis of cough presented in this section are based on data from other species. The author infers that similar events occur in 142
horses. Because differences exist among species regarding the cough reflex,[2] studies on horses will be required to 143
define the physiologic events of the cough reflex in this species.

3.8.1

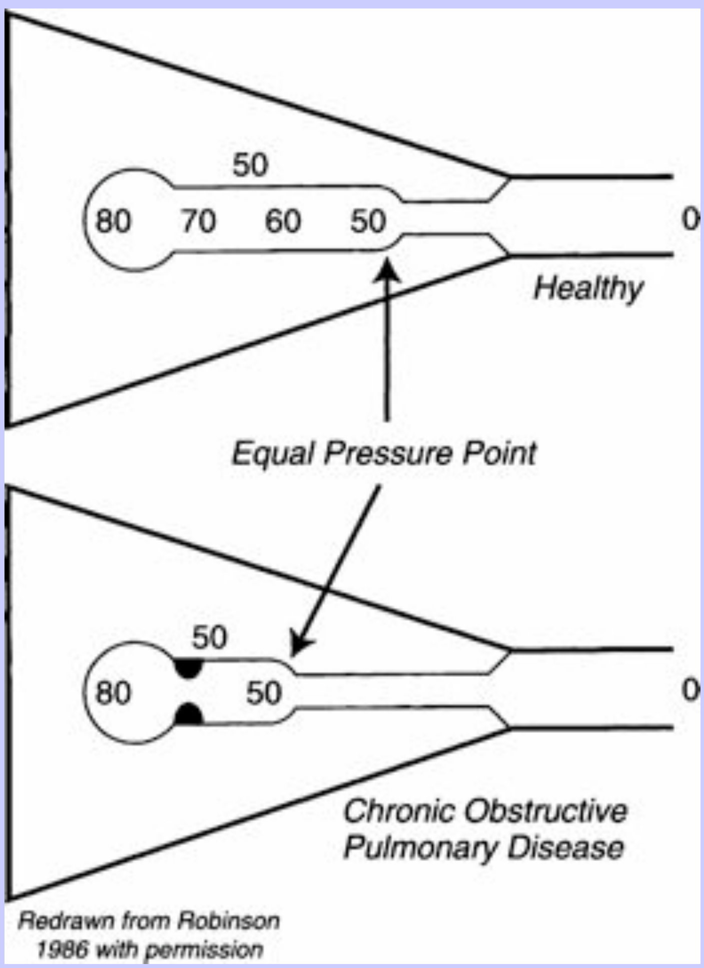
Cough Cycle

The cough cycle has four phases: inspiration, compression, expression, and relaxation.[1] Deep *inspiration,* which immediately precedes cough, increases lung volume. As lung volume increases, the ability to generate maximum expiratory airflow increases because of the greater force of contraction achieved by the muscles of respiration when their precontraction length increases and because of the greater elastic recoil pressure of the lung at high lung value.[3] Thus precough expansion of lung volume maximizes the velocity of expiratory airflow. Achievement of maximum expiratory airflow rates requires a relatively gentle expiratory effort, and airflow maxima are therefore independent of effort.[2]

After deep inspiration, the glottis closes. While the glottis remains closed, *compression* of the chest cavity occurs by contraction of the thoracic and abdominal musculature during an active expiratory effort. Compression of the chest results in an increase in pleural pressure from 50 to 100 mm Hg.[2] This increase in pleural pressure is transmitted to pressure in the intrathoracic airways and trachea. Intraalveolar pressures actually exceed intrapleural pressures by an amount equal to the elastic recoil pressure of the lung.[4]

Expression occurs when the glottis opens abruptly, thus producing a gradient in airway pressure (atmospheric at the pharynx and high in the alveoli), and air is expired forcefully. The occurrence of dynamic airway compression in larger airways maximizes the velocity of airflow toward the mouth (Figure 3.8-1). The intraairway pressures vary in the respiratory system according to the instantaneous transpulmonary pressure.[3] At the equal pressure point, the airway pressure equals the pleural pressure. Toward the mouth from the equal pressure point (downstream), the pleural pressure is greater than intrathoracic airway pressure, and the intrathoracic airways therefore are compressed dynamically. Partial collapse of the airways downstream of the equal pressure point maximizes airflow velocities in these airways by decreasing their diameter. At high lung volumes, the equal pressure point likely is in the larger airways and therefore only the intrathoracic trachea may be subject to dynamic compression and maximal airflow velocity.[4] Maximum airflow velocity produces high shearing forces that dislodge mucus and debris from airway walls, thus facilitating expectoration. Cough is therefore most effective as a defense mechanism for clearing the larger airways in healthy animals. Removal of noxious substances from the smaller peripheral airways depends on the presence of mucus in the airways, and irritants that stimulate cough also may stimulate mucus production.[5]

Figure 3.8-1 Dynamic airway compression during cough or maximum expiratory airflow. Lungs are represented at total lung capacity. When chronic obstructive pulmonary disease is present, the equal pressure point moves toward the alveoli. This peripheral migration of the equal pressure point results in dynamic compression in more peripheral airways during cough than would be found in a healthy individual. (Redrawn from Robinson NE: Pathophysiology of coughing. In Proceedings of the thirty-second convention of the American Association of Equine Practitioners, Nashville, 1986, pp 291-297.)



In diseases characterized by increased resistance in small peripheral airways caused by partial obstruction (e.g., chronic obstructive pulmonary disease [COPD]), maximal expiratory flow rates are reduced. When small airways are obstructed partially, the equal pressure point moves toward the periphery of the lung during

coughing because pressures in airways downstream of the partial obstruction are lower than are pressures in those airways in healthy lungs (see Figure 3.8-1). This shift in the equal pressure point subjects more peripheral airways to dynamic compression. Coughing is likely to be less effective as a clearance mechanism when obstructive diseases of the small airways are present. Bronchodilator therapy may increase the effectiveness of cough in such patients by increasing expiratory airflow rates.[4]

The sound of cough is generated by vibration of laryngeal and pharyngeal structures caused by the rapid expulsion of air immediately after opening of the glottis,[3] by narrowing and deformation of airways, and by 143
vibration of surrounding lung tissue. Variations in the sound of cough most likely relate to the quantity and 144
quality of mucus in the airway.[6]

At the end of cough, *relaxation* occurs. Intrapleural pressure falls, and the muscles of expiration relax. Transient bronchodilation occurs.[1]

3.8.2 Neural Basis of the Cough Reflex

The afferent input for the cough reflex is carried predominantly in the vagus nerves, and the cough reflex depends uniquely on vagal afferents in the species studied.[5,7,8] Sensory myelinated nerves in the larynx respond to mechanical and chemical irritation and mediate cough and changes in airway diameter.[7] Debate continues about the identity of receptors that initiate cough in the lower airways; however, all the receptors described is this section likely contribute to the cough response.[8] *Rapidly adapting receptors* are located in the airway mucosa in the region of the carina and are stimulated primarily by mechanical deformation produced, for example, by inhaled particles, mucus, or cellular debris accumulating near the carina. Chemical irritants (e.g., ammonia fumes, ozone, and inflammatory mediators) evoke cough by stimulation of receptors located in the peripheral airways. *Pulmonary C fibers* may mediate a chemically evoked cough, although this issue still is debated. Chemical mediators known to stimulate pulmonary C fibers and cough when inhaled as aerosols by human beings include bronchodilator prostaglandins, bradykinin, and capsaicin.[8] Forced expiration during coughing may be facilitated by the modulating effects of information from these receptors on central respiratory neurons.

Bronchoconstriction is a constant component of cough,[3,6] and stimuli of cough also may cause bronchoconstriction; however, cough and bronchoconstriction are separate airway reflexes. Inhalation of dust and irritant gases causes reflex bronchoconstriction in the species studied. Reflex bronchoconstriction has a slow onset and is long lasting compared with the cough reflex.[9] Bronchoconstriction may increase the efficiency of cough by decreasing airway diameter and therefore increasing airflow velocity. In some cases, bronchodilating drugs may suppress the cough reflex by desensitizing airway receptors that elicit cough.[6]

Sensory nerves mediating bronchoconstriction and cough are distributed unevenly along the airways.[7] Laryngeal receptors and sensory nerves in the extrapulmonary airways may be more sensitive to mechanical stimuli, whereas intrapulmonary receptors may respond preferentially to chemical mediators and irritants.

Little is known about the brainstem neuronal pathways of the cough reflex. In the cat, the cough center is reported to be in the medulla at the level of the obex, alongside the solitary nucleus of the vagus and close to the expiratory neurons of the respiratory center. On the motor side of the cough reflex, the vagal, phrenic, intercostal, and lumbar nerves and motor portions of the trigeminal, facial, hypoglossal, and accessory nerves are

distributed to the striated and smooth muscles of respiration, the vocal fold abductors and adductors, and glands of the respiratory tract.[3]

3.8.3 Stimuli of Cough

Cough may be stimulated by airway smooth muscle contraction (bronchoconstriction), excessive mucus production, presence of inhaled particles in the airways, release of inflammatory mediators (infectious diseases), exposure to cold or hot air, intramural or extramural pressure or tension on the airways (tumor, granuloma, abscess, or decreased pulmonary compliance caused by restrictive disease such as interstitial fibrosis or pleuritis), sloughing of airway epithelial cells, and enhanced epithelial permeability (pulmonary edema).[5] Epithelial sloughing and enhanced epithelial permeability theoretically increase the accessibility of cough receptors to the mechanical or chemical agents that stimulate them. Loss of the integrity of the epithelial lining of the respiratory tract is a common feature in many respiratory diseases associated with cough (infectious diseases); however, a cause-and-effect relationship between alterations in respiratory epithelium and cough has not been established.[5]

Diseases of the respiratory tract may alter the sensitivity of the cough reflex.[5] For example, viral diseases may increase the responsiveness of cough receptors to stimuli.

3.8.4 Deleterious Consequences of Cough

Although cough is an important defense mechanism of the respiratory system that promotes expectoration of inhaled noxious substances and voluminous airway secretions, cough may lose its original defensive function and may contribute to the morbidity and discomfort associated with bronchopulmonary disease.[8] This is especially true when the effort to cough is intense and when multiple coughs occur sequentially. Chronic coughing is exhausting and, especially in foals, may decrease food intake. Paroxysmal or persistent cough may impair respiration. Coughing may have profound effects on the cardiovascular system. During the deep inspiratory phase of cough, the rise in intraabdominal pressure because of contraction of the diaphragm and the fall in intrathoracic pressure combine to aspirate blood from the vena cava to fill the right atrium and ventricle abruptly.[3] Because the pleural pressure decreases, the pulmonary artery pressure also decreases. During the expiratory phase of cough, an initial increase in systemic arterial blood pressure and a simultaneous and commensurate increase in cerebral venous and cerebrospinal fluid pressures occur. However, venous return to the heart soon decreases and within a few heartbeats, filling of the heart and stroke volume decrease.[2,3] Hypotension ensues. Falling arterial blood pressure in the face of high cerebral venous pressures reduces the effective perfusion pressure of the brain. Cerebral hypoperfusion and anoxia may occur. Cough-induced syncope has been reported in human beings[2] and in dogs.[10]

In chronic cough, bronchial muscular hypertrophy may develop. Bronchial mucosal edema or emphysema may accompany chronic cough. During cough inspiration, inflammatory debris may be aspirated into previously uncontaminated areas of the lung. Cough in dogs has been associated with pneumothorax (from rupture of preexisting pulmonary bullae) and lung lobe torsion.[11] Rib and vertebral fractures have been reported in human beings with powerful coughs but have not been reported in horses.[2,3]

3.8.5 Clinical Approach to the Coughing Horse

Cough is a common sign of respiratory disease in horses (Figure 3.8-2). Cough is an indication of mechanical or irritant stimulation of cough receptors for which the potential causes are diverse. Many clinical approaches exist for anatomic localization of the origin of the cough stimulus in respiratory disease and for discovery of the cause. All methods have in common a systematic and thorough evaluation of the history and physical examination of the patient. To aid the clinician in formulating a rational approach to diagnosis, diseases associated with cough may be grouped according to those characterized by fever (current or historical) and those characterized by lack of an elevated body temperature. The clinician should keep in mind that exceptions to generalizations always occur concerning disease processes, and the following discussion therefore serves only as a guide to develop a logical approach to differenting diseases characterized by cough.

3.8.5.1 COUGH WITH FEVER

Horses with cough and fever should have a thorough physical examination (see Chapter 7 for a complete description of a physical examination for horses with respiratory disease). A minimum laboratory database for the coughing horse with fever should include the results of a hemogram and a fibrinogen determination. The clinician carefully should auscultate the thorax of the horse in a quiet room with the horse breathing quietly. If the horse is not dyspneic or hypoxemic, the clinician also should undertake auscultation during forced breathing. A plastic bag loosely held over the nostrils of the horse forces the horse to increase tidal volume and respiratory rate. This maneuver causes many horses with exudate in the airways to cough, and deep breathing may be frankly painful for some horses with pleuropneumonia. Auscultation during forced breathing is not necessary in horses with obviously abnormal lung sounds during quiet breathing and is not advisable in horses with pneumonia (especially aspiration pneumonia) or in horses with foreign material in the trachea. Crackles and wheezes heard repeatedly during the inspiratory and early expiratory phases of breathing suggest that pulmonary parenchymal disease is present. Accentuated normal bronchovesicular sounds sometimes are present in horses with pulmonary consolidation, because of referral of sounds from the aerated lung. Absence of lung sounds in dependent portions of the thorax indicates that pulmonary consolidation, atelectasis, or fluid in the pleural cavity may be present. Thoracic percussion and sonographic evaluation are particularly helpful in documenting the presence of fluid in the pleural cavity. Ultrasonography also may show pleural irregularities and superficial parenchymal abscessation, atelectasis, or consolidation. Thoracic radiographs are especially helpful in demonstrating deeper parenchymal disease. Many equine practitioners do not have access to thoracic radiography but can perform thoracic ultrasonography.

Abnormal lung sounds, percussion irregularities, and sonographic evidence of fluid or consolidation are indications for performing transtracheal aspiration (TTA) and bronchoalveolar lavage (BAL). When both procedures are to be performed on the same patient, the clinician should perform TTA first to obtain a sample for culture before the airway is contaminated by the BAL tube. Many practitioners prefer to obtain TTA samples transendoscopically to avoid percutaneous aspiration. Despite the development of guarded culture swabs for transendoscopic use, this technique does not always prevent contamination of lower airway fluid samples. One study demonstrated that *Pseudomonas* spp. and anaerobic bacteria in cultures of tracheal fluid obtained transendoscopically should be viewed as potential contaminants.[12]

Cytologic evaluation of the TTA/BAL, indicating an increase in polymorphonuclear leukocytes (PMNs), is consistent with parenchymal disease. Some PMNs may be degenerate. Although some clinicians feel that PMNs may be seen in the tracheal aspirates of normal horses, few PMNs are found in bronchoalveolar lavage

fluids from healthy horses (4.4 ± 3.3 cells to 8.9 ± 1.2 cells/μl).[13] How well the results of cytologic evaluation of BAL fluids represent the environment of the lower airways is a matter of some debate. Bronchoalveolar lavage fluids are harvested from a focal area of the lung. If parenchymal disease is not generalized, bronchoalveolar lavage may miss the diseased region. Results of BAL fluid analysis are normal in some horses with pneumonia and pleuropneumonia. Transtracheal wash fluid consists of secretions from both lungs, and TTA cytologic examination was abnormal in all horses with pneumonia and pleuropneumonia in one study.[14] The prevalence of PMNs in TTA fluid from horses without lower respiratory tract disease has not been determined.

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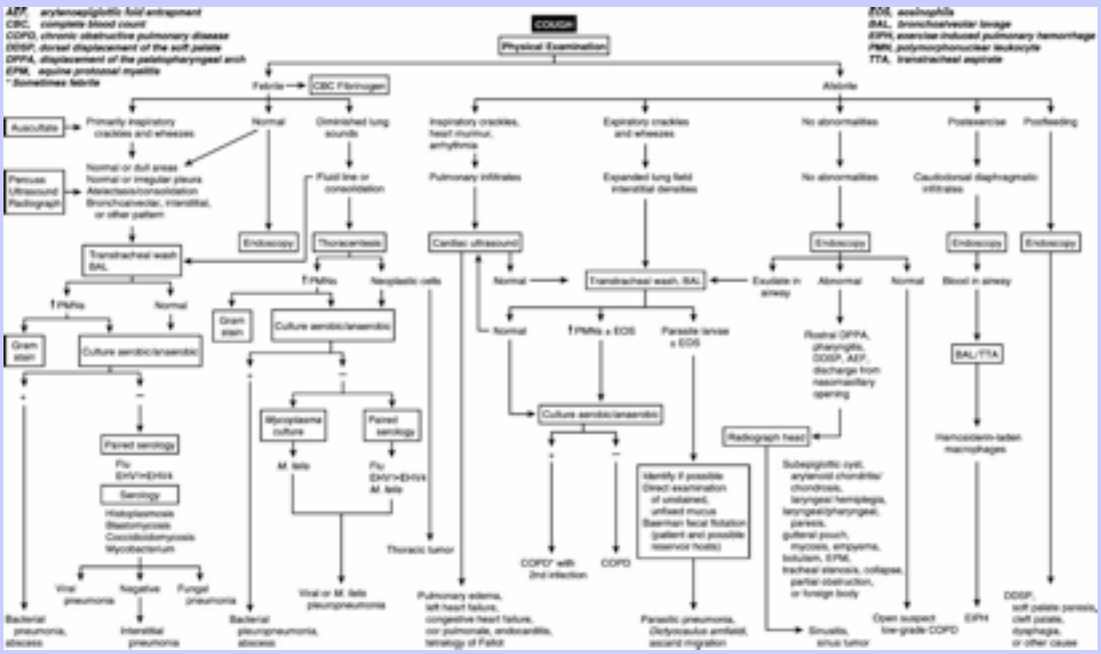
Figure 3.8-2 Approach to cough. *EHV1, 4,* Equine herpesvirus 1 or 4.



The presence of degenerate PMNs and extracellular or intracellular bacteria in TTA/BAL fluid is consistent with the diagnosis of a septic process. The clinician should evaluate a Gram stain to guide the initial choice of antimicrobial agents while awaiting results of culture and sensitivity determinations. Growth of aerobic or anaerobic bacteria in a culture of TTA fluid confirms the presence of bacterial pneumonia if clinical and radiographic findings are also consistent with this disease process. Contamination of cultures of airway secretions obtained via TTA occasionally may occur. Lack of growth of bacterial pathogens from TTA fluid suggests that viral, interstitial, or fungal pneumonia might be present. These possibilities should be investigated by evaluating paired serum samples taken 10 to 14 days apart for influenza virus, equine herpesvirus 1 (EHV1), EHV4, rhinovirus, and equine viral arteritis. Serologic testing for histoplasmosis, blastomycosis, coccidioidomycosis (southwestern United States especially), and possibly mycobacteria should be evaluated. Fungal cultures of tracheal fluid should be evaluated when other more common causes of pneumonia have been ruled out and if the clinical signs of the patient are consistent with this diagnosis. Negative results on serologic tests and fungal cultures in patients with a significant interstitial pattern on thoracic radiographs should prompt consideration of the diagnosis of interstitial pneumonia, a condition for which the inciting cause has not been established and for which the prognosis is grave.

Percussion, radiographic, or ultrasonic evidence of increased intrapleural fluid is an indication for thoracocentesis. Many horses with bacterial pleuropneumonia have elevated pleural fluid PMN concentrations, and PMNs may be degenerate. Intracellular or extracellular bacteria may be seen on cytologic evaluation. Occasionally, frankly neoplastic cells may be identified in thoracic fluid (usually squamous cells or lymphocytes). Many cytologists are uncomfortable diagnosing thoracic neoplasia based solely on an evaluation of pleural fluid. Thoracic fluid should be cultured aerobically and anaerobically. A positive culture identifies the cause of bacterial pleuritis; however, often pleural fluid cultures may be negative. Cultures of TTA fluid are more likely to be positive in horses with pleuropneumonia, and TTA cultures should be performed routinely for these patients. Primary viral pleuritis, although rare in the author's experience, has been reported in horses, and paired serologic examinations for influenza virus and EHV1/EHV4 may be helpful when cultures are negative. One case of pleuritis caused by *Mycoplasma felis* has been reported.[15] Culture of pleural fluid and paired serologic examinations for this organism should be performed in patients for which other tests have not proved diagnostic.

Intrathoracic neoplasms may cause cough with or without accompanying fever. Confirmation of a thoracic tumor may require an ultrasound-guided biopsy or an exploratory thoracotomy and a biopsy. Secondary bacterial pleuritis may complicate thoracic neoplasms, and aerobic and anaerobic cultures of thoracic fluid from patients suspected of having thoracic neoplasms should be performed.

Some febrile coughing horses have no abnormalities on auscultation, percussion, thoracic radiography, or ultrasound. In such patients, occult pulmonary disease may be present and TTA/BAL and culture of TTA fluid are indicated. Alternatively, such horses may have upper airway disease (sinusitis, sinus tumor, guttural pouch empyema), and an endoscopic evaluation is also indicated.

3.8.5.2 COUGH WITHOUT FEVER

When auscultation of the thorax demonstrates primarily expiratory crackles and wheezes, thoracic percussion often reveals a caudoventral expansion of the lung borders. These findings suggest that COPD may be present. Thoracic radiographs usually show increased interstitial densities; radiographs are useful to rule out occult underlying pulmonary disease (such as a well walled-off abscess) but are not required for diagnosis in most cases. TTA and BAL are indicated. Horses with COPD usually have an increase in well-preserved PMNs, and sometimes eosinophils, in TTA and BAL fluids. Growth of pathogens in aerobic or anaerobic culture of TTA fluid identifies secondary bacterial infection. No growth in cultures of TTA fluid is also consistent with the diagnosis of COPD. Occasionally, TTA/BAL fluids may contain parasite larvae or many eosinophils. If horses historically have been housed with donkeys or mules, one should suspect *Dictyocaulus arnfieldi* infestation. Coughing horses younger than 18 months of age with eosinophilic TTA fluid may be experiencing an aberrant migration of *Parascaris equorum* larvae. The clinician should attempt to identify the larvae, although this may be difficult. A direct cytologic evaluation of unfixed, unstained, or iodine-stained mucus may be helpful to identify larvae of *D. arnfieldi*. The clinician should perform a Baermann flotation on feces from the patient and potential reservoir hosts, but the test may not demonstrate ascarid larvae, because pulmonary migration may occur early in the prepatent period.[16] The diagnosis of pulmonary ascarid migration is based on ruling out other causes of pneumonia.

When TTA/BAL fluids have no abnormal cells, cultures still should be assessed. For afebrile coughing horses 147
with thoracic auscultation findings of inspiratory crackles and wheezes and cardiac murmur or arrhythmia, one 148
should take thoracic radiographs. The presence of diffuse pulmonary infiltrates in a bronchoalveolar pattern suggests that pulmonary edema may be present. A complete ultrasonic evaluation of the heart is indicated.

Some coughing, afebrile horses have no abnormalities on auscultation or percussion, and endoscopy of the upper airway and trachea is indicated. Some horses have endoscopic evidence of exudate in the trachea and likely have low-grade COPD. The clinician should take thoracic radiographs of these horses if possible and perform TTA/BAL testing followed by culture of TTA fluid. A transtracheal aspirate should not be obtained immediately after tracheoscopy because bacteria on the endoscope may contaminate airway cultures.

In other patients, cough may be a symptom of upper airway obstructive disease (dorsal displacement of the soft palate, rostral displacement of the palatopharyngeal arch, arytenoepiglottic fold entrapment, subepiglottic cyst, arytenoid chondritis/chondrosis, laryngeal hemiplegia, or tracheal stenosis, collapse, or partial obstruction) or maxillary or frontal sinusitis with discharge into the nasal passages via the nasomaxillary opening or laryngeal/pharyngeal paresis. The latter may be a symptom of guttural pouch mycosis, empyema, or systemic disease (e.g., botulism or equine protozoal myelitis). Cough also may be a symptom of a tracheal foreign body (e.g., a twig or TTA catheter) in the airway. One should suspect horses with cough but no abnormalities on endoscopic examination of having low-grade COPD.

Cough after exercise or feeding also should prompt an endoscopic evaluation. Evidence of hemorrhage in the trachea after exercise indicates that exercise-induced pulmonary hemorrhage is likely. This diagnosis can be confirmed by finding hemosiderin-laden macrophages in BAL or TTA fluid. Thoracic radiographs may show interstitial densities and pleural thickening in the caudodorsal lung field. Postprandial cough may be associated with soft palate paresis, dorsal displacement of the soft palate, cleft palate (neonates and foals), or dysphagia of any cause.

A detailed description of diagnostic and therapeutic strategies for diseases of the respiratory system can be found in Chapter 7.

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3.9

3.9—Changes in Body Temperature

Melissa T. Hines

Assessment of body temperature is an essential part of every physical examination. As with all mammalian species, 148

horses normally maintain their core body temperature within a narrow range despite extremes in environmental 149 conditions. The core temperature may vary by approximately 1° C (2° F) between individuals. In adult horses, the average normal body temperature is 38.0° C (100.5° F), whereas in neonatal foals the temperature tends to be slightly higher, ranging from 37.8° to 38.9° C (100.0° to 102.0° F). A diurnal variation of up to 1° C (2° F) may occur, with the low point typically in the morning and the peak in the late afternoon.

3.9.1

Control of Body Temperature

The set-point is the crucial temperature that the body attempts to maintain, primarily via neuronal control operating through temperature centers in the hypothalamus.[1,2] Peripheral and central thermoreceptors sense changes in ambient and core body temperatures and activate feedback mechanisms that bring the temperature back to the set-point. Specifically, the anterior hypothalamic-preoptic area contains large numbers of heat-sensitive neurons and lower numbers of cold-sensitive neurons that function as temperature detectors. Peripheral receptors, which are generally most sensitive to low temperatures, are located in the skin and in some deep tissues, such as the spinal cord, abdominal viscera, and around certain great veins. The anterior hypothalamic-preoptic area and the peripheral receptors transmit signals into the posterior hypothalamic area, subsequently activating autonomic and behavioral effector responses to regulate body temperature.

When the body temperature is too high, heat loss increases and heat production diminishes. Increasing blood flow to the skin is an effective mechanism for heat transfer from the body core to the surface. In response to changes in core body temperature and environmental temperature, the sympathetic nervous system regulates the degree of vasoconstriction and thus the amount of blood flow. Heat is lost from body surfaces to the surroundings by several physical mechanisms, including radiation, conduction, and convection. Evaporation is

also an important mechanism of heat loss in horses.[3] The rate of sweating controls to some extent the amount of evaporative heat loss. However, even when the animal is not sweating, water evaporates insensibly from the skin and lungs, causing continual heat loss. In horses, evaporative heat loss, primarily though increased sweating but also through increased respiration, becomes more important as the ambient temperature rises and during exercise.[3,4] In addition to increased heat loss when the body temperature rises, the horse also decreases temperature further by inhibiting means of heat production, such as shivering, and by behavioral responses, such as seeking shade, wind currents, and wading into water.

Mechanisms that increase body temperature come into play when the body temperature is too low.[2] Heat is conserved by stimulation of the posterior hypothalamic sympathetic centers leading to cutaneous vasoconstriction and piloerection. Heat production also increases and may occur through increased muscle activity ranging from inapparent contractions to generalized shivering. Shivering may increase heat production by 4 to 5 times baseline. The primary motor center for shivering is in the posterior hypothalamus, which normally is stimulated by cold signals from the peripheral receptors and to some extent the anterior hypothalamic-preoptic area. Signals from heat sensitive neurons in the anterior-hypothalamic-preoptic area inhibit the center. Digestion of food also contributes to total body heat. Sympathetic stimulation may increase the rate of cellular metabolism, increasing heat production by chemical thermogenesis. Cooling also increases the production of thyrotropin-releasing hormone, ultimately increasing thyroid hormones and cellular metabolism, and further contributing to chemical thermogenesis. In addition to these physiologic adaptations, behavioral responses to conserve heat also occur, such as adopting a huddled stance, aggregating in groups, and seeking shelter.

3.9.2 Conditions of Increased Body Temperature

Elevation of the body temperature above normal is one of the most common clinical problems encountered, and although classically associated with infection, a variety of disorders may cause increased body temperature. One should distinguish between conditions of hyperthermia, in which the temperature set-point is unaltered, and true fever, in which the set-point actually increases.

3.9.2.1 HYPERTHERMIA

The body temperature may become elevated without an increase in the set-point when a loss of equilibrium occurs in the heat balance equation. Increased heat production or absorption of heat beyond the ability of the body to dissipate heat may occur. In some conditions, impaired heat loss also may occur. Hyperthermic conditions include problems such as exercise-related hyperthermia, heat stroke, malignant hyperthermia, anhidrosis, central nervous system disorders, and reactions to certain toxins or drugs. In general, these conditions do not respond to treatment with antipyretic drugs.

3.9.2.2 EXERCISE-RELATED HYPERTHERMIA

During sustained or high-intensity exercise, increased heat production is associated with muscular activity.[3,4]
The heat produced may exceed the ability of the body to lose heat, resulting in an increased core body 149
temperature. Typically, the temperature returns to normal with rest as heat loss mechanisms remain activated. 150
Elevated temperature also may occur with the intense muscle activity associated with generalized seizures.

3.9.2.3
HEAT STROKE

Heat stroke occurs when the body temperature rises above a critical temperature, leading to multisystemic problems. In horses, signs of heat stroke may develop when the body temperature is above 41.5° C (107° F), which most often occurs in association with exercise in environmentally stressful conditions. Although horses can acclimatize to various weather conditions to some extent, the efficiency of evaporative heat loss may be compromised significantly in hot, humid weather.[4,5] Susceptibility to heat stroke may increase if sweating leads to dehydration and electrolyte imbalances. Once the body temperature reaches the critical point, the homeostatic mechanisms of thermoregulation fail, resulting in peripheral vasoconstriction, decreased cardiac output, and decreased blood pressure. Affected horses are lethargic, with weak flaccid muscles. Prostration, circulatory shock, disseminated intravascular coagulation, multiple organ failure, and death may occur.

3.9.2.4
ANDHIDROSIS

Especially in hot, humid climates, horses may develop anhidrosis, which is characterized by a partial or total loss of the ability to sweat.[6] Because of the resulting impaired heat loss, hyperthermia may develop. Clinical signs of poor performance, increased respiratory rate, and poor hair coat also are observable.

3.9.2.5
MALIGNANT HYPERTHERMIA

Malignant hyperthermia encompasses a group of inherited skeletal muscle disorders in which calcium metabolism is altered.[7] Although the condition is most common in human beings and pigs, it has been reported in several species, including horses.[8,9] The disorder is characterized by a hypermetabolic state of muscle that generally is induced by halogenated inhalation anesthetics, depolarizing skeletal muscle relaxants, and occasionally local anesthetics or stress. Clinical signs include a rapid increase in core body temperature, skeletal muscle rigidity, and tachycardia. Affected animals may develop significant acidosis and muscle necrosis and in some cases may die. In pigs, malignant hyperthermia has been linked to a single point mutation in the gene for the skeletal muscle ryanodine receptor, but a genetic basis has not yet been established in horses.[7]

3.9.2.6
CENTRAL NERVOUS SYSTEM DISORDERS

Any condition affecting those areas of the hypothalamus involved in thermoregulation may alter the body temperature, with hyperthermia being more common than hypothermia.[1,2] Thus central hyperthermia occurs in association with a variety of conditions, including hemorrhage, neoplasms or abscesses, infectious/inflammatory changes, and degenerative disorders. Central hyperthermia usually is characterized by a lack of any diurnal variation, absence of sweating, resistance to antipyretic drugs, and excessive response to external cooling.

3.9.2.7
CERTAIN TOXINS OR DRUGS

Occasionally, hyperthermia has been associated with toxins or drugs. Exposure to compounds that act to uncouple oxidative phosphorylation, such as the wood preservative pentachlorophenol, potentially could cause a significant rise in body temperature.[10] Foals treated with the antibiotic erythromycin are at risk of

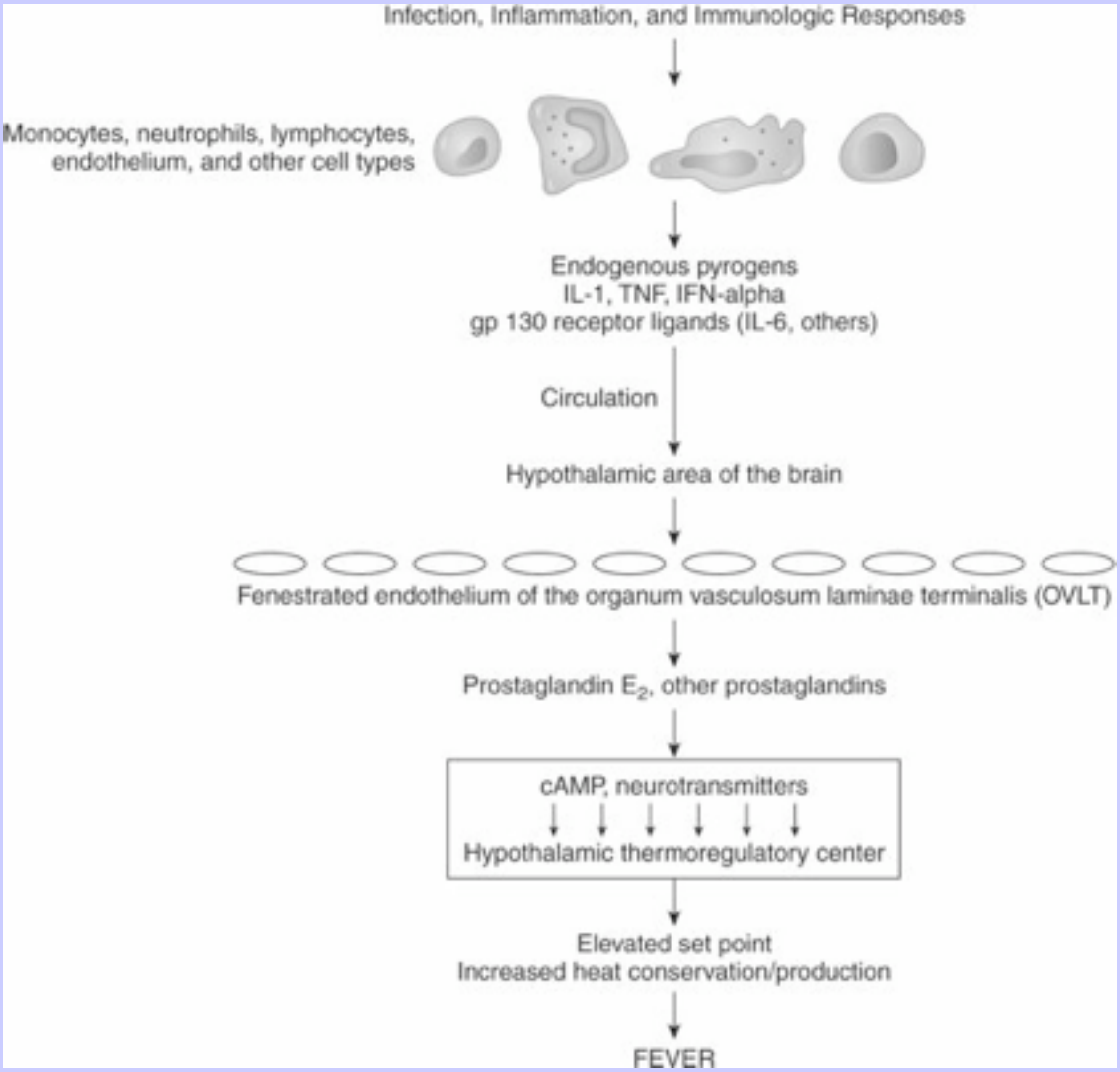
developing hyperthermia.[11] Such predisposition has been attributed to a reaction to the erythromycin itself or to an alteration of the thermoregulatoy system of the foal by mechanisms not yet described. Environmental conditions may exacerbate the development of hyperthermia, with foals exposed to high ambient temperatures and direct sunlight being at greatest risk.

3.9.3 Pathogenesis of True Fever

In true fever the set-point for the desired core body temperature increases and then is maintained by the same mechanisms that maintain the normal body temperature. Although primarily associated with infectious diseases, fever is also a prominent component of many inflammatory, immunologic, and neoplastic conditions. Although the pathogenesis of the febrile response is complex, essentially all of these conditions initiate fever by stimulating the release of endogenous pyrogens (Figure 3.9-1).

Endogenous pyrogens are substances with the biologic property of fever induction.[12,13] Initially endogenous pyrogen was assumed to be a single molecule produced by leukocytes, thus the name leukocytic or granulocytic pyrogen. Now, multiple cytokines are known to act as pyrogens, and a variety of cell types produce them, with monocytes and macrophages predominating. Currently, the following cytokines are thought to be intrinsically pyrogenic in that they produce a rapid-onset fever via direct action on the hypothalamus without requiring formation of another cytokine: interleukin-1α (IL-1α) and IL-1β, tumor necrosis factors (TNF) α and β, interferon-α, and IL-6. IL-1α and IL-1β and TNF-α appear to be among the most potent pyrogens. Many endogenous pyrogens use the cell-signaling apparatus gp130. Cytokines that act through this receptor include
IL-6, IL-11, oncostatin M, ciliary neurotrophic factor, cardiotropin-1, and leukemic inhibitory factor. From a 150
clinical standpoint, several pyrogenic cytokines are produced during most febrile diseases and contribute to the 151
febrile response.

Figure 3.9-1 Schematic representation of the pathogenesis of fever. *cAMP,* Cyclic adenosine monophosphate; *IFN,* interferon; *IL,* interleukin; *TNF,* tumor necrosis factor.



The precise mechanism of action of pyrogenic cytokines in the central nervous system is still unclear. Endogenous pyrogens probably act on the circumventricular organs or organum vasculosum laminae terminalis (OVLT), a rich vascular network associated with neurons of the preoptic anterior hypothalamus.[13–15] Ablation of the OVLT prevents fever after a peripheral injection of endogenous pyrogens but has no effect when endogenous pyrogens are injected directly into the brain tissue.[14] In the region of the OVLT the blood-brain barrier is minimal, and endothelial cells lining this region may allow direct movement of endogenous pyrogens into the brain or they may release arachidonic acid metabolites in response to endogenous pyrogens, which then move into the brain. The production of arachidonic acid metabolites, particularly prostaglandin E_2 via the cyclooxygenase 2 (COX-2) pathway is clearly important in the pathogenesis of fever, because COX inhibitors,

and specifically COX-2 inhibitors, effectively reduce the febrile response but have no effect on the normal body temperature. The prostaglandins do not act directly but initiate neuronal signaling by producing a cascade of changes in cyclic nucleotides, calcium, and monoamines leading to a higher set-point in the hypothalamic thermoregulatory center.

Physiologic mechanisms exist to control the febrile response and prevent extremes that are incompatible with life. Multiple feedback mechanisms limit the activity of the pyrogenic cytokines and many endogenous cryogens or antipyretics have been identified.[16,17] For example, IL-10, which can be induced by pyrogenic cytokines, inhibits further production of IL-1 and TNF. Arginine vasopressin and α-melanocyte-stimulating hormone act within the brain to decrease fever.[16–19] When administered to human beings, α-melanocyte-stimulating hormone is a much more potent antipyretic than acetaminophen. Nitric oxide also has been shown to have an antipyretic role, mediated by cyclic guanosine monophosphate, in the anterior hypothalamic-preoptic region.[20] 151

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The cytokines that act as endogenous pyrogens have a variety of biologic effects. Therefore the onset of fever is accompanied by several hematologic, immunologic, and metabolic changes referred to as the acute phase response. In particular, IL-6 and IL-11 induce the synthesis of acute phase proteins by hepatocytes, including fibrinogen, C-reactive protein, haptoglobin, and others. Similarly, hypoferremia, hypozincemia, and hypercupremia are cytokine mediated, as is the activation of lymphocytes, which in turn produce additional cytokines.

Pyrogenic cytokines, particularly IL-1 and TNF-α cause membrane perturbation with an increase in phospholipases and the production of arachidonic acid.[12,13] The subsequent production of mediators depends on the metabolic pathways for arachidonic acid in the target tissue. Prostaglandins induced by endogenous pyrogens stimulate the muscle catabolism associated with fever and induce collagenase synthesis from synovial cells, contributing to the muscle and joint pain often seen with fever. Local tissue responses to IL-1β and TNF-α may stimulate afferent neural impulses that lead to behavioral responses associated with fever, such as lethargy and anorexia. As expected, treatment with COX inhibitors can diminish many of the signs of fever.

3.9.4 Effects of Fever

Fever is a normal physiologic response with beneficial and adverse effects to the animal. With the exception of some viral infections, the elevation in temperature is generally not high enough to affect pathogens directly. However, studies on bacterial infections in several species have demonstrated an increase in survival with fever, which is thought to be caused primarily by enhanced host defenses.[21–23] In addition, the concentration of iron, which is required by many bacteria for multiplication, decreases during the acute phase response.[24–26] If the temperature becomes extremely high, many of the beneficial effects are reversed.[2,27,28] In rabbits the severity of bacterial infection increases when the body temperature in more than 3° C (5° F) above normal. The increased catabolism, variable anorexia, and increased metabolic rate can lead to muscle wasting and weakness when fever is prolonged. Although seizures induced by fever are uncommon in horses, they can be seen in neonates when the temperature is above 42° C (108° F).[29] In debilitated animals, prolonged fever has been associated with cardiovascular failure.

3.9.5 Approach to Fever

Increased body temperature is a common clinical sign with diverse causes (Figure 3.9-2). Fortunately, in many cases the cause may be readily apparent based on the signalment, history, and physical examination. Conditions

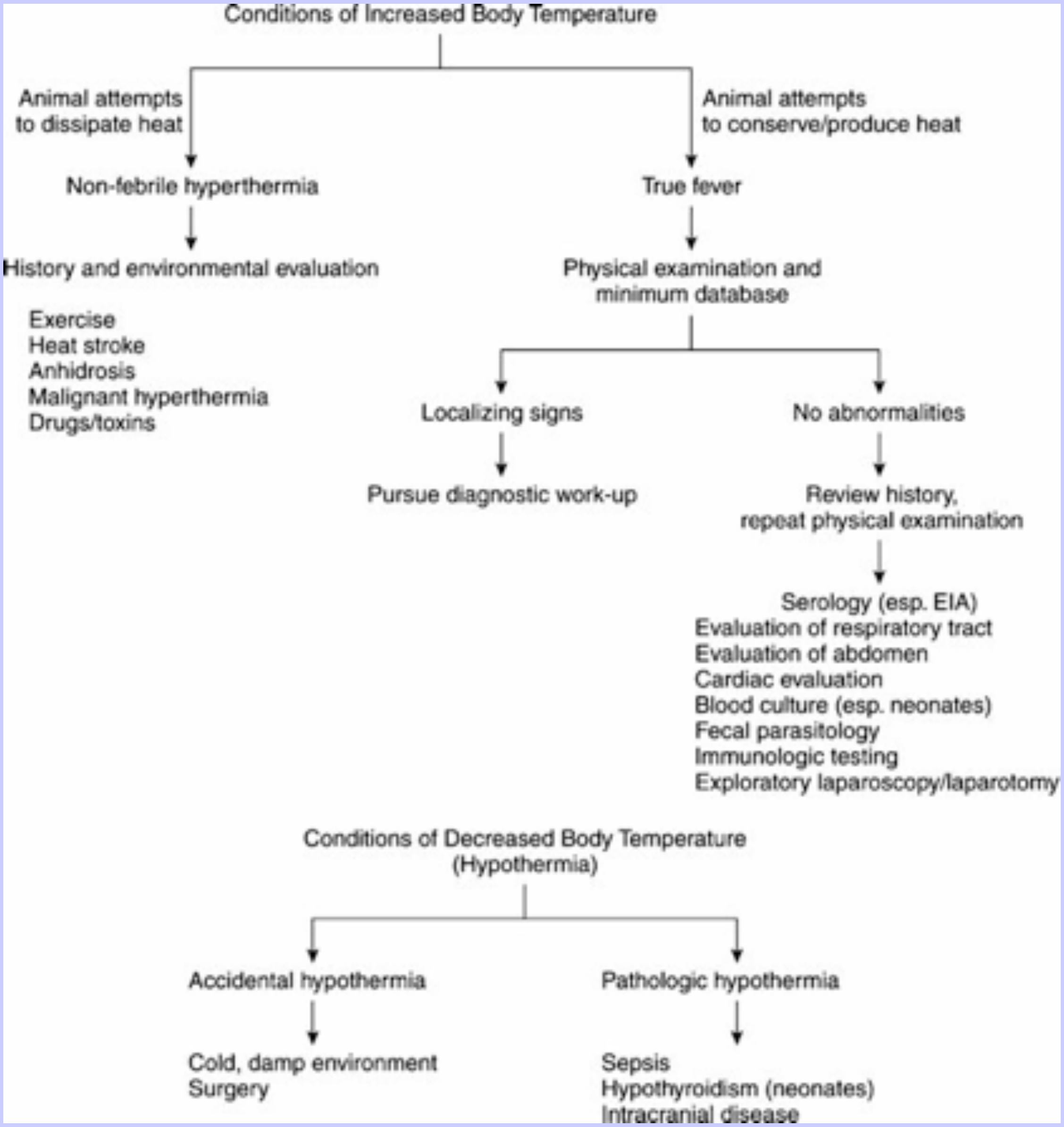
of increased temperature such as exercise-related hyperthermia and malignant hyperthermia are often apparent from the history. Infectious diseases remain the most common cause of fever, and often localizing clinical signs such as nasal discharge or diarrhea aid in the diagnosis. In other cases, an increased temperature may be one component of another obvious condition, such as neoplasm, immune-mediated disease, or a drug reaction.

3.9.6 Fever of Unknown Origin

Fever of unknown origin exists when fever is prolonged with no other specific signs. In many cases, the cause is a common disease with an unusual presentation. The specific criteria used to define fever of unknown origin in the horse in a review of 63 cases included the following: (1) illness of at least 3 weeks' duration associated with nonspecific signs, (2) body temperature of at least 38.6° C (101.5° F) on several occasions, and (3) no clear diagnosis after an initial complete blood count and serum biochemical profile.[30] The most common cause was found to be infection, which was responsible for 43% of the cases. Other causes included neoplasms in 22% of cases, immune-mediated diseases in 6.5%, and miscellaneous diseases including toxic hepatopathy, parasitism, and others in 19%. In 9.5% of cases, no diagnosis was made. Therefore diagnosis of fever of unknown origin requires a systematic approach with emphasis on the evaluation of infectious disease.

The clinician can have the body temperature taken twice daily over a period of time to document fever and identify any pattern. Although some inconsistencies in the precise terminology used to define patterns of fever exist, *intermittent fevers* generally are characterized by recurring paroxysms of elevated temperature followed by periods of normal temperature, such as those fevers that demonstrate diurnal variation. Intermittent fevers most often are associated with infectious causes, particularly viral infections, although they may be seen with a variety of other conditions. In most cases of intermittent fever the temperature tends to peak in the late afternoon or evening, though this is not always the case. *Remittent fevers* are those in which diurnal variation is exaggerated without a return to normal body temperature or those with a cyclic pattern in which the temperature elevation lasts for several days, such as may be seen with equine infectious anemia virus. *Biphasic fevers,* in which an initial rise in body temperature precedes a period of normal temperature and then a second rise, are characteristic of certain diseases such as equine monocytic ehrlichiosis (Potomac horse fever). Sustained fevers are those in which the elevation of temperature is consistent. 152

Figure 3.9-2 Approach to changes in body temperature.



A complete history is important when one is investigating fever of unknown origin. Any exposure to *Streptococcus equi* ssp. *equi* (strangles) may be significant because of the association of this organism with internal abscessation. Travel history may be relevant, especially regarding diseases with a geographic influence such as babesiosis and coccidioidomycosis.

The clinician always should perform a careful physical examination, including rectal palpation. Repeating the physical examination may yield new information. The clinician also should perform a complete neurologic examination, because disorders of the central nervous system may cause aberrations in temperature through pyrogenic cytokines or in some cases through direct effects on thermoregulatory centers.

Ancillary diagnostic tests usually are required to diagnose fever of unknown origin. A minimum database, including complete blood count, fibrinogen, biochemical profile with bile acids, and urinalysis should be performed. Hemoparasites occasionally may be seen on the blood smear, but the apparent absence of organisms does not rule out a parasitemia that is below readily detectable limits. Abnormalities consistent with chronic infection or inflammation, including anemia, hyperfibrinogenemia, and hyperglobulinemia are common but nonspecific findings. If an elevation of serum protein occurs, further assessment by serum protein electrophoresis and specific immunoglobulin quantitation may be indicated. A monoclonal gammopathy is characteristic of plasma cell myeloma and other tumors of the reticuloendothelial system, both of which may initiate fever directly and increase susceptibility to bacterial infection. In general, immunodeficiencies may be associated with chronic infections. If the serum protein is low, one should investigate the causes of hypoproteinemia, including 153
decreased production because of significant hepatic disease, increased gastrointestinal or renal loss, or loss into a 154
third space. The presence of hypercalcemia can be helpful in establishing a diagnosis, because in horses, hypercalcemia most often is linked with renal disease or neoplasms.

Infections of the respiratory tract and abdomen frequently are associated with fever of unknown origin in the horse, and therefore one should evalute these systems thoroughly. Careful auscultation of the thorax using a rebreathing bag should be performed at rest and, if possible, after exercise. Endoscopy, including examination of the guttural pouches can be useful. Diagnostic imaging of the thorax, including radiographs and ultrasound, often is indicated. The clinician also should include thoracocentesis in evaluation of the thorax, for abnormalities occasionally are apparent even without increases in the volume of pleural fluid. Pleuroscopy, which allows direct visual examination of the pleural space and which may facilitate biopsy of any masses, can be helpful in establishing a diagnosis, especially when neoplasia is suspected.

Peritonitis and abdominal abscessation are common causes of fever of unknown origin, and one should include abdominocentesis in the diagnostic plan. The peritoneal fluid should be evaluated for protein, cellularity, and cell morphology, and culture should be performed. One should remember that although many neoplastic conditions involve the abdomen, neoplastic cells are not always observed in the peritoneal fluid. In cases of gastric squamous cell carcinoma, gastroscopy is helpful in establishing the diagnosis. Radiographs of the abdomen may be useful, especially in neonates, and ultrasound of the abdomen may help to identify fluid for collection or abnormalities that indicate further evaluation, such as abdominal masses or pathologic liver conditions.

Gastrointestinal parasitism is a common clinical problem in the horse, although it is associated only occasionally with fever. However, one should examine feces for parasite ova in horses with fever of unknown origin. In cases of suspected gastrointestinal protein loss, diarrhea, or melena, one should consider diagnostic procedures such as fecal culture, polymerase chain reaction for *Salmonella,* rectal mucosal biopsy, or absorption tests.

Bacterial endocarditis can cause a fever of unknown origin, although the condition is not as common in horses as in some other species. In the study by Mair, Taylor, and Pinsent, the authors identified endocarditis in 3 of 63 cases of fever of unknown origin.[30] In each case a murmur they did not identify initially became apparent several weeks after the onset of illness. Therefore a thorough cardiac evaluation, including echocardiography, is indicated.

Blood cultures are generally most useful in neonates but can yield valuable information in adult horses with fever as well. Ideally, one should collect three to five samples at least 45 minutes apart when the horse is not in a regimen of antibiotic therapy. Sampling just before and during a temperature rise is most likely to yield a positive culture.

The clinician should consider equine infectious anemia as a differential diagnosis for horses with fever of unknown origin and should perform a serologic examination. Recently, a serologic test for detection of antibodies to the M protein of *Streptococcus equi* ssp *equi* was developed as an aid in the diagnosis of internal abscessation.[31] Serologic tests for equine babesiosis, brucellosis, and coccidioidomycosis are also available.

Immune-mediated disorders such as autoimmune hemolytic anemia, immune-mediated thrombocytopenia, systemic lupus erythematosis, vasculitides, and rheumatoid arthritis have been implicated as causes of fever of unknown origin, but more commonly in human beings and small animals than in horses. However, appropriate diagnostic tests, such as the Coombs' test, skin biopsy, and antinuclear antibody testing may be useful in some cases.

Exploratory laparoscopy or laparotomy is indicated when abdominal involvement is evident or the animal is becoming progressively debilitated. Occasionally, bone marrow aspiration may be useful, particularly in those cases with persistent abnormalities in circulating cell populations. In cases in which a specific diagnosis has not been made, therapeutic trials with antimicrobials may help, and in cases of suspected immune-mediated disease, corticosteroids may help.

3.9.7 Hypothermia

Hypothermia occurs when the core body temperature drops below accepted normal values. In clinical cases, hypothermia can be characterized as accidental or pathologic (see Figure 3.9-2). In accidental hypothermia a spontaneous decrease in the core body temperature occurs independent of actual disruption to the thermoregulatory system. These cases often can be identified from the history. Mild accidental hypothermia sometimes occurs with surgical procedures. Most often, accidental hypothermia is associated with exposure to cold or cold, damp environments, which can lead to severe hypothermia and death. Neonates are particularly susceptible to hypothermia, although central thermoregulation through the hypothalamus is normal.[32,33] Sick foals often decrease their activity and nutritional intake and have alterations in circulation. They also have a large ratio of surface area to body weight, enhancing heat loss. Geriatric and otherwise debilitated animals are also at increased risk of hypothermia.

One should consider pathologic causes of hypothermia when no clear reason for accidental hypothermia is evident. Pathologic hypothermia occurs in association with disorders that decrease metabolic activity or directly affect the thermoregulatory center and occurs with endocrine disorders, sepsis, and intracranial disease. In horses, hypothyroidism is probably an uncommon clinical problem; however, impaired thermoregulation has been seen in foals with congenital hypothyroidism.[34] Lesions of the thyroid gland also have been associated with hypothermia in donkeys.[35] Hypothermia has been observed with septicemia and shock, especially in neonates, in which 24% of septic foals were found to have a decreased body temperature.[36] 154 155

The ability to generate heat through shivering is impaired or lost when the body temperature becomes too low. The animal experiences a decrease in the metabolic rate of most tissues. Heart rate, cardiac output, glomerular filtration, and blood pressure may decrease.

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3.10

3.10—Diarrhea

Melissa T. Hines

Diarrhea, defined as an increase in the frequency, fluidity, or volume of bowel movements, is a commonly encountered clinical problem in the horse. Diarrhea may occur as a primary disease of the gastrointestinal tract or as a secondary response to another disease process, such as sepsis, endotoxemia, or hepatic disease.

The function of the equine gastrointestinal tract is complex and involves maintenance of normal fluid balance and digestion and absorption.[1–3] As a result of dietary intake and endogenous secretions, normally a large volume of fluid enters the gastrointestinal tract, most of which is reabsorbed. In the adult horse absorption occurs predominantly in the large bowel, where a volume of water approximately equal to the total extracellular fluid volume of the animal, or about 100 L, is recovered during the course of the day. Because the large colon is the primary site of water resorption, most significant diarrheal disease in the adult horse involves the colon. In young foals, however, small intestinal disorders such as rotaviral infection also may result in diarrhea.[4]

A second critical function of the large bowel is that of microbial digestion of carbohydrates and, to some extent, protein or nonprotein nitrogen.[1–3] Microbial fermentation of carbohydrates in the cecum and colon results primarily in the production of volatile fatty acids, which are absorbed readily, providing up to 75% of the energy requirement of the horse. Therefore maintaining a stable environment for the microbial population is important. In general, efficient function of the large bowel requires mechanisms that limit the rate of digesta passage, provide optimal conditions for microbial digestion, and allow for efficient transport of solutes and water.

The characteristics of normal equine feces vary somewhat with diet. Generally, equine feces are tan, brown, or greenish, and although approximately 75% water, they are well formed. An adult horse on a diet of grass hay and approximately 3 lb of oats per day produces about 20 to 28 g of feces per kilogram of body mass per day or about 11 to 13 kg of feces per day.[5] In cases of diarrhea, the amount of feces may increase up to tenfold, with horses producing more than 200 g/kg/day, or more than 90 L of diarrhea. As a result, diarrhea can cause significant losses of electrolytes and water and significant systemic acid-base imbalances. However, despite large water losses, horses with chronic diarrhea seldom develop severe dehydration or electrolyte abnormalities because they compensate for increased fecal losses.

3.10.1 Mechanisms of Diarrhea

Inflammation within the bowel plays a central role in the pathogenesis of diarrhea. Several basic mechanisms of diarrhea have been described, and in most diarrheal diseases, more than one mechanism is involved. These mechanisms include the following:

1. *Malabsorption:* Malabsorption results from a decrease in the functional absorptive surface area of the gastrointestinal tract. Villus atrophy in the small intestine, seen with rotaviral enteritis and infiltrative bowel disease, can result in malabsorption because of the loss of functional epithelium and maldigestion caused by decreased production of digestive enzymes. A number of insults to the colon result in inflammation and disruption of absorptive cells and tight junctions, leading to decreased absorptive capacity and decreased ability to retain absorbed fluid, that is, increased loss. Several inflammatory mediators, such as histamines and prostaglandins, contribute to the colonic inflammation. These mediators are produced primarily by inflammatory cells in the lamina propria and inhibit absorption through a variety of mechanisms.[6–10]

2. *Increased secretion:* The increased secretion of solutes and water by the inflamed colon can contribute significantly to the development of diarrhea. Although the precise mechanisms of secretion in the equine colon are not understood fully, active secretion and passive fluid loss occur.[6–12] Control of active secretion is complex, involving two primary pathways: first, the activation of adenyl cyclase, resulting in an increase of intracellular cyclic adenosine monophosphate concentrations, and second, the activation of calcium channels, leading to increased intracellular calcium concentrations.[11,12] Cyclic adenosine monophosphate and calcium stimulate specific secretory activities, primarily through chloride channels. In some cases of diarrhea, bacterial enterotoxins such as those produced by certain strains of *Escherichia coli* and *Salmonella* stimulate adenyl cyclase activity, thus increasing active secretion. This is true hypersecretory diarrhea. Also, a number of inflammatory mediators produced by the inflamed colon, particularly prostaglandin E, increase intracellular concentrations of cyclic adenosine monophosphate and to some extent calcium, thereby increasing active secretion by mucosal cells.[11–13] Inflammation also enhances passive fluid loss through a number of factors, such as changes in hydrostatic pressure in the colonic capillaries, mucosal damage, and loss of tight junctions. In cases of severe mucosal injury, the loss

of protein can decrease vascular oncotic pressure and further potentiate fluid exchange across the endothelium.

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3. *Decreased transit time (abnormal motility):* Progressive motility must be present for diarrhea to occur. Primary motility disorders causing diarrhea are not well recognized, although diarrhea associated with stress or excitement may represent this phenomenon. Inflammation is known to influence gastrointestinal motility, in addition to altering absorption and secretion. However, the precise significance of the altered motility in the pathogenesis of diarrhea is not clear. Sufficient retention time and thorough mixing are required for digestion and absorption of nutrients and fluid to occur, and decreased intestinal transit time has been recognized in association with many gastrointestinal diseases, including infectious diarrhea. Absorption of endotoxin and the release of inflammatory mediators, including prostaglandins, disrupts normal motility patterns.[14] In some cases of acute colitis, a period of ileus may occur without diarrhea. With diarrheal diseases, the elimination of gut contents is part of the normal host defense mechanism, and thus decreasing motility is not indicated in most cases.

4. *Osmotic overload:* Any increase in osmotically active particles within the intestinal lumen can result in diarrhea. The increase can be associated with the administration or ingestion of osmotically active substances such as magnesium sulfate. The increase also may be associated with overloading of the intestine with carbohydrates or occasionally lipids beyond the amount that can be digested and absorbed. Therefore sudden dietary changes that result in significant shifts in gut flora and changes in fermentation or gastrointestinal diseases that result in malabsorption or maldigestion also may result in an osmotic diarrhea. In foals the loss of villus epithelial cells in the small intestine associated with disorders such as rotavirus infection and clostridiosis may lead not only to malabsorption but also to maldigestion caused by the decreased production of lactase.[4,15] The resulting lactose intolerance allows excess lactose to enter the large intestine, increasing the osmotic load.

5. *Increased hydraulic pressure from the blood to the lumen:* This mechanism of diarrhea is more common in chronic conditions, such as congestive heart failure or inflammatory bowel disease. The condition may result from decreased oncotic pressure associated with hypoproteinemia, increased capillary hydrostatic pressure (as in heart failure), or decreased lymphatic drainage associated with inflammation of lymphatics and lymph nodes.

Understanding the mechanisms of diarrhea can be helpful in directing therapy. However, one must remember that most disorders that cause diarrhea, whether infectious or noninfectious, do so through inflammatory mechanisms resulting in multiple functional alterations.

TABLE 3.10-1 Differential Diagnoses for Acute Diarrhea in Adult Horses

CAUSES	MAJOR DIAGNOSTIC TEST(S)
COMMON	
Salmonellosis	Fecal culture or polymerase chain reaction (PCR), culture of rectal mucosal biopsy
Potomac horse fever (equine monocytic ehrlichiosis)	PCR (feces, peripheral blood), paired serologic tests
Clostridiosis (*Clostridium difficile, C.perfringens*)	Fecal culture, toxin analysis
Antibiotic-associated diarrhea	History
Nonsteroidal antiinflammatory toxicity (primarily right dorsal colitis)	History and supportive clinicopathologic findings, ultrasonography, exploratory surgery with biopsy
Undiagnosed	Other conditions ruled out
LESS COMMON	
Cantharidin toxicity	
Parasitism (strongylosis, cyathostomiasis, other)	
Aeromonas, Campylobacter	
Sand	
Carbohydrate overload	
Arsenic toxicity, other toxicities	
Thromboembolic disease	
Anaphylaxis	

3.10.2 Diagnostic Approach to the Patient With Diarrhea

Diarrhea is a common, and sometimes fatal, clinical problem of adult horses and foals. A number of specific
causes for acute and chronic diarrhea have been identified (Tables 3.10-1, 3.10-2, and 3.10-3). A comprehensive 157
evaluation may help in establishing a diagnosis and developing a treatment plan (Box 3.10-1). However, even in 158
severe cases a definitive diagnosis often is not made, making the problem particularly frustrating.[16,17]

TABLE 3.10-2 Differential Diagnoses for Chronic Diarrhea in Adult Horses

CAUSE OF DIARRHEA	MAJOR DIAGNOSTIC TEST(S)
Chronic salmonellosis	Fecal culture or polymerase chain reaction, culture of rectal mucosal biopsy
Sand	Fecal sedimentation
Parasitism (strongylosis, cyathostomiasis)	Fecal egg count, empirical deworming
Nonsteroidal antiinflammatory toxicity (primarily right dorsal colitis)	History and supportive clinicopathologic findings, ultrasonography, exploratory surgery with biopsy
Inflammatory or infiltrative disorders	Histopathologic exam, absorption tests (supportive but nonspecific)
Inflammatory bowel disease (granulomatous, lymphocytic-plasmacytic, or eosinophilic enterocolitis)	
Mucosal lymphosarcoma	
Amyloidosis	
Dietary: abnormal fermentation	History
Neoplasms: lymphosarcoma, squamous cell carcinoma	Histopathologic exam
Peritonitis, abdominal abscessation	Peritoneal fluid analysis, ultrasound, exploratory surgery
Nongastrointestinal causes (chronic liver disease, congestive heart failure, renal disease)	Physical exam, clinicopathologic findings

3.10.2.1

HISTORY AND PHYSICAL EXAMINATION

One should consider the signalment and history carefully when evaluating a patient with diarrhea. Age is particularly important because several disorders, such as foal heat diarrhea and rotavirus, are age related. The genetic background also may be significant, because diarrhea has been associated with certain heritable immunodeficiencies, and granulomatous bowel disease has been identified in three sibling horses.[18–20] Establishing whether the diarrhea is acute or chronic is important. Other historical questions of particular relevance include dietary changes, deworming program, involvement of single versus multiple animals, exposure to sand, and the use of medications, especially antibiotics and nonsteroidal antiinflammatory drugs.[21–23] Other concurrent diseases, stress, possible exposure to toxins, weight loss, water consumption, and salt availability also may be significant. The information obtained helps to prioritize differential diagnoses and direct further testing.

TABLE 3.10-3 Differential Diagnoses for Diarrhea in Foals

CAUSE OF DIARRHEA	MAJOR DIAGNOSTIC TEST(S)
Salmonellosis	Fecal culture or polymerase chain reaction (PCR)
Clostridiosis (*Clostridium difficile, C.perfringens*)	Fecal culture, toxin analysis
Endotoxemia, gram-negative septicemia	Blood culture, physical exam, complete blood count, sepsis score
Antibiotic-associated diarrhea	History
Foal heat diarrhea	History, physical exam
Viral: rotavirus; rarely coronavirus or adenovirus	Electron microscopy, enzyme immunoassay
Protozoan: cryptosporidiosis	Fecal analysis
Secondary lactose intolerance	Oral lactose tolerance test, response to therapy
Rhodococcus equi	Culture, PCR
Lawsonia intracellulare	Fecal PCR, serologic testing
Gastric ulcer disease syndrome	Gastric endoscopy
Strongyloides westeri	Fecal egg count
Sand	Fecal sedimentation

The clinician should perform a complete physical examination. The body condition of the horse and the presence of any edema should be noted. The presence of fever, dehydration, or signs of endotoxemia may help in assessing the severity of the disease and differentiating the cause, because some causes of diarrhea are not associated typically with systemic signs of illness. Careful evaluation of the abdomen should be performed. Visible abdominal distention is often an indication of large intestinal distention, which may occur in 158
association with acute colitis. However, distention also may be visible with extreme dilation of multiple loops 159
of small intestine. Careful auscultation of the abdomen can be useful in assessing motility. Generally, progressive borborygmi heard about every 3 to 4 minutes on both sides of the abdomen suggests normal motility of the cecum and colon. Auscultation behind the xiphoid process may help to identify the presence of sand or gravel if one hears particles grinding together during contractions of the colon.[24] Particularly in foals, transabdominal palpation and ballottement may be useful to identify increased abdominal fluid or large masses near the body wall. Transrectal palpation can be helpful in assessing the size of intestinal segments, consistency of contents, and wall thickness as well as in identifying masses, enlarged lymph nodes, or mesenteric arteritis.

3.10.2.1.1

BOX 3.10-1 OUTLINE OF DIAGNOSTIC APPROACH TO DIARRHEA

I. Signalment, history, and physical examination

II. Clinical pathology

1. Minimum database: complete blood count, fibrinogen, and serum chemistry profile

   a. Assess hydration, acid-base status, electrolyte abnormalities, and protein status.

b. Assess renal and hepatic function.

c. Assess endotoxemia.

2. Serum protein electrophoresis and immunoglobulin quantitation

3. Serologic testing: *Ehrlichia risticii* and *Lawsonia intracellulare*

4. Peritoneal fluid analysis

III. Evaluation of feces

1. Gross appearance: severity, hemorrhage, odor, and presence of sand

2. Direct smear: evaluation of protozoan populations and presence of leukocytes and epithelial cells

3. Parasite evaluation: including evaluation for *Cryptosporidium parvum,* especially in foals

4. Evaluation of bacterial pathogens

a. Gram stain and spore stain

b. Aerobic and anaerobic culture (culture of multiple samples or rectal mucosal biopsy for *Salmonella*)

c. Clostridial toxin analysis

d. Polymerase chain reaction: *Salmonella, E. risticii,* and L. intracellulare

5. Foals: evaluation of viral pathogens, primarily rotavirus (electron microscopy and enzyme immunoassay)

IV. Diagnostic imaging: radiography and ultrasonography

V. Endoscopic examination: stomach, rectum, and descending colon

VI. Absorption tests (glucose or xylose absorption): primarily for chronic protein-losing enteropathy

VII. Histopathologic examination

VIII. Toxin evaluation: cantharidin in urine or gastrointestinal contents, arsenic in liver, or other

IX. Response to therapy

3.10.2.2

CLINICAL PATHOLOGY

Routine analysis of blood work rarely identifies a specific cause of diarrhea but can be important in directing appropriate supportive care and may help to establish whether diarrhea is caused by another condition, such as hepatic or renal disease. Some important parameters to evaluate include the presence of leukopenia, particularly neutropenia with a left shift and toxic changes in the white blood cells. These abnormalities

suggest endotoxemia, which also may be associated with thrombocytopenia and coagulopathies. One also should evaluate the concentration of protein, as well as the albumin/globulin ratio. Significant hypoproteinemia, especially hypoalbuminemia caused primarily by protein loss, may occur with acute and chronic diarrhea. Hyperglobulinemia may indicate a chronic inflammatory condition. Disturbances in acid-base balance, especially metabolic acidosis, and electrolyte abnormalities frequently occur in cases of acute diarrhea but are uncommon in chronic diarrhea. Because of the dehydration frequently seen with acute diarrhea, prerenal azotemia is common and is important to recognize because some therapies, especially nonsteroidal antiinflammatory medications, may worsen the condition. In a study of 122 horses with acute diarrhea, horses with azotemia and clinicopathologic findings consistent with hemoconcentration and hypoproteinemia were less likely to survive.[17]

The diagnostic and prognostic value of serum protein electrophoresis has been evaluated in horses with chronic diarrhea.[25] Significantly higher levels of β_1-globulin were found in horses with larval cyathostomiais than in other horses, and such values in conjunction with a decreased albumin were helpful in diagnosing intestinal parasitism. However, a normal β_1-globulin concentration was not a reliable indicator of the absence of the disease. Significantly lower albumin concentrations and significantly higher α_2-globulin concentrations were found in horses that did not survive, suggesting that these parameters are nonspecific indicators of the severity of inflammatory changes within the intestinal wall. Parasitic infections, particularly strongylosis, also may be associated with elevated serum concentrations of immunoglobulin G(T).[26]

Infrequently, immunodeficiencies are associated with diarrhea.[18,19] Therefore in some cases, further evaluation of immune status may be indicated and may include specific immunoglobulin quantitation, 159
evaluation of specific lymphocyte subsets, or functional assays. One should consider genetic testing for severe 160
combined immunodeficiency in sick foals of Arabian breeding.

Analysis of peritoneal fluid may be useful in some cases of diarrhea. Abnormalities in the peritoneal fluid may reflect the severity of inflammation and in some cases may help to establish a specific diagnosis. Increases in protein and sometimes nucleated cell count may be seen in association with ulcerative colitis.[23] In cases of bacterial peritonitis, one may find organisms on cytologic examination or culture. Occasionally, one may identify neoplastic cells in the peritoneal fluid, although their absence does not rule out the presence of neoplasia.

3.10.2.3 EVALUATION OF FECES

Evaluation of the feces may yield important information in cases of diarrhea. Even the gross appearance of the feces can be helpful. For example, profuse, watery diarrhea is not generally consistent with a diagnosis of right dorsal colitis. Frank blood in the feces suggests bleeding into the distal colon from mucosal damage. Hemorrhagic, foul-smelling feces often are seen in association with clostridial diarrhea. One also can assess the feces for the presence of occult blood, which indicates bleeding from any source. Although excess sand in the feces is readily apparent in some cases, other cases require mixing the feces in a rectal sleeve with water and allowing the sand to settle.

Microscopic examination of the feces for evidence of parasitism and evaluation of viable protozoal populations also may be useful. A direct smear of fresh feces allows for observation of the motility of ciliates and can be used as a screen for the presence of ova and oocysts, although more sensitive techniques, including fecal flotation and sedimentation, are recommended for evaluation of parasitism. Ideally, a quantitative method that allows for estimation of the number of eggs per gram of feces, such as McMaster's or Stolley's, is

recommended. However, one must remember that fecal examination for parasites sometimes can be misleading, giving false-negative results. *Cryptosporidium parvum* infection can be difficult to diagnose, but oocysts can be detected in the feces by acid-fast staining or by immunofluorescence assay.[27]

Fecal samples also can be examined microscopically for leukocytes and epithelial cells. In general the cellularity increases with the severity of diarrhea. Fecal leukocytes and epithelial cells are increased in salmonellosis, but are not specific for this disorder.[28] More than 10 leukocytes per high-power field may indicate salmonellosis.

Evaluation of the feces for infectious agents is essential in the diagnostic evaluation of horses with diarrhea. *Salmonella* and *Clostridium* species are among the most common causes of bacterial diarrhea in horses. Other less common bacterial agents include *Campylobacter* spp., *Aeromonas* spp., and particularly in weanling age 160
foals, *Lawsonia intracellulare*.[29,30] Although primarily a respiratory pathogen, *Rhodococcus equi* also can 161
cause diarrhea, particularly in foals 2 to 4 months of age.[31] *Escherichia coli* is an uncommon cause of diarrhea in foals, unlike in calves and piglets. However, enterotoxigenic strains, characterized by the presence of virulence factors, have been identified in foals. Gram stain and spore stain of fecal smears can help to identify and quantitate the bacterial populations present, particularly clostridial species. However, although large numbers of gram-positive rods or spores have been identified in foals with clostridial enterocolitis, the results of direct staining may be misleading.[32,33] In one study, *Clostridium perfringens* was cultured from 59% of samples in which no gram-positive rods were visible. Some clostridial strains also are likely part of the normal microflora.[34] Large numbers of yeast in the feces should alert the clinician to the possibility of candidiasis, especially in compromised neonatal foals.

Fecal culture is used commonly to establish a diagnosis in cases of bacterial diarrhea. When culturing feces, especially if an outside laboratory is used, one must consider proper sample handling, particularly for anaerobic clostridia.[35] *Salmonella* spp. are one of the most significant bacterial pathogens in equine feces.[36] Although the number of *Salmonella* spp. organisms isolated from the feces of horses with clinical salmonellosis is generally greater than from horses with asymptomatic infections, the volume of feces in horses with profuse diarrhea may decrease recovery. Culture of multiple fecal samples, typically five, is recommended to increase the sensitivity. Culture of a rectal mucosal biopsy or rectal scraping is an alternative to fecal cultures and may increase sensitivity, because *Salmonella* spp. are intracellular organisms. Identifying clostridial species requires anaerobic culture. However, evaluating the presence of toxin in cases of suspected clostridial diarrhea also is critical, because *Clostridium* spp., particularly *C. perfringens* type A, may be present in normal equine feces.[34] Depending on the clostridial species and the laboratory, toxin can be assessed by detecting preformed toxin in the feces, toxin being produced by the isolate in culture, or the toxin gene in the isolate.[32–35]

An increasing number of polymerase chain reaction (PCR) assays are available for detecting causative agents of equine diarrhea. In comparing a PCR with microbial culture for detection of salmonellae in equine feces and environmental samples, the PCR method was found to be more sensitive and more rapid and required submission of fewer samples.[37,38] Currently, PCR is also available for detection of *Ehrlichia risticii,* the causative agent of Potomac horse fever, in feces and peripheral blood.[39,40] Fecal PCR analysis also has been shown to be useful in documenting equine proliferative enteropathy caused by *Lawsonia intracellulare*.[30] Serologic methods, evaluating the presence of antibodies, are additional diagnostic tests used for diagnosis of *Ehrlichia* and *Lawsonia*.[30,40]

Rotaviral infection is associated with diarrhea in foals and is most common in foals from 1 to 4 weeks of age.[4,41] One generally makes a diagnosis by detecting the virus by electron microscopy or the viral antigen by enzyme immunoassay (Rotazyme, Abbot Laboratories, North Chicago, Illinois), which is generally more sensitive than direct electron microscopy.[42] Coronavirus appears to have a low prevalence in foals but has been isolated from a horse with diarrhea.[43]

Less commonly used tests include evaluation of fecal osmolality and electrolyte concentrations (sodium and potassium). If the concentration of sodium plus potassium is much less than the osmolality, the result indicates the presence of osmotically active nonelectrolytes, confirming an osmotic diarrhea.

3.10.2.4 DIAGNOSTIC IMAGING

Diagnostic imaging, although particularly useful in foals, also can be valuable in adult horses. In foals, radiographs can detect gas distention in the lumen of the gastrointestinal tract, and the gas pattern may help to differentiate ileus from mechanical obstruction. Occasionally, gas may be seen within the bowel wall in severe cases of clostridial necrotizing enterocolitis. In adult horses, abdominal radiography is limited somewhat by having the proper facilities and equipment to perform the procedure safely. However, radiographs can be effective in identifying radiodense material, such as enteroliths and sand. Ultrasonography can be used in horses of all ages to evaluate the amount and character of the peritoneal fluid, masses, intestinal distention, and wall thickness. In cases of right dorsal colitis, the diagnosis has been supported by ultrasonographic evidence of thickening of the right dorsal colon. Altough isotope-labeled white blood cell scintigraphic scans also may help identify colonic ulcerations, the availability and sensitivity of the procedure are limited.

3.10.2.5 OTHER DIAGNOSTICS

Endoscopic examination of the stomach and proximal duodenum may reveal the presence of neoplasms or ulceration. Diarrhea and inappetance are common clinical signs in symptomatic foals with ulceration of the squamous gastric mucosa. Endoscopy also can be used for inspection of the mucosa of the rectum and descending colon, allowing for evaluation of mural masses or mucosal inflammation.

Absorption tests are used primarily in cases of chronic diarrhea or weight loss to evaluate the small intestinal absorptive capacity. Oral glucose and oral xylose absorption tests have been used.[44,45] Although the plasma concentration of glucose may reflect glucose metabolism as well as absorption from the gastrointestinal tract, the assay has been shown to be reliable in the diagnosis of significant malabsorptive conditions. Xylose is influenced less by the metabolic status of the horse, but the compound is more expensive than glucose, and the assay is not available in many laboratories. Results of both assays are nonspecific, but abnormal results support malabsorption and may indicate the necessity of biopsy.

Diagnosing neoplasms and chronic inflammatory or infiltrative disorders often requires histopathologic examination. A rectal mucosal biopsy is easy to collect and also can be cultured, but the area that can be reached for biopsy is limited. Laparoscopy allows for visualization of the abdomen and certain biopsies. One can obtain full thickness intestinal biopsy during exploratory celiotomy.

Diarrhea is a component of the clinical syndrome associated with several toxins. Cantharidin (blister beetle toxin) can be detected in urine or gastrointestinal contents.[46,47] One can measure lead in the blood and liver, selenium in the blood and liver, or arsenic in the liver if they are suspected.[47,48] One should consider oleander

toxicity in horses with diarrhea, arrhythmias, and renal disease, especially if exposure is possible.[47] Oleandrin is detectable in urine and gastrointestinal contents.

3.10.2.6 EVALUATION OF RESPONSE TO THERAPY

Evaluating the response to empirical therapy may be helpful in some cases of chronic, undiagnosed diarrhea. Dietary changes may decrease diarrhea in some cases, and often a diet of grass hay alone is recommended. In cases in which right dorsal colitis is suspected but cannot be confirmed, using pelleted feed may be beneficial. Addition of psyllium mucilloid and corn oil to the diet also may be beneficial in right dorsal colitis. Psyllium mucilloid also has been used in cases in which sand was suspected as contributing to the diarrhea. Any medications that the horse has been receiving, especially nonsteroidal antiinflammatory drugs or antibiotics, should be discontinued in case they are contributing to the diarrhea.

Transfaunation can be used in an attempt to restore normal flora. Fresh colonic or cecal contents are considered the best source of organisms, but feces can be used. A number of commercial probiotics are available, but their efficacy has not yet been established.

A course of corticosteroids can be tried in cases of chronic diarrhea in which infectious causes have been ruled out. Treatment with a larvicidal anthelmintic may be beneficial in some cases, and sometimes is used with corticosteroids. Some horses with chronic diarrhea have responded to iodochlorhydroxyquin (10 g/450 kg/day for 2 weeks). This drug sometimes has been used concurrently with trimethoprim-sulfa. Occasionally, transfusion with plasma seems to suppress diarrhea in young horses. 161

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3.11

3.11—Clinical Assessment of Poor Performance

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Any decrease in performance may be critical to the equine athlete. Numerous factors influence performance, including genetics, training, desire, and overall health. Peak athletic performance requires optimal function of all body systems, particularly those involved in locomotion and oxygen transport.

3.11.1 Approach to Poor Performance

Determining the cause of poor performance in those horses without overt clinical disease often is challenging.[1–4] In a study by Martin, Reef, Parente et al. of 348 cases of poor performance, a definitive diagnosis was established in 73.5% of cases after in-depth examination, which included the use of a high-speed treadmill.[3] Subtle abnormalities may be sufficient to impair performance, and in some cases, problems may be evident only during exercise, contributing to the difficulty of making a diagnosis. Additionally, multiple problems may occur concurrently. In a study by Morris and Seeherman of 275 racehorses with a history of poor racing performance, 84% were found to have more than one abnormality.[2] Therefore determining the actual clinical significance of any given problem may be difficult.

Equine athletes presented for poor performance should undergo a comprehensive evaluation, the basic components of which include a history, detailed physical examination, and laboratory screening. The clinician should emphasize examination of the respiratory, musculoskeletal, and cardiovascular systems, because these systems most often are linked to performance problems. In many cases, standardized exercise testing, generally on a high-speed treadmill, is critical in identifying the problem. Endoscopic examination of the upper airways during exercise has proved particularly useful.

3.11.2 History

Obtaining a complete history is a fundamental part of evaluating poor performance. The clinician should establish the use of the horse, the time in training, and the specifics of the training program. Determining whether the horse has never performed as expected or has experienced a decline in the level of performance is crucial. If the horse has never performed as expected, one should consider a lack of ability, congenital abnormalities, or training problems. A change in performance, either sudden or insidious, often is associated with an acquired problem. The clinician should characterize specifically the decline in performance, including the intensity of exercise at which signs are observed and whether performance is abnormal from the onset of exercise or declines during an exercise bout. In those cases in which performance drops off during exercise, the clinician should determine whether the decline is acute or gradual and whether any other signs such as stridor are associated with it.

Other elements of the history with particular relevance to athletic performance include any previous respiratory disease, respiratory noise, or respiratory distress associated with exercise. Any change in gait also may be significant. Establishing the feeding practices, changes in appetite or body condition, the type of tack used, and whether sweating is appropriate is important. The clinician should determine the response to any medications that have been used, such as phenylbutazone or furosemide. The information obtained in the history may help direct the investigation.

3.11.3 General Physical Examination and Laboratory Screening

The clinician should perform a complete physical examination in all cases. Hematologic testing and a biochemical profile are indicated, although in most horses presented for poor performance without obvious clinical abnormalities, routine evaluation of a single sample is within normal limits. Because exercise can induce some changes in laboratory parameters, such as an increase in the packed cell volume and neutrophil count, 163
considering the time of sample collection relative to exercise is important.[5–7] Potentially significant findings 164

include changes consistent with chronic inflammation, such as anemia, hyperglobulinemia, and possibly hyperfibrinogenemia. Subclinical infections may have only slight alterations in the leukocyte count and differential. Viral infections, especially in the early stages, may be associated with a leukopenia and neutropenia. A decrease in the neutrophil-to-lymphocyte ratio has been associated with overtraining, although this is not a reliable correlation.[7]

Horses at rest normally maintain a significant proportion of red blood cells and hemoglobin in the splenic reserve.[5,6,8] Thus although total body hemoglobin increases in response to training and may correlate with performance, such cannot be determined from a resting sample. Special techniques must be used to document total red cell mass or hemoglobin.[8,9] Anemia can decrease the oxygen-carrying capacity during exercise, resulting in suboptimal performance.

Signs of organ dysfunction in horses presented for poor performance are not common findings. Muscle enzymes may be elevated, although many cases of myopathy are subclinical and require evaluation of muscle enzymes after exercise.[10] Much attention has been paid to the importance of electrolytes and exercise; however, abnormalities seldom are found. In general, circulating electrolyte concentrations are regulated tightly and may not reflect closely the total body electrolyte status.[11] However, a concentration of potassium consistently below 3 mEq/L may suggest a potassium deficit. Chronic electrolyte deficiencies may be detected by performing renal fractional excretion of electrolytes.

3.11.4 Evaluation of the Respiratory System

The clinician should give careful attention to examining the respiratory tract, because abnormalities of this system frequently influence performance. The examination should include evaluation of air flow from the nares and percussion of the sinuses, as well as assessment of any cough or nasal discharge. Careful palpation of the larynx may reveal an increase in prominence of the muscular process of the left arytenoid cartilage resulting from a loss of mass of the left dorsal cricoarytenoid muscle associated with idiopathic hemiplegia. The clinician can use the laryngeal adductor response test, or slap test, to evaluate adduction of the arytenoid cartilages by slapping the withers during expiration and evaluating movement of the contralateral arytenoid by endoscopy or palpation. The clinician should perform a thorough auscultation of the trachea and lungs. Having the horse rebreathe from a plastic bag placed over the nostrils increases the respiratory rate and tidal volume, accentuating sounds. In addition to auscultation, one should note the character and pattern of respiration, including the presence of any abdominal component, and the recovery time. Percussion of the thorax may be useful in establishing the lung border and any dull or hyperresonant areas, as well as in detecting pleural pain.

Dynamic obstruction of the airway is among the most common causes of poor performance in the equine athlete.[2,3,12–14] In the study by Morris and Seeherman of 275 racehorses evaluated for poor performance, 40% were found to have dynamic obstruction.[2] Similarly, in the study by Martin, Reef, Parente et al. of 348 racehorses and show horses with poor performance, 148 (42.6%) had dynamic obstruction of the airways.[3] Of these 148 affected horses, 39 were found to have multiple airway abnormalities. An additional 22 horses had dynamic airway obstruction concurrently with a cardiac arrhythmia. In both studies of poor performance the most common conditions causing airway obstruction were dorsal displacement of the soft palate and idiopathic left laryngeal hemiplegia with arytenoid collapse. Other conditions diagnosed included dynamic pharyngeal collapse, epiglottic entrapment, subepiglottic cyst, rostral displacement of the palatopharyngeal arch, and redundant alar folds. An important note is that many of the horses with airway obstruction did not have a history of abnormal respiratory noise and did not have abnormalities at rest. Also, not all abnormalities observed at rest caused

obstruction. Therefore these studies emphasize the importance of treadmill videoendoscopy as a component of the evaluation of poor performance. In most cases, the clinician should perform a treadmill videoendoscopy regardless of the history and physical examination findings.

Endoscopy also can be useful in identifying respiratory problems other than dynamic airway collapse. For example, one can identify narrowing of the ventral nasal meatus associated with sinusitis, nasal masses, and pharyngitis. If the endoscope is sufficiently long, tracheal injury and secretions in the lower respiratory tract can be visualized. Sampling of airway secretions by bronchoalveolar lavage may aid in the diagnosis of low-grade respiratory infections, small airway inflammatory disease, or exercise-induced pulmonary hemorrhage. In some cases, evidence of inflammation and retropharyngeal lymphadenopathy on endoscopic examination of the guttural pouches has been associated with dorsal displacement of the soft palate, which may result from 164
neuropathy of the pharyngeal branch of the vagus nerve.[15] 165

Radiographs and ultrasound may be indicated on evaluation of the respiratory system of horses with poor performance, especially in those horses with evidence of lower respiratory tract disease. Radiographs also can be useful in assessing upper respiratory disorders, allowing for the evaluation of soft tissue masses or fluid accumulations. In addition, sometimes one can identify abnormalities of the pharyngeal and laryngeal structures such as thickening of the soft palate or hypoplasia of the epiglottis.

3.11.5 Evaluation of the Cardiovascular System

Any decrease in cardiac output potentially can limit performance, making thorough evaluation of the cardiovascular system essential. On basic physical examination, the clinician should evalutate the mucous membrane color, capillary refill time, and arterial and venous peripheral pulses, although finding abnormalities in these parameters in horses presented for decreased performance is uncommon. One should perform careful auscultation of the heart on both sides of the thorax to evaluate the cardiac rhythm and murmurs. Many horses have murmurs that are of little clinical significance.[2,16] In the study by Martin, Reef, Parente et al., 102 of the 348 horses were found to have murmurs, the most common being mitral regurgitation.[3] In all cases the murmur was determined to be clinically unimportant.

The clinician can use electrocardiography to evaluate the cardiac rhythm further, and ideally should perform the procedure before, during, and after exercise using radiotelemetry. Cardiac arrhythmias were the only abnormality found in 33 of the 348 horses evaluated by Martin, Reef, Parente et al. and were found in conjunction with dynamic airway obstruction in 22 horses.[3] However, in the study by Morris and Seeherman, arrhythmias were noted in just 2 of 275 horses.[2] The most frequent arrhythmias observed include atrial and ventricular premature depolarizations. Ventricular tachycardia and paroxysmal atrial fibrillation also have been noted. Changes in the T wave, once thought to be related to poor performance, and second-degree atrioventricular block have been found to have no effect on exercise capacity.[17]

Echocardiography before and after exercise helps to evaluate cardiac function. Martin, Reef, Parente et al. found decreased fractional shortening indicating left ventricular dysfunction after exercise in 19 horses, only 8 of which had echocardiographic changes at rest.[3] Six of the 19 horses had clinically significant arrhythmias. Myocardial disease may contribute to left ventricular dysfunction and arrhythmias. Elevations in myocardial fractions of creatine kinase, lactate dehydrogenase, and troponin support myocardial disease but are not present in all cases.

3.11.6 Evaluation of the Musculoskeletal System

A surprising number of horses presented for poor performance are found to be lame, even when lameness is not part of the presenting complaint.[1,2,4] Therefore the clinician should perform a complete lameness examination in all cases. In some horses presented for poor performance, the gait asymmetry may be subtle and only discernable at high speed, making diagnosis by traditional methods difficult. In these cases, gait analysis on the treadmill and advanced diagnostic techniques such as nuclear scintigraphy, thermography, and computed tomography or magnetic resonance imaging may be useful. One also should perform a neurologic examination to identify any deficits that could contribute to poor performance.

Myopathy can lead to decreased performance. In many cases the condition is subclinical and requires an exercise challenge test to make the diagnosis.[3,10] One should measure creatine kinase before exercise and ideally 4 to 6 hours after an exercise bout consisting of 15 to 30 minutes at the trot. In normal horses, this light exercise rarely causes more than a threefold increase in creatine kinase. An increase of fivefold or more indicates exertional rhabdomyolysis. A muscle biopsy can help to define the myopathy. In the study by Martin, Reef, Parente et al., 10 of 348 horses developed clinical exertional rhabdomyolysis after exercise, and an additional 53 demonstrated subclinical myopathy as demonstrated by increased creatine kinase levels after exercise.[3]

3.11.7 Exercise Testing

Exercise testing provides a mechanism for evaluating a range of body systems under standard exercise conditions. In particular, measurements of cardiorespiratory and metabolic function taken during an exercise test provide information about the capacity and efficiency of key body systems involved in energy production. From a clinical standpoint, exercise testing is generally most useful in assessing the effect on performance of abnormalities found on a physical examination. Testing also may help to establish the reason for reduced athletic capacity in horses that have no abnormalities on basic examinations. Exercise testing can be done in the field, which mimics the condition in which the horse actually performs. However, most testing is currently done on a treadmill, which provides more standard conditions and an opportunity to perform a greater range of measurements. The specific protocol used for exercise testing may vary somewhat.[1,18,19] Occasionally a high-speed test is performed in which the horse is accelerated rapidly to maximum speed and run to fatigue. However, the most common type of test is an incremental test in which the speed increases every 1 to 2 minutes until the horse reaches fatigue, allowing for the generation of data during submaximal and maximal exercise. In most cases the test is performed with the treadmill at a slope of 10%. This slope is not so steep as to be completely 165
unrepresentative of normal exercise, and yet it ensures that maximum intensity exercise can be performed 166
without reaching speeds that may be too fast for horse safety. Some parameters that can be assessed in an exercise test include heart rate, blood lactate level, arterial blood gases, total red cell volume, stride length, and oxygen uptake. As previously discussed, treadmill videoendoscopy is often valuable.

3.11.7.1 HEART RATE DURING EXERCISE

Evaluation of the heart rate during exercise provides an indirect index of cardiovascular capacity and function. Several heart rate monitors are available.[20] Radiotelemetry also can be used to evaluate the heart rate and rhythm, particularly at the end of exercise. Because the stroke volume does not change greatly with increasing exercise speed, the heart rate provides a guide to changes in cardiac output. In general, a linear increase in heart rate occurs with increasing exercise speed up to the point at which the maximal heart rate is reached.[21–]

[23] The maximal heart rate (HRmax) is identified when no further increase in heart rate occurs despite an increase in exercise speed. The HRmax does not change with training state, although the speed at which it is reached increases with increasing fitness.

One reference point for comparison of cardiovascular capacity is the treadmill speed at a heart rate of 200 bpm (V_{200}). At a heart rate of 200 bpm, most horses are close to the point of onset of blood lactate accumulation. The V_{200} can be calculated by linear regression analysis or plotted using measurements taken at three to four submaximal exercise speeds, without the horse reaching maximal exercise. One should take care when using the V_{200} to assess exercise capacity, because at a heart rate of 200, horses may be exercising at different proportions of their HRmax and therefore their maximal oxygen uptake (VO_{2max}). In general, however, horses with the highest cardiovascular and metabolic capacities have the highest V_{200} values; that is, the better horses reach a heart rate of 200 at higher speeds than those with a lower exercise capacity. The V_{200} increases with training and can be useful for monitoring changes in fitness. The better quality Thoroughbreds have a V_{200} of 8 to 9 m/sec in an exercise test with the treadmill set at a 10% slope. Values less than 7 m/sec are abnormal and if found in a fit horse indicate decreased cardiac capacity.

Another measurement of cardiovascular capacity is the treadmill speed at which the horse reaches HRmax, known as V_{HRmax}. This value correlates with VO_{2max} and exercise capacity but requires the horse to exercise up to maximal speeds so that a plateau in heart rate can be identified.

Heart rate measurements are helpful in determining the actual significance of cardiac abnormalities such as murmurs and arrhythmias. In horses with functional cardiac disease the reduced stroke volume necessitates higher heart rates to maintain adequate cardiac output. Also, studies in Standardbred racehorses have suggested that horses with musculoskeletal problems have an increased V_{200} and that monitoring the V_{200} may help to identify subclinical lameness.

3.11.7.2 BLOOD OR PLASMA LACTATE MEASUREMENT

Exercising muscles produce lactate to some extent during all intensities of exercise, but production increases exponentially with the intensity of exercise.[23–25] As exercise becomes more intense, the aerobic energy contribution becomes insufficient to meet total energy requirements, and increased anaerobic metabolism results in increased lactate production. Lactate diffuses from muscle to blood, and therefore blood or plasma concentrations of lactate reflect muscle lactate. Some evidence suggests that whole blood concentrations most accurately measure lactate accumulation, because red blood cells actively take up lactate.[25–28]

The rate of increase of lactate in the blood may be used as an indirect indicator of cardiovascular and metabolic capacity. Horses with the highest aerobic capacities because of a high maximal cardiac output tend to have lower lactate values at submaximal exercise intensities than those with lower aerobic capacities. Lactate values can be used to compare horses or to evaluate training in the same horse. The treadmill speed at which a plasma lactate of 4 mmol/L (V_{LA4}) is reached is one measure of lactate production, and a high value reflects good aerobic capacity. The V_{LA4} has been used to monitor changes in fitness. In fit Thoroughbred horses 3 years of age and over, values for V_{LA4} range from 8.0 to 9.5 m/sec. Horses that are not fit or have respiratory disease have lower values. Another useful reference is the blood or plasma lactate at conclusion of the 10 m/sec exercise step of the incremental test, and highly fit, athletic horses usually have values less than 5 mmol/L. High-quality sprint horses, which perform largely under anaerobic conditions and have a high anaerobic capacity, may have high peak lactate values.

3.11.7.3 OXYGEN UPTAKE

The measurement of oxygen uptake (VO_2) is critical to assessing athletic performance.[21,22] The VO_{2max} has been used as a key indicator of exercise capacity in human athletes since the 1950s. As the VO_2 increases linearly with increasing treadmill speed, VO_{2max} can be identified when VO_2 reaches a plateau despite an increase in speed. The Thoroughbred horse has VO_{2max} values that are higher than those of many other mammalian species when expressed on a mass-specific basis. The major factor responsible for the high 166
VO_{2max} in athletic horses is their high oxygen-carrying capacity, which arises from a high maximum stroke 167
volume and to some extent a large arteriovenous oxygen content difference. The VO_{2max} is a good index of changes in fitness and a measurement of exercise capacity in performance horses.

3.11.7.4 MAXIMUM OXYGEN PULSE

The oxygen pulse is defined as the VO_2/heart rate and is expressed as ml/kg/beat. This value provides an indication of the maximum stroke volume, and in high-quality horses, values range from 0.66 to 0.76 ml/kg/beat. Those horses with cardiac problems resulting in low cardiac outputs and individuals with low VO_{2max} values usually have values in the range of 0.5 to 0.56 ml/kg/beat. The maximum oxygen pulse also has been shown to correlate with treadmill total run time.

3.11.7.5 ARTERIAL BLOOD GAS ANALYSIS DURING EXERCISE

Arterial blood gas analysis during exercise may be indicated, especially in horses in which respiratory disorders are the suspected cause of poor performance. For an accurate blood gas analysis, one should take into account the temperature of the blood because it may reach 42° C during maximal exercise. At exercise intensities above 65% VO_{2max}, athletic horses become hypoxemic, although the extent varies between individuals.[29–32] Horses with low VO_{2max} values do not necessarily have a significant decrease in arterial oxygen tension.

3.11.7.6 HEMATOCRIT AND TOTAL RED CELL VOLUME DURING EXERCISE

The total volume of red cells is a major determinant of oxygen-carrying capacity, and therefore measurement of red cell volume can give some index of exercise capacity. A postexercise packed cell volume test is not a reliable indicator of total red cell volume primarily because of plasma volume variations, but it does provide a rough estimate of total circulating red cells.

One can make an accurate determination of red cell volume by techniques that use dye dilution following mobilization of the splenic erythrocyte pool to measure the plasma volume. Although total red cell volume increases with training, some evidence indicates that Standardbred racehorses with overtraining syndrome may develop an abnormal red cell hypervolemia that contributes to poor performance.[9]

3.11.7.7 PEAK RUNNING SPEED AND TOTAL RUN TIME

The peak treadmill running speed and the total run time may indicate exercise capacity. In some studies of human athletes, the peak treadmill running speed during an exercise test was shown to be a predictor of performance. Athletic Thoroughbred racehorses can complete 60 seconds at 13 m/sec during an incremental exercise test at a 10% slope.

3.11.7.8 STRIDE LENGTH

Athletic horses are thought to have better stride characteristics.[4,33] Some studies have shown a correlation between maximum stride length and the treadmill run time. An accelerometric device has been used to provide quantitative information about locomotory variables that may be useful in evaluating performance.[33]

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4 # CHAPTER 4 PHARMACOLOGIC PRINCIPLES

Patricia M. Dowling

4.1 ## 4.1—Introduction to Clinical Pharmacology

Patricia M. Dowling

Drug administration is a daily income-generating activity in veterinary practice. Before administering a drug, the veterinarian must select a safe and efficacious dosage regimen based on the physiology of the animal and the nature and formulation of the drug. If the ultimate goal of drug therapy is to improve or cure the condition of the animal, then the veterinarian is responsible for ensuring that the selected drug will be efficacious with minimal toxicity or adverse reactions in the patient and will not result in violative residual drug residues in the tissues of food animals. Individual animals of various ages and species may vary widely in their handling of an administered drug. Given that most veterinary practitioners deal with a number of different animal species and frequently administer more than one drug at time, the great potential for error in therapy and adverse drug interactions is obvious. A basic understanding of pharmacokinetics and the effects of pathophysiology on drug disposition enables the clinician to choose optimal therapy.

4.1.1 ### Pharmacokinetics

Pharmacokinetics is the mathematics of drug dosage determination and involves mathematic evaluation of the rates of drug absorption, distribution throughout the body, metabolism, and ultimate excretion from the body. Researchers usually perform basic pharmacokinetic studies in healthy animals. Unfortunately, veterinarians often do not administer drugs to normal, healthy animals. Dosage regimens derived from studies in healthy animals may not be accurate for diseased animals. Clinical pharmacokinetics is the study of the effects of disease states or other variables (age, sex, and pregnancy) on the pharmacokinetics of drugs in animals. Clinical pharmacokinetics guides veterinarians appropriately to adjust dosage regimens determined in healthy animals to optimize treatment of diseased animals.

4.1.2 ### Plasma Drug Concentrations as Therapeutic Guidelines

Most pharmacokinetic information is derived from plasma drug concentrations, even though pharmacologic action depends on drug concentration at a particular effector site, which is often a specific drug receptor. In reality, measurement of drug concentration at the receptor site is not practical. Instead, plasma (or serum) drug concentrations are measured and assumed to represent drug concentrations in target tissues. Tissue fluids or plasma perfuse most cells in the body, and drug concentrations usually reach equilibrium between tissue fluids and the blood. For most drugs, the pharmacologic action correlates well with the drug concentration in the blood. Therefore measured plasma drug concentration is assumed to represent drug concentration at the receptor sites in the tissues.

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4.1.3 ### Variation Between Drug Dose and Plasma Drug Concentration

Drug dosages needed for a therapeutic effect differ widely among individuals. The usual dose has no effect in some individuals, causes serious toxicity in others, and produces an optimal effect in a few. Several factors affect the relationship between the dosage of a drug and its concentration in plasma: its bioavailability, the body size

and fluid composition of the animal, variability in drug distribution within the body, and variability in rates of metabolism and excretion. Genetic differences in metabolism and excretion, environmental factors, disease alterations of system function and concurrent administration of other drugs affect these factors. Therefore plasma concentration of drug is not a perfect index of pharmacologic response. However, pharmacologic response relates more closely to plasma drug concentration than to drug dose. The veterinarian should never make therapeutic decisions based on the plasma drug concentration alone. One always should use knowledge of plasma drug concentration with careful medical observation and judgment.

4.1.4 Definitions in Pharmacokinetics

Pharmacokinetic information aids in determining drug dosage regimens in clinical patients. To understand how one derives drug dosage regimens and adjusts for different disease states, one necessarily must understand some basic pharmacokinetic terms. Mathematic *models* provide equations to describe drug concentration as a function of time. With an *open model*, the drug is eliminated from the body. An open model describes the fate of most drugs. With a *closed model*, the drug is recirculated within the body, as with a drug that undergoes enterohepatic recirculation. In pharmacokinetic models, a series of *compartments* that communicate reversibly with each other represent the body. A *compartment* is a tissue or group of tissues with similar blood flow and drug affinity. The drug is assumed to be distributed uniformly within a compartment. Drugs move dynamically in and out of compartments. Rate constants represent the entry and exit of drugs from each compartment. The *central compartment* is the highly perfused tissues that equilibrate rapidly with the drug. Overall drug elimination occurs only from the central compartment, because the kidneys and liver are well perfused tissues. The *peripheral compartment* is the less perfused tissues such as muscle and connective tissues. The *deep compartment* consists of slowly perfused tissues or depot tissues such as fat and bone. The presence of a deep compartment for drug distribution is important for toxins and drug residues. Most drugs in clinical use are described by one or two compartment models. Models with more than three compartments are not physiologically relevant. Describing drug disposition with compartment models creates differential equations to describe drug concentration changes in each compartment and provides a visual representation of the rate processes between compartments.

4.1.5 Rates and Orders of Reactions

The drug absorption or elimination rate is the speed with which the specified process occurs. If the amount of drug in the body (C) decreases over time (t), then the elimination rate is expressed as follows:

$$-\Delta C/\Delta t$$

The absorption and elimination rate of a drug is determined experimentally by measuring the drug concentration in the body at given time intervals. *Rate constants* relate the observed rate of a kinetic process to the drug concentration that controls the process. The elimination rate constant (K) is equal to the rate of drug elimination divided by the amount of drug in the body. The absorption rate constant (Ka) describes the rate of drug absorption into the central compartment. *Reaction order* refers to the way that drug concentration influences reaction rate.

With a *zero order* reaction the amount of drug changes at a constant time interval, regardless of the drug concentration. The rate of drug elimination is as follows:

$$\Delta C/\Delta t = -K_0$$

where K_0 is the zero order rate constant in milligrams per milliliter minute. A graph of drug concentration versus time on regular graph paper for a zero order reaction produces a straight line (Figure 4.1-1), described by the following equation:

$$C = -K_0 t + C_0$$

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where C is the drug concentration at any time (t) and C_0 is the drug concentration at time zero. For most drugs, zero order elimination only occurs when elimination mechanisms become saturated (Figure 4.1-2). Renal tubular secretion and bile secretion of drugs are potentially saturable processes. The most well-known zero order reaction is the oxidation of ethanol in human beings. Ethanol has a low molecular weight (46 d) relative to most drugs (>300 d). The alcohol dehydrogenase system becomes saturated with small amounts of ethanol. To achieve mild intoxication (1 mg/ml) throughout a 75-kg person requires an intake of about 56 ml of absolute alcohol (or 4 oz of whiskey, vodka, or gin). The maximum amount of alcohol that can be eliminated is 10 ml/hr, therefore totally eliminating the original 56 ml takes 5 hours. Therefore to maintain a constant level of mild intoxication requires only 10 ml of ethanol or 25 ml of liquor per hour.

Figure 4.1-1 Drug concentration versus time for a zero order reaction produces a straight line.

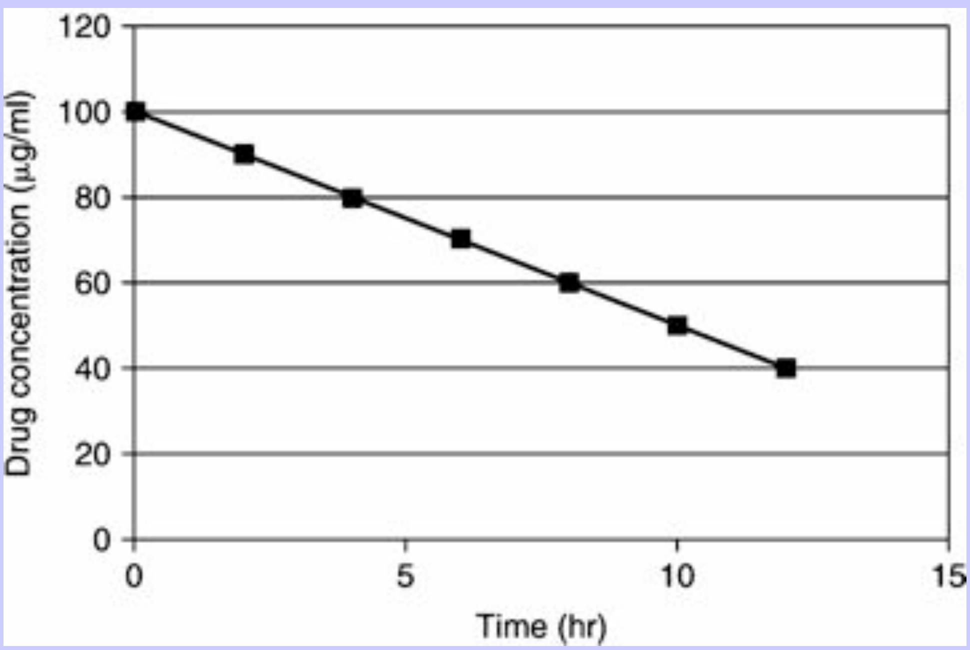
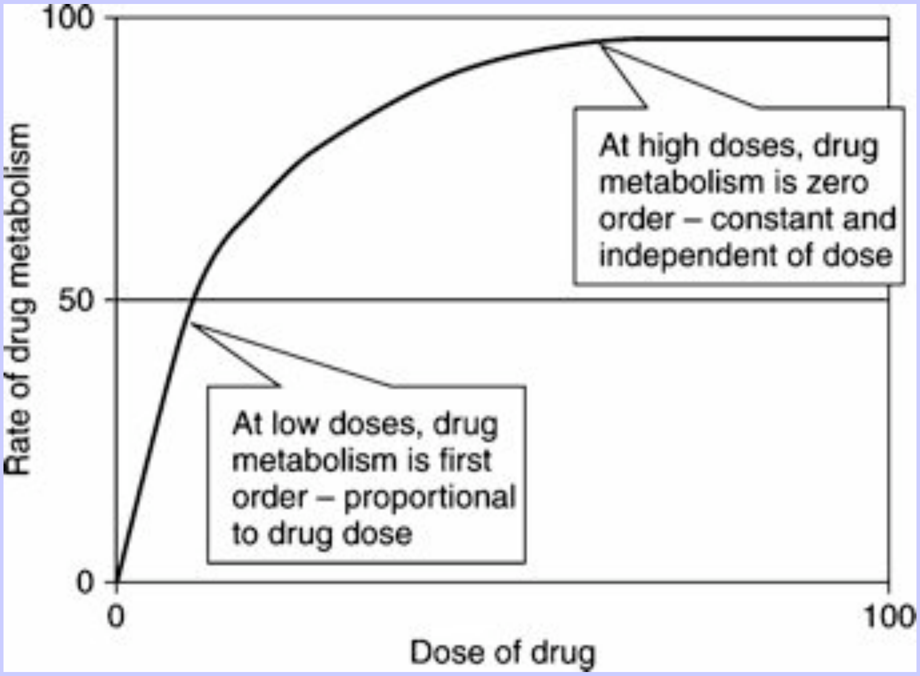


Figure 4.1-2 The effect of drug dose on drug metabolism.



With a *first order* reaction the amount of drug changes at a rate proportional to the amount of drug remaining. The first order elimination rate is expressed as follows:

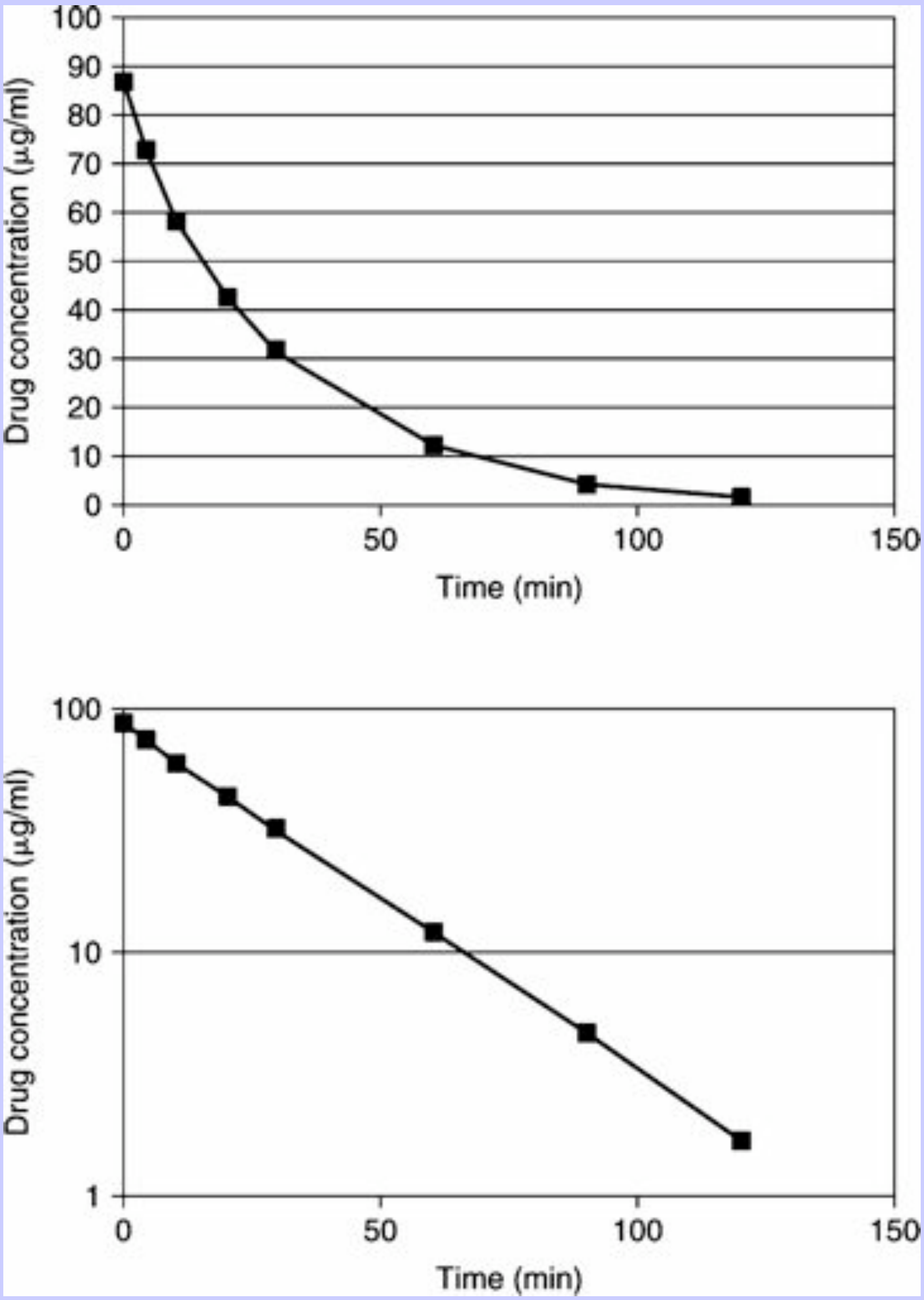
$$\Delta C/\Delta t = -KC$$

where K is the first order rate constant expressed in units of time^{-1} (minute^{-1} or hour^{-1}). K defines the fraction of drug eliminated from the body per unit time. C is the plasma drug concentration at any time (t). Although K remains constant, the rate ($\Delta C/\Delta t$) always changes, because C always decreases. A graph of drug concentration versus time for a first order reaction produces an exponential curve on regular graph paper but produces a straight line on semilogarithmic graph paper (Figure 4.1-3), as described by the following equation:

$$C = C_0e^{-Kt}$$

where C is drug concentration at any time (t), K is the first order rate constant in minutes or hours, and C_0 is the drug concentration at time zero (the moment of injection). Most drugs are absorbed and eliminated by first order processes. Glomerular filtration by the kidney is a first order process.

Figure 4.1-3 Drug concentration versus time for a first order reaction produces an exponential curve on regular graph paper but produces a straight line on semilogarithmic graph paper.



4.1.6 Clinical Application of Compartmental Modeling and Rates and Orders of Reactions

The aforementioned concepts can be combined to describe mathematically the changes in the drug concentration in the body over time. Drug disposition described by a one-compartment open model with intravenous injection and first order elimination (Figure 4.1-4) means that the body acts as one homogeneous compartment. The concentration of a drug in one part of the body is assumed to be proportional to its concentration in any other 171
part. A one-compartment open model with first order absorption and elimination describes many drugs 172
administered by routes other than intravenous, such as oral, subcutaneous, intramuscular, or intradermal (Figure 4.1-5). With a two-compartment open model with intravenous injection and first order elimination, the model assumes the body acts as two compartments: the central compartment (blood and highly vascularized tissues) and a peripheral compartment (less vascularized tissues). This model describes most drugs administered in veterinary medicine. Elimination is considered to occur only from the central compartment because the liver and kidneys are highly vascularized tissues. The plasma concentration versus time graph does not produce a straight line on semilogarithmic paper (Figure 4.1-6) but can be broken into two sections and described by the following biexponential equation:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where C is the concentration at any time (t), A is the y-intercept of the first portion of the curve extrapolated to zero and α is the slope of the line, whereas B is the y-intercept of the latter portion of the curve extrapolated to zero and β is its slope. The rate constants K_{12} and K_{21} describe movement of drug between the central and peripheral compartments.

Figure 4.1-4 Graphic representation of a one-compartment open model with intravenous administration and first order elimination.

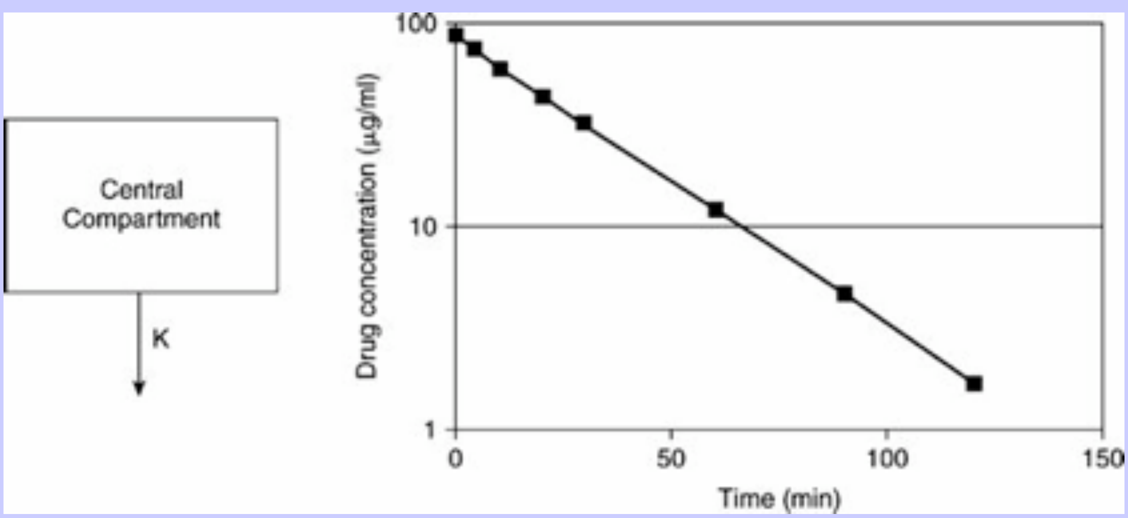
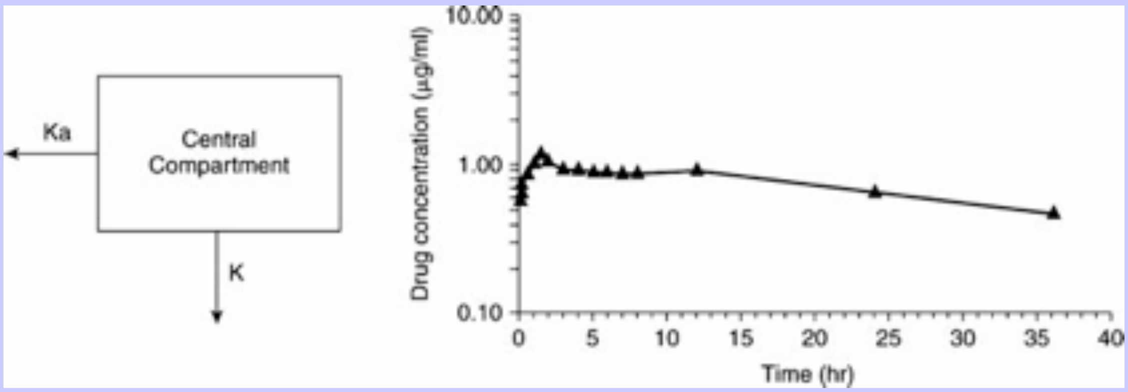


Figure 4.1-5 Plasma concentration versus time graph after intramuscular administration of long-acting oxytetracycline product to a horse, demonstrating a one-compartment open model with first order absorption and elimination.

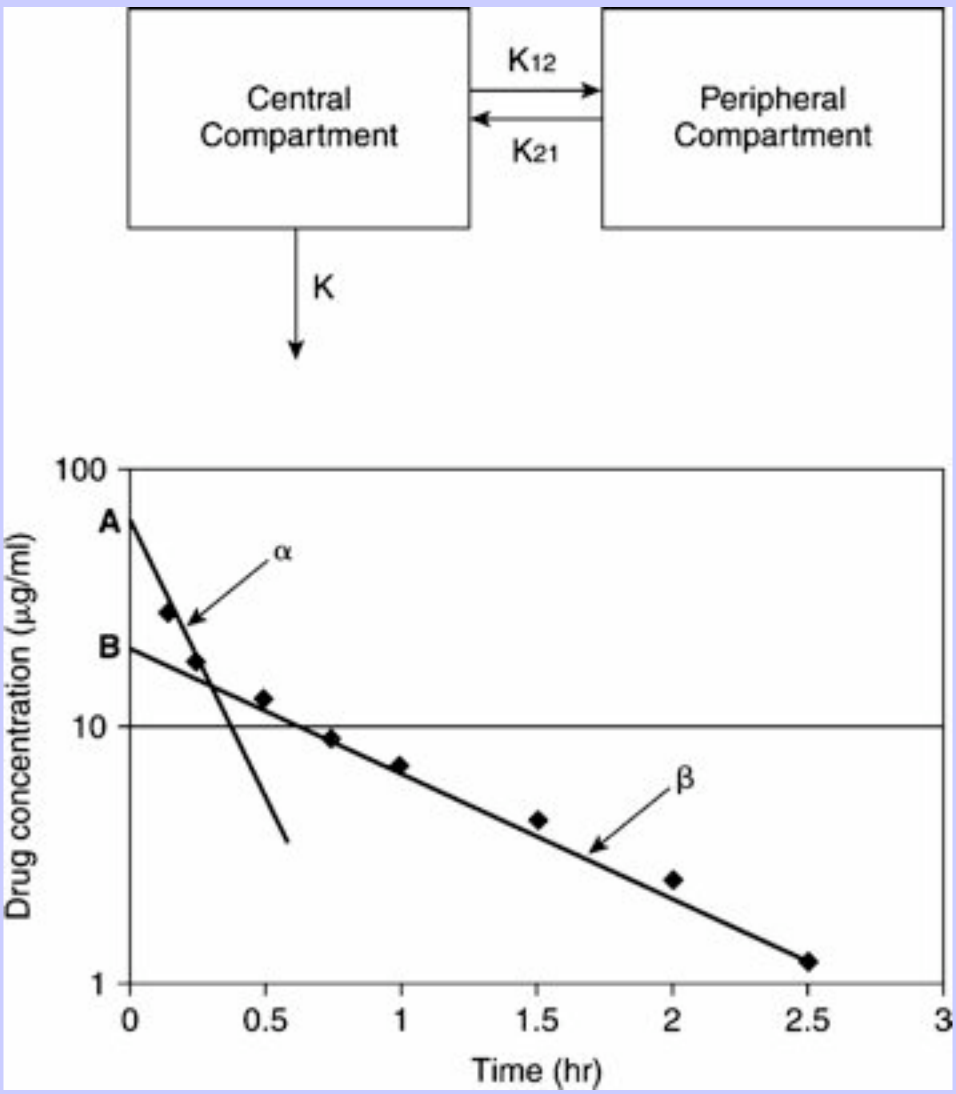


For some concentration versus time data the line can be broken into three or more straight lines and can be described mathematically with three or more exponential terms. Theoretically, drug distribution in the body can be described by as many compartments as are different tissues, but for all practical purposes, models of more than three compartments are not necessary. Drugs that are described by three-compartment models usually have some tissue site where the drug is sequestered and slowly eliminated from the body, such as the aminoglycosides, which sequester in the renal tubular epithelial cells.

4.1.7 Distribution of Drugs in the Body

The *volume of distribution* (Vd) of a drug is the mathematic term used to describe the apparent volume of the 172
body in which a drug is dissolved. The numerical value of Vd indicates the distribution of the drug in the body. 173
The ability of the drug to cross biologic membranes and reach tissues outside the vascular system determines drug distribution. The physical characteristics of the drug molecule such as ionization, lipid solubility, molecular size, and degree of protein binding determine the ability of the drug to cross biologic membranes.

Figure 4.1-6 A two-compartment open model with intravenous injection and first order elimination assumes the body acts as two compartments: the central compartment (blood and highly vascularized tissues) and a peripheral compartment (less vascularized tissues). The plasma concentration versus time graph does not produce a straight line on semilogarithmic paper but can be broken into two sections and described by a biexponential equation.



The value of Vd relates to the dose of drug administered and the amount of drug measured in a blood sample by the following equation:

$$Vd = Dose/C_0$$

where C_0 is the concentration at time zero, extrapolated from the plasma concentration versus time graph. A drug administered intravenously rapidly distributes in the extracellular fluid (ECF). A drug that does not cross lipid membranes readily will be confined mainly to the ECF. A plasma sample therefore will have a high drug concentration. The higher the measured concentration in relation to the original dose, the lower the numerical value for Vd. Drugs such as the β-lactam and aminoglycoside antibiotics are poorly lipid soluble and so remain predominantly in the ECF and have low values for Vd.

Some drugs readily cross lipid membranes and distribute into tissues. A plasma sample will have a low drug concentration in proportion to the original dose and therefore will have a high numerical value for Vd. Given the limitations in measuring drug concentrations at time zero and using the formula given previously, highly lipid soluble drugs can have measured plasma drug concentrations low enough to result in a value of Vd that is greater than 1 L/kg. Comparing the Vd of a drug to the distribution of water in the body to get an idea of the distribution of the drug is useful. Drugs with a Vd value of less than 0.3 L/kg are confined predominantly to the ECF, whereas drugs with a Vd value greater than 1 L/kg are highly lipid soluble (Box 4.1-1). In general, the higher the value of Vd, the more likely the drug molecules will reach sequestered sites such as the brain and cerebrospinal fluid, the prostate and other sex organs, the eye and the mammary gland. Although the value of Vd is useful to predict the extent of distribution of a drug, it does not confirm penetration of a drug to specific tissues. In the pharmacokinetic literature, several different terms for Vd occur, such as volume of distribution at steady state or volume of distribution by area. These values differ slightly, but they all indicate the ability of the drug to cross membrane barriers and can be considered interchangeable.

4.1.7.1 BOX 4.1-1 VOLUME OF DISTRIBUTION (Vd) OF VARIOUS DRUGS

4.1.7.1.1 Low Vd Drugs (<0.3 L/kg)

Aminoglycosides

β-Lactams

Nonsteroidal antiinflammatory drugs

4.1.7.1.2 Medium Vd Drugs (0.3-1 L/kg)

Florfenicol

Phenobarbital

Sulfonamides

4.1.7.1.3 High Vd Drugs (>1 L/kg)

Chloramphenicol

Fluoroquinolones

Macrolides

Metronidazole

Rifampin

Tetracyclines

Trimethoprim

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4.1.7.2 CONDITIONS THAT AFFECT VOLUME OF DISTRIBUTION

The Vd is constant for any drug and only changes with physiologic or pathologic conditions that change the distribution of the drug. Clinically, any condition that changes ECF volume dramatically affects the plasma concentrations of drugs with low Vd values. Changes in body water status typically do not affect drugs with high Vd values significantly. Many medical conditions affect the distribution of a low-Vd drug in a patient. Neonates have a higher percentage of body water than an adult animal (80% versus 60% total body water). The extra 20% is confined primarily to the ECF. Many conditions in horses are characterized by volume contraction and dehydration, which primarily affect the ECF. Changes in acid-base balance and alterations in protein binding also can affect the Vd of a drug.

4.1.7.3 BIOAVAILABILITY

Bioavailability (F) is a measure of the systemic availability of a drug administered by a route other than intravenous. One determines bioavailability by comparing the area under the plasma drug concentration curve versus time (AUC, area under the curve) for the extravascular formulation to the AUC for the intravenous formulation. AUC is calculated by computer or by the *trapezoidal method* in which the entire curve is divided into trapezoids and the area of each trapezoid is calculated and summed to give the AUC. For an orally administered drug, bioavailability is calculated as follows:

$$F = (AUC_{oral}/AUC_{iv}) \times 100 = \%\ \text{Bioavailable}$$

If F is significantly less than 100%, the drug dose must be increased to achieve systemic drug concentrations similar to the following intravenous formulation:

$$\text{Adjusted dose} = \text{Dose}_{iv}/F$$

If the oral formulation of a drug has a bioavailability of 50%, one must double the drug dose to achieve the same concentrations in plasma as achieved using the intravenous formulation. Poor oral bioavailability is a major limitation of many drugs administered to horses. If the bioavailability is low, the oral route of administration may not be feasible.

4.1.7.4 LIPID SOLUBILITY AND DRUG IONIZATION (THE pH-PARTITION HYPOTHESIS)

The degree of lipid solubility determines how readily a drug crosses biologic membranes. Drugs are classified as lipid soluble (or nonpolar) versus water soluble (or polar). Highly lipophilic drugs diffuse easily across almost all tissue membranes. Most of the drugs used in equine practice exist as weak acids or weak bases. Their lipid solubility depends greatly on their degree of ionization (charged state). An *ionized* drug is hydrophilic and poorly lipid soluble. A *nonionized* drug is lipophilic and can cross biologic membranes. The

degree of ionization for a weak acid or weak base depends on the pK_{α} of the drug and the *pH* of the surrounding fluid and is calculated from the *Henderson-Hasselbach* equations.

For a weak acid the equation is as follows:

$$pH = pK_a + Log(ionized\ drug/nonionized\ drug)$$

For a weak base the equation is as follows:

$$pH = pK_a + Log(nonionized\ drug/ionized\ drug)$$

When the pH is equal to the pK_a of the drug, then the drug is 50% ionized and 50% nonionized (log 1 = 0).

Although one can calculate the precise proportions of ionized versus nonionized drug from the Henderson-Hasselbach equations, one can understand the relevance of the equations simply by remembering *"like is nonionized in like."* For example, a weak acid is most nonionized in an acidic environment, so aspirin is most nonionized in the stomach and is absorbed readily. The fluid of most sequestered sites in the body (cerebrospinal fluid, accessory sex gland fluid, milk, and abscesses) has a pH more acidic than plasma. In cattle with mastitis, weak acid antibiotics are administered by intramammary infusion, whereas weak bases are administered parenterally. This makes sense from the pH-partition concept. Milk is more acidic than plasma. Weak bases in the plasma are highly nonionized and readily cross into the mammary gland, and then as the equilibrium shifts, they become ion-trapped in the more acidic milk. The fraction of nonionized drug in the mammary gland is available to cross the bacterial membrane for antimicrobial action. Weak acids are highly ionized in plasma and therefore do not penetrate into the mammary gland well and so typically are administered by local infusion.

Typically, drugs that are weak acids have low Vd values, whereas weak bases have high Vd values (Box 4.1-2). Amphoteric drugs such as the fluoroquinolones and tetracyclines have acidic and basic groups on their chemical structures. These drugs have a pH range in which they are maximally nonionized. For example, enrofloxacin is most lipid soluble (nonionized) in the pH range of 6 to 8 and so is lipid soluble at most physiologic pHs. In acidic urine, significant ionization occurs that reduces enrofloxacin antibacterial activity, but this reduction in activity is offset by the high concentration of enrofloxacin achieved in urine and so is of no clinical importance. Despite being weak bases, the aminoglycosides are large, hydrophilic molecules and are highly ionized at physiologic pHs. Therefore, parenterally administered aminoglycosides do not achieve therapeutic concentrations in milk, accessory sex gland fluids, abscesses, or cerebrospinal fluid. 174

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4.1.7.4.1 BOX 4.1-2 DRUGS CLASSIFIED BY pH

4.1.7.4.1.1 Acidic Drugs

Cephalosporins

Nonsteroidal antiinflammatory drugs

Penicillins

Sulfonamides

4.1.7.4.1.2

Basic Drugs

Aminoglycosides

Chloramphenicol

Macrolides

Trimethoprim

4.1.7.4.1.3

Amphoteric Drugs

Fluoroquinolones

Tetracyclines

4.1.7.5

DRUG-PROTEIN BINDING

Many drugs in circulation are bound to plasma proteins, mainly albumin and acute phase proteins. Bound drug is too large to pass through biologic membranes, so only free drug is available for delivery to the tissues and to produce the desired pharmacologic action. Equilibrium exists between free and bound drug, however, just as in the relationship of ionized and nonionized drug. The degree of protein binding is only clinically significant with those drugs that are more than 90% protein bound (Box 4.1-3). For these drugs, any condition that changes the amount of bound drug causes significant increases in the amount of free drug available for pharmacologic action. In addition, increasing the concentration of free drug also transiently increases the elimination of the drug as more drug enters the organs of elimination (liver and kidney), so the absolute increase in drug available for pharmacologic (or toxic) effect is somewhat lessened but still may cause clinical problems. Some of the conditions characterized by alterations in plasma proteins include hepatic insufficiency, renal failure, protein-losing enteropathies, parasitism, and burns.

4.1.7.5.1

BOX 4.1-3 EXAMPLES OF HIGHLY PROTEIN-BOUND DRUGS (>90%)

Aspirin

Ceftiofur

Diazepam

Flunixin meglumine

Furosemide

Phenylbutazone

Propranolol

Quinidine

Warfarin

Many drug interactions occur as a consequence of protein binding. If when a second highly protein-bound drug is administered, it uses the same binding site as the first drug, it can displace the first drug and increase the amount of the first drug available for pharmacologic action. The phenylbutazone-warfarin interaction is well known[1]; however, no adverse effects occur from administering aspirin and ceftiofur concurrently, because they bind to different sites.[2]

4.1.8 Drug Elimination From the Body

Drug elimination refers to the irreversible removal of drug from the body by all routes of elimination. Elimination may be divided into two major components: excretion and biotransformation. Drug excretion is the removal of the intact drug. Most drugs are excreted by the kidney into the urine. Other pathways include the excretion of drug into bile, sweat, saliva, or milk. Biotransformation (drug metabolism) converts the drug in the body to a metabolite that is excreted more readily. Enzymes involved in biotransformation are located mainly in the liver. Other tissues such as the kidney, lung, small intestine, and skin contain biotransformation enzymes.

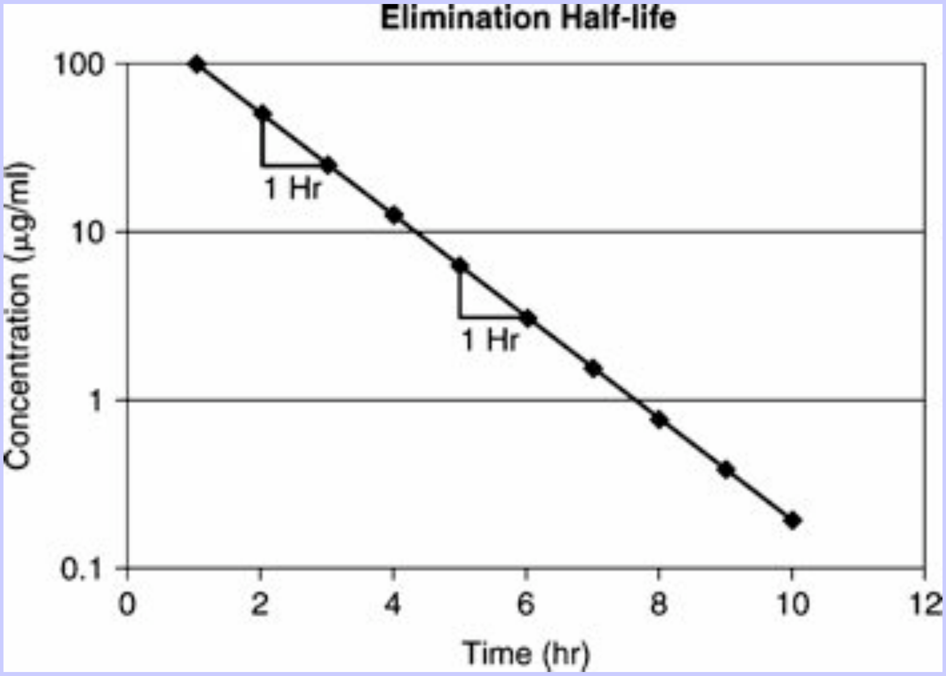
4.1.9 Elimination Rate Constant and Elimination Half-Life

The rate of elimination for most drugs is a first order process. The elimination rate constant K represents the sum of drug elimination by excretion and metabolism. Drug elimination is considered always to occur from the central compartment because the liver and kidney are well perfused tissues. The elimination rate constant is used to calculate the half-life ($T_{1/2}$) of the drug, the time required for drug concentration to decrease by one half (Figure 4.1-7). For first order reactions, $T_{1/2}$ is constant across the plasma concentration versus time curve and is calculated from the following equation:

$$T_{1/2} = 0.693/K$$

where 0.693 = ln2 (the natural logarithm of 2). Mean residence time is the equivalent of $T_{1/2}$ when pharmacokinetics are calculated using statistical moment theory. Mean residence time is actually the time the 175
drug concentration takes to decrease by 63.2%, so the mean residence time value is slightly greater than $T_{1/2}$. The 176
$T_{1/2}$ determines the drug dosage interval, how long a toxic or pharmacologic effect persists, and drug withdrawal times for food animals or performance horses. One should note that elimination of 99.9% of a drug from the plasma requires 10 half-lifes (Table 4.1-1). Knowing the $T_{1/2}$ of a drug gives the clinician some idea of the withdrawal time of the drug and propensity to cause violative drug residues. For drugs that undergo hepatic metabolism (chloramphenicol) or drugs that sequester in specific tissues (aminoglycosides, tetracyclines), simply multiplying the $T_{1/2}$ by a factor of 10 for a withdrawal time may not be sufficient to prevent a violation. One also should note that doubling a drug dose does not double the withdrawal time; it merely adds one half-life to the withdrawal time (Figure 4.1-8).

Figure 4.1-7 For a drug with first order elimination and a half-life of 1 hour, the plasma drug concentration decreases by 50% every hour.



4.1.9.1

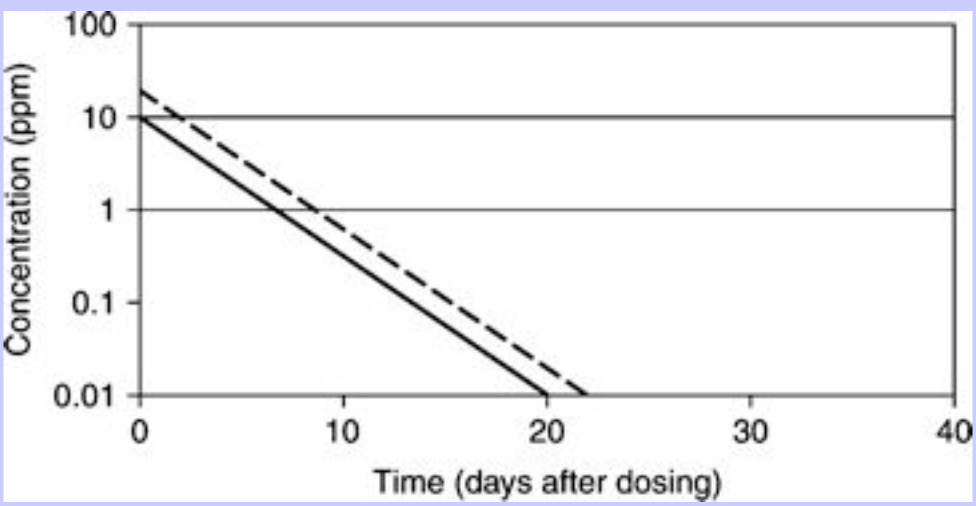
FLIP-FLOP KINETICS

Long-acting drug formulations are often products with carriers that cause them to be absorbed slowly into the systemic circulation. Therefore the drug absorption rate limits the drug elimination rate. The value for K (the elimination rate constant) calculated from the plasma concentration versus time curve is actually the value for Ka (the absorption rate constant). The easiest way to identify “flip-flop” kinetics is to compare the plasma concentration versus time curve for the extravascular route of administration with the curve after the drug is given intravenously (Figure 4.1-9). If the elimination phases of the curves are not parallel, then delayed absorption is prolonging elimination and the flip-flop phenomenon has occurred.

TABLE 4.1-1 Half-Life Elimination of a Drug

NUMBER OF HALF-LIVES	FRACTION OF DRUG REMAINING
0	100%
1	50%
2	25%
3	12.5%
4	6.25%
5	3.125%
6	1.56%
7	0.78%
8	0.39%
9	0.195%
10	0.0975%

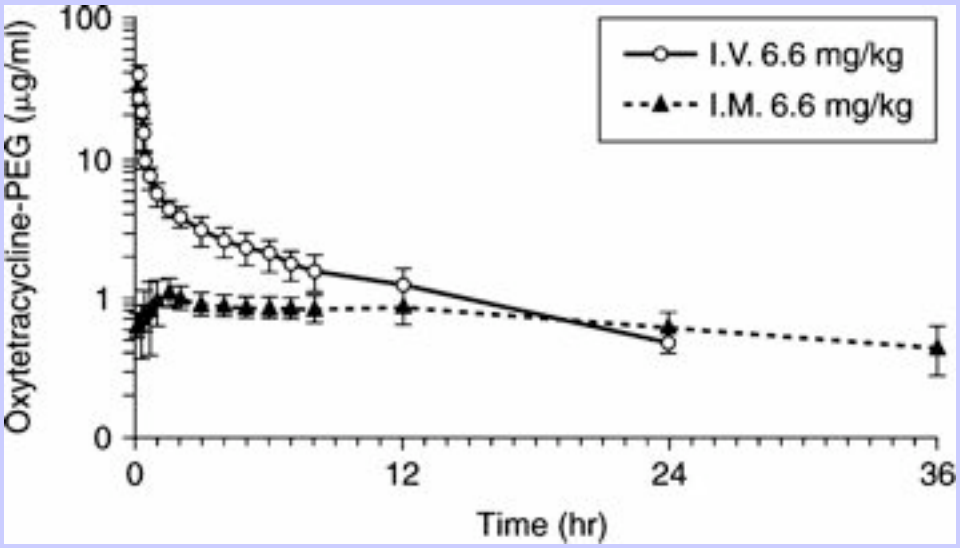
Figure 4.1-8 Doubling a drug dose only adds one half-life to its withdrawal time. Withdrawal times are based on maximum residue limits. For this drug with a half-life of 24 hours, doubling the dose to reach a plasma concentration of 20 ppm results in 21 days instead of 20 days to reach the maximum residue limit of 0.01 ppm.



For a two-compartment model, β (from the equation $C = Ae^{-\alpha t} + Be^{-\beta t}$) is the drug elimination rate constant from the entire body once the drug has reached equilibrium between the two compartments. Therefore, β is used to calculate the elimination half-life:

$$T_{1/2} = 0.693/\beta$$

Figure 4.1-9 Plasma concentration versus time graph for long-acting oxytetracycline demonstrating flip-flop kinetics. The intramuscular route demonstrates the prolonged elimination that results from delayed absorption from the injection site.



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4.1.9.2 CLEARANCE

The parameter clearance (Cl) measures drug elimination from the body without reference to the mechanism of elimination. Cl is the total drug clearance and is the sum of renal clearance (Cl_R), hepatic clearance (Cl_H), and all other elimination mechanisms. The body is considered a compartment of fluid with a definite volume (Vd) in which a drug is dissolved. Cl is the volume of fluid containing drug that is cleared of drug per unit of time (milliliter per minute per kilogram):

$$Cl = (K)(Vd)$$

$$Cl = (0.693/T_{1/2})(Vd)$$

4.1.9.3 RENAL CLEARANCE OF DRUGS

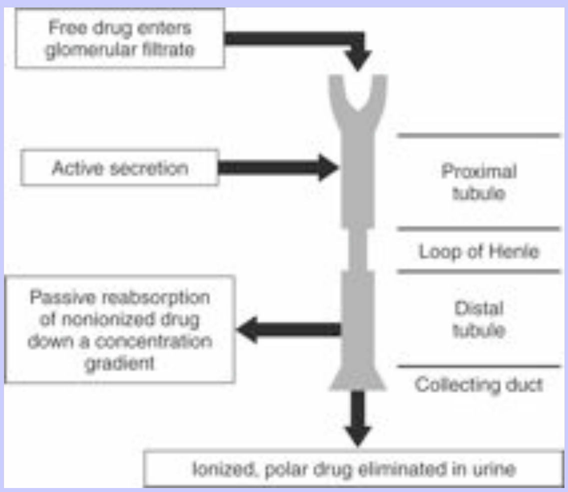
Renal excretion is the major route of elimination from the body for most drugs. Drug disposition by the kidneys includes glomerular filtration, active tubular secretion, and tubular reabsorption (Figure 4.1-10), such that renal drug clearance is defined by the following equation:

$$Cl_R = Cl_F + Cl_S - FR$$

where Cl_R is the total renal clearance, Cl_F is the clearance attributed to glomerular filtration, Cl_S is the clearance attributed to active tubular secretion, and FR is the fraction reabsorbed from the tubule back to circulation.

Glomerular filtration (Cl_F) occurs with small molecules (<300 molecular weight) of free drug (not bound to plasma proteins). Large molecules or protein-bound drugs are not filtered at the glomerulus because of size and electric hindrance. The kidneys receive approximately 25% of cardiac output, so the major driving force for glomerular filtration is the hydrostatic pressure within the glomerular capillaries. Glomerular filtration rate is estimated by measuring a substance or drug that is eliminated only by glomerular filtration, such as creatinine or insulin.

Figure 4.1-10 Movement of drugs in the renal tubule.



If Cl_R is greater than Cl_F, then some tubular secretion is occurring. Active tubular secretion is a carrier-mediated transport system, located in the proximal renal tubule. Such secretion requires energy input because drug is moved against a concentration gradient. Two active tubular secretion systems have been identified: anion secretion for acids and cation secretion for bases. Drugs with similar structures may compete with each other for the same transport system. For example, probenecid competes with penicillin or the fluoroquinolones for the same transport system, effectively decreasing Cl of these antimicrobials.[3] In patients with reduced functional renal tissue, remaining transport systems become saturated easily and drug accumulation occurs.

If Cl_R is less than glomerular filtration rate, then tubular reabsorption of drug is occurring. Tubular reabsorption is an active process for endogenous compounds (vitamins, electrolytes, glucose) and is a passive process for most drugs. Tubular reabsorption occurs along the entire nephron but primarily in the distal renal tubule. Factors that affect reabsorption include the pK_a of the drug, urine pH, lipid solubility, drug size, and urine flow. Drug reabsorption depends highly on ionization, which is determined by the pK_a of the drug and the pH of the urine. According to the Henderson-Hasselbach equation, a drug that is a weak base will be nonionized in alkaline urine and a weak acid will be ionized in alkaline urine. The nonionized form of the drug is more lipid soluble and has greater reabsorption. The pK_a of a drug is constant, but urinary pH is highly

variable in animals and varies with the diet, drug intake, time of day, and systemic acidosis/alkalosis. Species differences can have a major influence on the renal excretion of ionized drugs. Carnivores (with a urine pH of 5.5 to 7.0) have a greater renal excretion of basic drugs than herbivores (with a urine pH of 7.0 to 8.0).

4.1.9.4 HEPATIC CLEARANCE OF DRUGS

Nonrenal drug elimination is assumed to be caused primarily by biotransformation (hepatic metabolism) and biliary excretion. Clearance of a drug by the liver is determined by hepatic blood flow (Q_H) and the intrinsic ability of the liver to extract the drug (extraction ratio or ER_H):

$$Cl_H = (Q_H)(ER_H)$$

Drugs with a high extraction ratio (approaching 1) have Cl_H equal to the hepatic blood flow. These drugs are called *high clearance drugs*. Examples of drugs with high ER_H are lidocaine, propranolol, and isoproterenol. 177

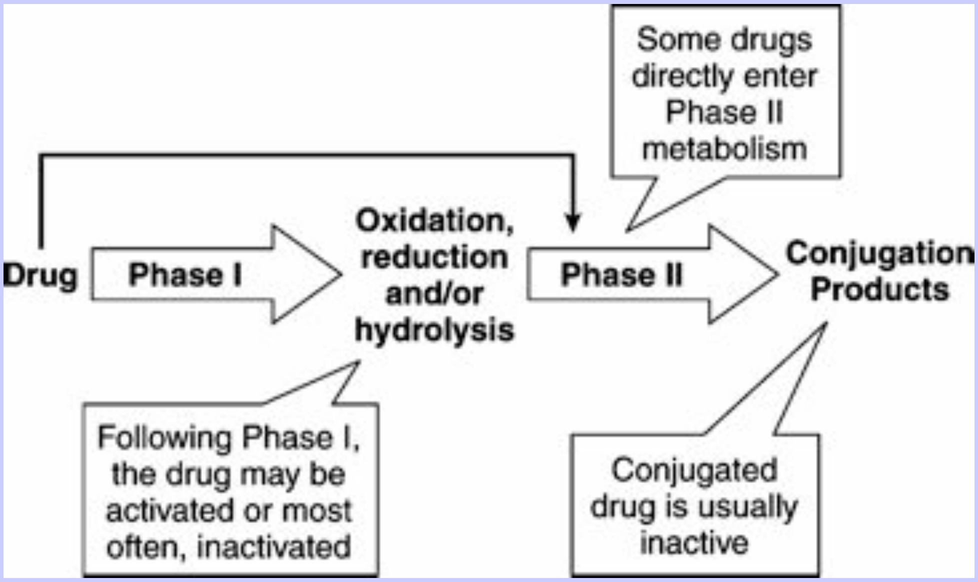
Changes in hepatic blood flow greatly influence clearance of drugs with high ER_H. Lidocaine, an antiarrhythmic drug, has an average Cl of 21 ml/min/kg after intravenous administration in dogs. Hepatic plasma flow in dogs is 20 to 26 ml/min/kg. Lidocaine is not eliminated by any other route; therefore the clearance of lidocaine is almost identical to hepatic blood flow.[4] Drugs that are administered orally and are absorbed across the intestinal mucosa must first pass through the liver via the portal circulation before being distributed to the rest of the body. Most of a drug with a high ER_H is cleared in one pass through the liver; this is called the *first pass effect*, and it limits the oral administration of many drugs. 178

Drugs that have a low hepatic extraction rate ($ER_H \leq 0.2$) are not affected greatly by changes in hepatic blood flow. However, changes in the hepatic microsomal enzyme systems and protein binding do affect their clearance. A first pass effect does not interfere with the systemic availability of these drugs. Drugs with a low ER_H include chloramphenicol, phenylbutazone, phenobarbital, and digoxin.

4.1.9.5 BIOTRANSFORMATION (HEPATIC METABOLISM) OF DRUGS

Metabolism is necessary for removal of lipophilic drugs from the body (Figure 4.1-11). Biotransformation depends on the chemical composition of the liver, activity of major drug metabolism enzymes, hepatic volume (perfusion rate), drug accessibility to and extraction by hepatic metabolic sites, and physicochemical properties of the drug. Biotransformation of a parent drugs results in metabolites that may be active or inactive themselves. A *prodrug* is a drug administered in an inactive form that must be biotransformed to its active form. Drug metabolic pathways have been divided into *phase I* and *phase II* reactions. Phase I reactions (oxidation, reduction, hydrolysis, hydration, dehioacetylation, and isomerization) typically add functional groups to the drug molecule necessary for phase II reactions. Phase II reactions (glucuronidation, glucosidation, sulfation, methylation, acetylation, amino acid conjugation, glutathione conjugation, and fatty acid conjugation) typically include conjugation reactions that increase the water solubility of the drug, facilitating its excretion from the body.

Figure 4.1-11 Hepatic metabolism of drugs.



Among the reactions catalyzed by drug metabolism enzymes, the cytochrome P-450 mixed-function oxidase system is the most intensively studied. This reaction catalyzes the hydroxylation of hundreds of structurally diverse drugs the only common characteristic of which is high lipid solubility. Species differences in drug metabolic rate are the primary source of variation in drug activity and toxicity. Cats have a poor ability to glucuronidate drugs, pigs are deficient in sulfate conjugation, and dogs are poor acetylators.

4.1.9.6

INDUCTION AND INHIBITION OF METABOLISM

Enzyme induction or inhibition by other drugs or chemicals can affect metabolism of drugs substantially (Box 4.1-4). In some cases the drug itself may alter its own metabolic rate by induction or inhibition. Many drugs are capable of inducing enzyme activity, thereby increasing the rate of metabolism and hepatic clearance of concurrently administered drugs, typically resulting in a decreased pharmacologic effect. Enzyme induction typically occurs slowly, requiring several weeks to reach maximum effect. Increased hepatic RNA and protein synthesis and increased hepatic weight accompany induction. Enzyme induction is important in the 178
pathogenesis of hepatotoxicity and therapeutic failure of many drugs. Phenobarbital is a potent enzyme 179
inducer, known for hepatotoxicity and for inducing its own metabolism.

4.1.9.6.1

BOX 4.1-4 DRUGS THAT AFFECT ENZYME FUNCTION

4.1.9.6.1.1

Enzyme Inducers

Chlorinated hydrocarbons

Griseofulvin

Phenobarbital

Phenylbutazone

Phenytoin

Rifampin

4.1.9.6.1.2

Enzyme Inhibitors

Chloramphenicol

Cimetidine

Erythromycin

Fluoroquinolones

Ketoconazole

Phenylbutazone

Prednisolone

Quinidine

Drug-induced enzyme inhibition also occurs and typically results in prolonged clearance of a concurrently administered drug. The potential for toxicity or for an exaggerated pharmacologic response increases. In contrast to induction, inhibition occurs rapidly. Erythromycin and enrofloxacin are known inhibitors of the metabolism of theophylline; concurrent administration can cause seizures.[5,6]

4.1.9.7

KINETICS OF DRUG METABOLISM

The enzymes that catalyze drug metabolism typically obey Michaelis-Menten kinetics as a *first order reaction:*

$$V = \frac{V_{max}[C]}{K_m + [C]}$$

where V is the rate of drug metabolism, K_m is the Michaelis constant, and C is the drug concentration. In most clinical situations, the drug concentration is much less than the Michaelis constant, so the equation reduces to the following:

$$V = \frac{V_{max}[C]}{K_m}$$

that is, the rate of drug metabolism is directly proportional to the concentration of free drug, and first order kinetics are observed in that a constant fraction of drug is metabolized per unit of time.

With a few drugs—such as phenylbutazone, ethanol, and phenytoin—or if large doses of a drug are given, the drug concentrations achieved are much greater than K_m and the rate equation becomes the following:

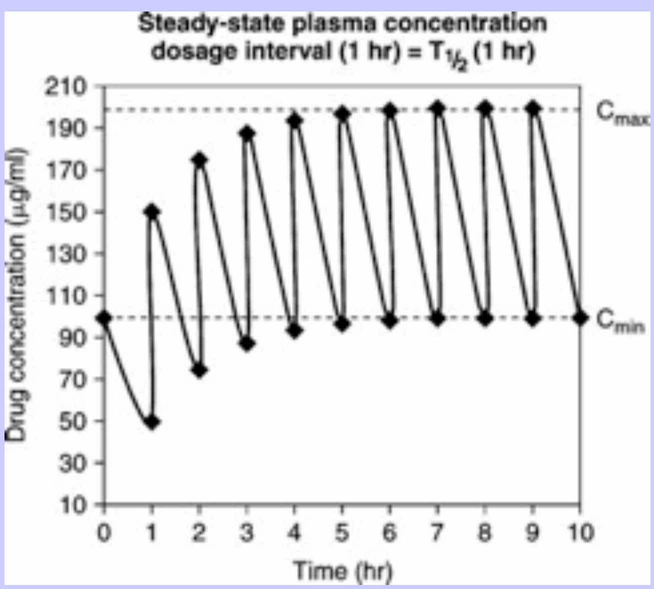
$$V = \frac{V_{max}[C]}{[C]} = V_{max}$$

The enzymes are saturated by the high free drug concentrations, and the rate of metabolism remains constant over time. This is *zero order kinetics* or *nonlinear kinetics*, and a constant amount of drug is metabolized per unit of time.

4.1.10 Drug Accumulation

Drugs often are given in multiple-dosage regimens. To predict plasma drug levels, one must decide whether successive doses of a drug will have any effect on the previous dose. The *principle of superposition* assumes that early doses of drug do not affect the pharmacokinetics of subsequent doses. For most drugs, as equal doses are given at a constant dosage interval, the plasma concentration–time curve plateaus to reach a steady state. At steady state the plasma drug concentrations fluctuate between a maximum concentration (C_{max} or peak) and minimum concentration (C_{min} or trough). Once the drug concentration reaches steady state, C_{max} and C_{min} are constant and remain unchanged from dose to dose (Figure 4.1-12). The time to steady state depends solely on the elimination $T_{½}$. Approximately six half-lives occur to reach 99% steady state levels. The drug dose and dosage frequency influence the values of C_{max} and C_{min} at steady state, whereas the dosage frequency and $T_{½}$ influence the fluctuation between C_{max} and C_{min}.

Figure 4.1-12 Plasma concentration versus time graph for an intravenous drug that produces a peak plasma concentration of 100 μg/ml after a single dose. After 6 hours (which equals six half-lives), the maximum and minimum serum drug concentration become constant and further drug accumulation does not occur. This is steady state.



4.1.10.1

CLINICAL CONSEQUENCES OF DOSAGE INTERVALS LESS THAN THE HALF-LIFE

Drugs such as phenobarbital, potassium bromide, phenylbutazone, and digoxin commonly are given at dosage intervals much shorter than their half-lives (Figure 4.1-13), resulting in a C_{max} at steady state that is greater than the peak concentration after a single dose. Minimal fluctuation occurs between C_{max} and C_{min}, and missing a single dose does not affect plasma concentrations greatly. A lag time to reach the desired plasma concentrations occurs at steady state, and a lag time for plasma concentrations to change in response to a dose change also occurs.

4.1.10.2

CLINICAL CONSEQUENCES OF DOSAGE INTERVALS GREATER THAN THE HALF-LIFE

Drugs such as intravenous formulations of penicillin and cephalosporins are administered at dosage intervals greater than the $T_{½}$ (Figure 4.1-14). As the dosage interval increases, C_{max} at steady state is closer in value to 179
the peak concentration of a single dose. If the dosage interval is greater than 10 half-lives (the time required to 180
eliminate 99.9% of the previous dose), drug accumulation essentially does not occur. Great fluctuation occurs between C_{max} and C_{min} (peak and trough), and missing a dose greatly affects plasma concentrations. However, minimal lag time to achieve the desired plasma concentration occurs.

Figure 4.1-13 Plasma concentration versus time graph for drug with a dosage interval (0.5 hour) less than the half-life (1 hour), demonstrating significant drug accumulation.

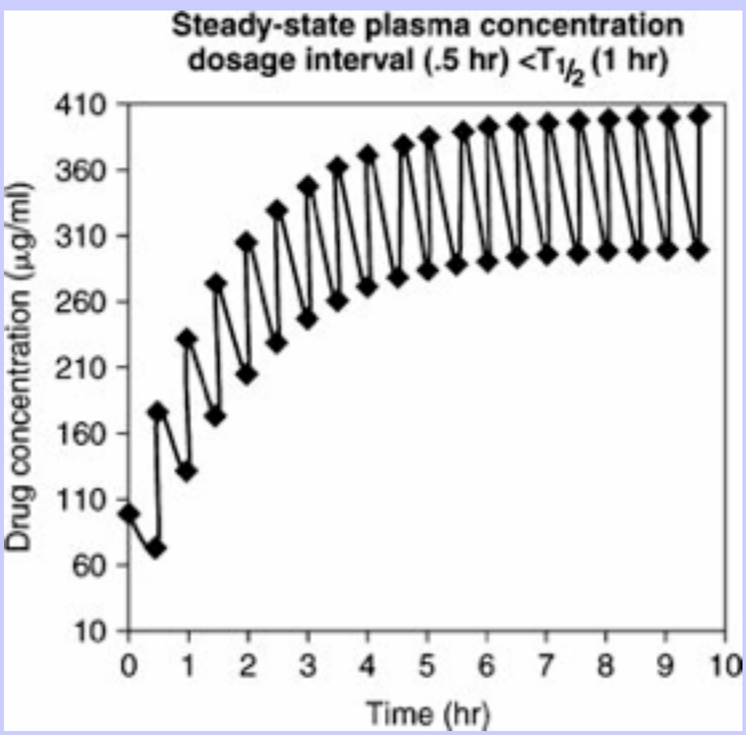
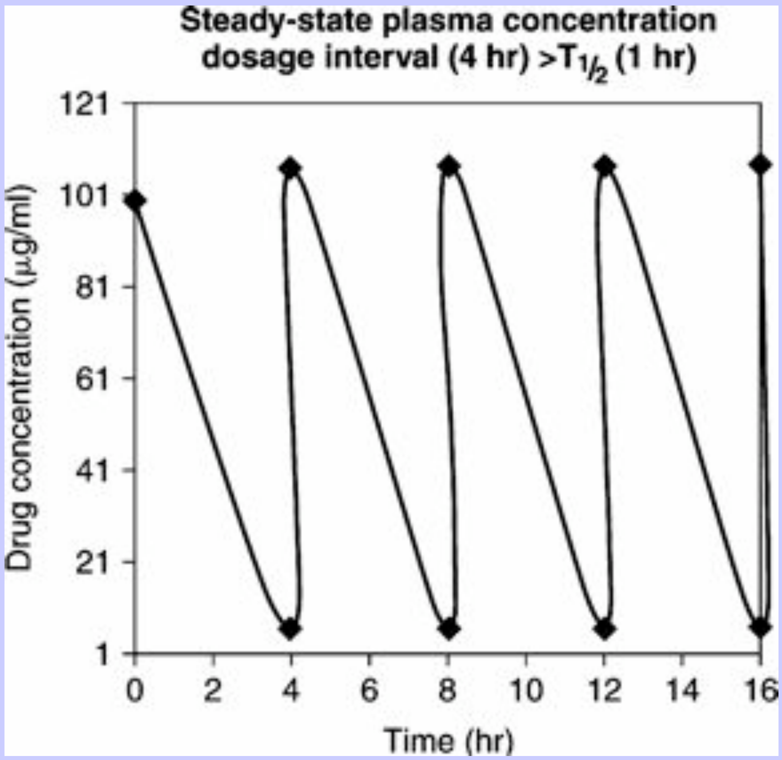


Figure 4.1-14 Plasma concentration versus time graph for a drug with a dosage interval (4 hours) greater than the half-life (1 hour), resulting in no significant drug accumulation.



4.1.11 Designing Drug Dosage Regimens

The success of drug therapy depends greatly on the dosage regimen design. Not all drugs need rigid individualization of the dosage regimen. In the case of antimicrobials with a broad safety range, such as the penicillins and cephalosporins, the dosage is not titrated precisely but rather is based on clinical judgment to maintain an effective plasma concentration above the minimum inhibitory concentration for the bacterial pathogen. For drugs with narrow therapeutic margins such as digoxin, the aminoglycosides, and theophylline, the individualization of the dosage regimen is important. The objective of the dosage regimen for these drugs is to produce a safe plasma drug concentration that does not exceed the minimum toxic concentration or fall below a critical minimum concentration at which the drug is not effective. Factors that influence the concentration of drug attained at the site of action include the dose administered, the route of administration, release and absorption of drug from dosage form, the extent of drug distribution, and the rate of drug elimination.

Age of the animal may have a profound effect on drug disposition (Table 4.1-2).[7] The definition of *geriatric* varies between species, and in small animals the definition varies between breeds. Body composition and regional blood flow change in aged animals. Cardiac output decreases, so regional and organ blood flow also decreases. These changes affect drug absorption, distribution, and elimination. Blood flow is redistributed preferentially to the brain and heart, so the risk of drug toxicity increases in these organs. Gastrointestinal

motility and absorptive capacity decrease. Hepatocyte number and function decrease along with hepatic and splanchnic blood flow. As renal blood flow decreases, glomerular filtration rate and active secretory capacity of the nephron decrease, resulting in decreased renal clearance of drugs. Lean body mass decreases, whereas fatty tissues increase. Increased body fat results in a decrease in total body water and cell mass. The plasma concentrations of water-soluble (low volume of distribution) drugs tend to increase, whereas the plasma concentrations of lipid-soluble (high volume of distribution) drugs tend to decrease. Serum albumin decreases, whereas γ-globulins increase, so that total plasma protein concentrations remain the same. Concentrations of unbound drug for drugs that normally bind to albumin increase, such as for the nonsteroidal antiinflammatory drugs.

TABLE 4.1-2 Age-Related Changes in Geriatric and Pediatric Patients

BODY PART/FUNCTION AFFECTED	GERIATRIC	PEDIATRIC
Organ blood flow	Decrease	Increase
Total body water	Decrease	Great increase
Body fat	Increase	Decrease
Serum proteins	Decrease in albumin	Decrease in albumin
	Increase in globulins	
Hepatic metabolism	Decrease	Great decrease

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The definition of *pediatric* also varies with species and age, but all the determinants of drug disposition are altered as the animal matures. Blood flow to the heart and brain is greater and faster, making the pediatric patient more susceptible to drug-induced cardiotoxicity and neurotoxicity. Gastrointestinal absorption tends to decrease because of decreased gastric emptying and decreased intestinal peristalsis. Absorption from intramuscular and subcutaneous sites changes as muscle mass and blood flow change. Neonates have less fat and greater total body water (primarily ECF) than adults. Therefore low Vd drugs distribute into a large volume, requiring an increase in the dose to avoid therapeutic failure. Because of low body fat, lipid soluble drugs have higher plasma concentrations. Drug elimination by hepatic metabolism and renal excretion is limited in pediatric animals.

The sick animal usually has impaired drug detoxifying ability. Hepatic damage reduces drug metabolism and may increase drug action, whereas renal damage and impaired excretion decrease drug clearance. Alterations in gastrointestinal motility affect drug absorption. Decreased plasma proteins increase free drug available for effect. Peripheral circulation decreases in shock and decreases absorption of intramuscularly and subcutaneously administered drugs.

4.1.11.1 PHARMACOKINETIC-BASED DOSAGE REGIMENS

A drug dose regimen is composed of a dose and a dosing frequency. Some drugs are given as single doses, so a specific plasma concentration is targeted. When giving multiple doses of a drug, one must consider the dosage frequency and drug accumulation. Simple calculations can be used to design a patient-specific dose regimen.

4.1.11.2
SINGLE-DOSE REGIMEN

A single drug dose has its duration of action determined by the size of the dose, the elimination rate constant, and the volume of distribution. To calculate a single dose of a drug or when assuming a once a day dosing, one needs only the Vd and the desired plasma concentration:

$$\mathrm{Dose} = (\mathrm{Vd})(C_0)$$

For drugs administered by routes other than intravenously, one must remember to correct for bioavailability:

$$\mathrm{Dose} = (\mathrm{Vd})(C_0)/(F)$$

4.1.11.3
CONTINUOUS INTRAVENOUS INFUSION RATE

When the desired response needs to be constant, a drug may be infused at a constant rate following the initial intravenous dose:

$$R = (C)(\mathrm{Vd})(K)$$

R (rate) is essentially the rate of drug loss from the body (milligrams per minute or per hour). So to maintain the established concentration of drug in the body, infusing the drug at the rate equal to its loss (K is the elimination rate) is necessary. For example, the vasopressive drugs dopamine and dobutamine have short elimination half-lives, so they must be administered by constant infusion.

4.1.11.4
MULTIPLE-DOSE REGIMEN

Continuous intravenous infusion offers the most precise control of drug levels in the body and is essential for precise control of drugs with a narrow safety margin or rapid elimination. Continuous intravenous infusion is not feasible for most drugs in veterinary medicine. Maintaining an average desired plasma concentration is possible by repeating doses at constant dosing intervals. Obviously, the highest plasma concentrations occur soon after drug administration and the lowest concentrations occur just before the next dose is administered. As long as the lowest concentration is acceptable for therapy, and the highest concentration does not cause toxicity, these variations in plasma concentration are acceptable.

$$\mathrm{Dose} = \frac{(C_{ave})(V_d)(\tau)}{(1.44)T_{1/2}}$$

where ι is the dosage interval and 1.44 is a constant to correct for logarithmic scale.

4.1.11.5
LOADING DOSE FOLLOWED BY MAINTENANCE DOSE REGIMEN

Drugs that have long elimination half-lives such as digoxin and the sulfonamides have a long lag time to acceptable drug concentrations; therefore they usually are given by a large loading dose followed by maintenance doses:

$$\mathrm{Dose}_L = \frac{\mathrm{Dose}_M}{1-e^{-K\tau}}$$

4.1.12 Therapeutic Drug Monitoring

The monitoring of plasma drug concentrations is valuable if a relationship exists between the plasma drug concentration and the desired clinical effect or between the plasma drug concentration and an adverse effect. Monitoring is particularly helpful in patients with gastrointestinal, cardiovascular, hepatic, or renal disease and also is useful when many drugs are being administered at the same time and may be altering the metabolic fate of each other. Therapeutic drug monitoring (TDM) is often valuable for regulating the dosage of drugs used chronically or prophylactically.

For those drugs in which plasma concentration and clinical effect are not related, one may monitor other 181
pharmacodynamic parameters. For example, one may measure clotting times in patients given anticoagulant 182
therapy. From the plasma drug concentration data and patient observations, the clinician then may adjust the dosage regimen. Therapeutic drug monitoring in veterinary medicine is done most commonly for digoxin, phenobarbital, gentamicin, and amikacin. The drugs for which TDM commonly is used are characterized by serious toxicity (digoxin, phenobarbital); a steep dose-response curve in which a small increase in dose can cause a significant increase or decrease in response (theophylline); significant pharmacokinetic variability between individual patients so that dose is poorly predictive of plasma drug concentration; easily saturated elimination mechanisms that lead to nonlinear kinetics; or the cost of therapy justifies confirming a desired plasma drug concentration (Table 4.1-3).

4.1.12.1 PERFORMANCE OF THERAPEUTIC DRUG MONITORING

The clinician should not submit samples for TDM until plasma drug concentrations have reached steady state in the patient, so most drug monitoring occurs after six elimination half-lives have passed. For conditions in which steady state concentrations must be reached immediately, the clinician can administer a loading dose. The risk of adverse drug reactions obviously increases, so the clinician can use TDM to determine proactively the proper maintenance dose. When administering a loading dose, the clinician should perform TDM after the loading dose to establish a baseline. Taking a second sample one drug half-life later ensures that the maintenance dose is able to maintain the concentrations achieved by the loading dose. If the drug concentrations at the second sample do not match the first sample, the clinician can adjust the maintenance dose at this time rather than waiting for steady state, with the risk of therapeutic failure or toxicity. The third time for TDM is at steady state.

TABLE 4.1-3 Recommendations for Therapeutic Drug Monitoring

DRUG	THERAPEUTIC RANGE	VOLUME OF DISTRIBUTION (L/kg)	HALF-LIFE	TIME TO STEADY STATE	SAMPLE COLLECTION
Amikacin	25 µg/ml peak	0.25	1–2 hours	1 day	Peak: 1 hour after administration
	<5 µg/ml trough				Trough: Just before next dose
					Collect sample in plastic only.
Bromide	Monotherapy:	–	24 days	4 months	Anytime
	2–3 µg/ml				
	(20–30 mmol/L)				
	With phenobarbitol:				
	1–2 g/ml				
	(10–20 mmol/L)				
Digoxin	0.9–3.0 ng/ml	6.7	23 hours	7 days	2–5 hours after dose Collect sample in glass only.
Gentamicin	10 µg/ml peak	0.3	1 hour	1 day	Peak: 1 hour after administration
	<2 g/ml trough				Trough: Just before next dose
Phenobarbital	14–45 µg/ml	0.96	24 hours	6 days	Anytime
	(70–170 µmol/L)				
Theophylline	10–20 µg/ml	0.8	13 hours	3 days	1–2 hours after dose

The number of samples collected for TDM depends on the drug, its $T_{½}$, and the reason for monitoring. Determining optimal aminoglycoside therapy requires C_{max} and C_{min} samples. No statistically significant difference occurs between C_{max} and C_{min} values for phenobarbital or potassium bromide in most animals when they are administered twice a day, so one can collect a single sample for TDM without regard for when the sample is taken during the dosing interval. However, one should collect C_{max} and C_{min} samples in any patient not responding as expected to therapy to determine if the individual has a shorter or longer elimination half-life than normal for the drug (Box 4.1-5). 182

4.1.12.1.1 BOX 4.1-5 INTERPRETING THE RESULTS OF THERAPEUTIC DRUG MONITORING

4.1.12.1.1.1 Plasma Concentrations Lower Than Anticipated

Poor compliance with regimen

Error in dosage regimen

Wrong drug product (controlled release instead of immediate release)

Poor bioavailability

Rapid elimination

Increased apparent volume of distribution

Steady state not reached

Poor timing of blood sample

4.1.12.1.1.2 Plasma Concentrations Higher Than Anticipated

Poor compliance with regimen

Error in dosage regimen

Rapid bioavailability

Decreased apparent volume of distribution

Slow elimination

4.1.12.1.1.3 Plasma Concentration Correct but Patient Does Not Respond to Therapy

Incorrect diagnosis of patient

Altered tissue receptor

Sensitivity (tolerance)

Drug interaction at receptor site

4.1.12.2 ADJUSTMENT OF DOSAGE REGIMENS

Adjusting the dosage regimen frequently is required as one administers drugs to diseased animals, because the dosage regimens have been worked out in healthy, normal animals. Adjustment is indicated when drug elimination or the volume of drug distribution alters significantly in the animal. In general the following are true:

- If the volume of distribution changes, one must change the drug dose.
- If the elimination half-life of the drug changes, one must change the dosing interval.

For drug dosage regimens determined by TDM, one makes modifications based on a percentage:

$$\text{New Dose} = \text{Old Dose} \times (\text{Target Concentration} \div \text{Measured Concentration})$$

4.1.13 Adjustments in Renal Failure

The ultimate route for drug elimination from the body is the kidney. Therefore that renal disease profoundly affects the disposition of drugs administered to animal in renal failure is not surprising. With reduced renal clearance, the parent drug and/or its metabolites may accumulate in the patient and cause toxicity. Loss of proteins and electrolytes in urine and the alterations in acid-base balance associated with renal failure affect the pharmacokinetics and pharmacodynamics of drugs. Enhanced drug activity or toxicity can occur because of synergy with uremic complications. Altogether these effects make determining safe and effective drug dosages difficult for veterinary patients in renal failure.

4.1.13.1 BIOAVAILABILITY AND ABSORPTION OF DRUGS

Uremic patients exhibit delayed gastric emptying, which may delay the oral absorption of some drugs.[8] Renal failure also may affect the absorptive capacity of the small intestine.[9] Gastrointestinal symptoms such as nausea, vomiting, and diarrhea are common in uremic patients and may affect drug absorption. Concomitant administration of antacids or phosphate-binding drugs, which commonly are administered to renal failure patients, may decrease drugs with pH-dependent bioavailability.[8] The fluoroquinolones, tetracyclines, ampicillin, and sulfonamides are examples of antimicrobials that have reduced bioavailability when administered with antacids.

4.1.13.2 DISTRIBUTION OF DRUGS

The Vd of many drugs changes in patients with renal failure. Fluid retention is often a characteristic of renal failure, and the consequent change in body water alters the Vd of drugs that are distributed predominantly to extracellular water, such as penicillins, cephalosporins, aminoglycosides, and nonsteroidal antiinflammatory drugs. A significant reduction in the degree of protein binding of many drugs also occurs, which is more than can be explained by the hypoalbuminemia that occurs in many glomerular diseases.[10] Conformational change in the albumin molecule is thought to be caused by uremic toxins that reduce the degree of drug binding. The accumulation of organic molecules that displace acidic drugs from their albumin binding sites also alters protein binding of these drugs. Protein binding of basic drugs tends to be normal in renal failure patients.[11] The increase in unbound drug increases the Vd, and drugs cleared only by glomerular filtration actually have an increased clearance rate. Although determining the absolute changes in protein binding in an individual patient is difficult, clinicians must realize that they can achieve therapeutic concentrations of many drugs in renal failure patients at lower than normal therapeutic concentrations of these drugs in normal animals.

Distributional changes also have been documented for drugs that are not highly protein bound.[9] Such changes may be due to changes in binding to tissues. The clinical significance of distributional changes in animals with 183
renal failure is not known, and dosage adjustments may be necessary only with drugs that are highly protein 184

bound (>99%) and are excreted primarily in the urine. Of the antimicrobials, only ceftiofur and some sulfonamides are this highly protein bound, but anticoagulants like warfarin (coumarin) and almost all nonsteroidal antiinflammatory drugs are highly protein bound. Because pharmacologic action is caused by the unbound, free drug fraction, small changes in binding of these drugs greatly increase the free drug available for such action and can have dramatic clinical consequences and should be considered when one develops treatment plans.

4.1.13.3

HEPATIC METABOLISM

Hepatic metabolism of some drugs is altered during renal insufficiency, and this effect varies considerably between species. Glycine conjugation, acetylation, and hydrolytic reactions generally are slowed in uremia. Uremia does not seem to affect glucuronide synthesis, sulfate conjugation, or methylation pathways.[12] The metabolism of cephalothin, cortisol, insulin, procaine, procainamide, salicylate, and some sulfonamides decreases in uremic human beings,[8] resulting in drug accumulation if the overall drug elimination rate decreases. The formation of renally eliminated drug metabolites is also important in renal failure patients, because some metabolites are pharmacologically active.[13] For example, enrofloxacin undergoes some hepatic metabolism to ciprofloxacin, which has greater antimicrobial activity than enrofloxacin against *Pseudomonas* spp. The high incidence of adverse drug reactions in renal failure patients is attributed in part to the accumulation of toxic metabolites. The acetylation metabolites of sulfonamides are not antimicrobially active but retain the toxicity of the parent drugs.

4.1.13.4

METABOLIC BALANCE

The uremic patient is often in a state of altered acid-base balance, electrolyte derangement, and fluid depletion. Administration of sodium- or potassium-containing antimicrobials such as sodium ampicillin or potassium penicillin may result in serious sodium overload and potassium induced neuronal disturbances. Administration of antacids, enemas, or laxatives may cause magnesium, aluminum, or phosphate intoxication. Acidosis, which occurs commonly in uremic patients, increases the free drug concentrations of some drugs such as salicylate and phenobarbital, thereby increasing drug concentrations in the central nervous system. Acidosis also increases ionic binding of the aminoglycosides, increasing accumulation in the renal tubular epithelium and enhancing nephrotoxicity. Uremic complications also may enhance drug toxicity. Uremia-induced functional changes in gastrointestinal and nervous system tissues allow adverse reactions to be induced more easily. The blood-brain barrier is altered in uremia, allowing greater drug concentrations in the central nervous system. The anabolic effect of tetracyclines and the catabolic effect of corticosteroids may worsen azotemia.[10]

4.1.13.5

DOSAGE ADJUSTMENTS

The goal of dosage adjustment is to provide a drug concentration–time profile in the renal failure patient that is as similar as possible to a normal patient. The best approach to modifying drug therapy in renal failure patients is to carry out therapeutic drug monitoring and adjust the dosage for each patient. This is possible with some drugs, such as gentamicin and amikacin, but is impractical and cost-prohibitive for most drugs used in veterinary practice. The best approach for most drugs is to estimate a corrected dose from available renal function tests and then to monitor the patient closely for evidence of efficacy or toxicity. For drugs eliminated primarily by renal mechanisms, creatinine clearance correlates well with drug clearance. Creatinine is an endogenous product of creatinine phosphate metabolism in muscle and is removed by glomerular filtration, and serum concentrations are relatively constant in healthy persons and animals. The elimination $T_{1/2}$ of a drug

eliminated in urine remains stable until creatinine clearance is reduced to 30% to 40% of normal, which is why drug dosage regimens typically are not adjusted until two thirds of renal function has been lost. In human patients, creatinine clearance is quantified by determining urinary creatinine excretion over 24 hours. The measured creatinine clearance then is used in formulae to make drug dosage adjustments. Unlike in human medicine, values for creatinine clearance are not usually available for veterinary patients. When creatinine clearance is not available, a single value of the patient's serum creatinine can be substituted into the formulae. However, the relationship between serum creatinine is not linear once serum creatinine is greater than 4 mg/dl, so the adjustment formulae are even less accurate for predicting an ideal dose adjustment.[10] These formulae do not account for changes in the volume of distribution, degree of protein binding, and nonrenal clearance mechanisms of the drug that may be caused by the renal dysfunction. Therefore one must regard these dosage adjustments as preliminary estimations to be followed by adjustments based on observed clinical response.

With the dose-reduction method, one adjusts the normal dosage regimen by reducing the drug dose and maintaining the drug dosing interval:

$$\text{Adjusted Dose} = \text{Normal Dose} \times (\text{Patient's Cr Clearance}/\text{Normal Cr Clearance})$$

or

$$\text{Adjusted Dose} = \text{Normal Dose} \times (\text{Normal Cr}/\text{Patient's Cr})$$

where Cr is creatinine. 184

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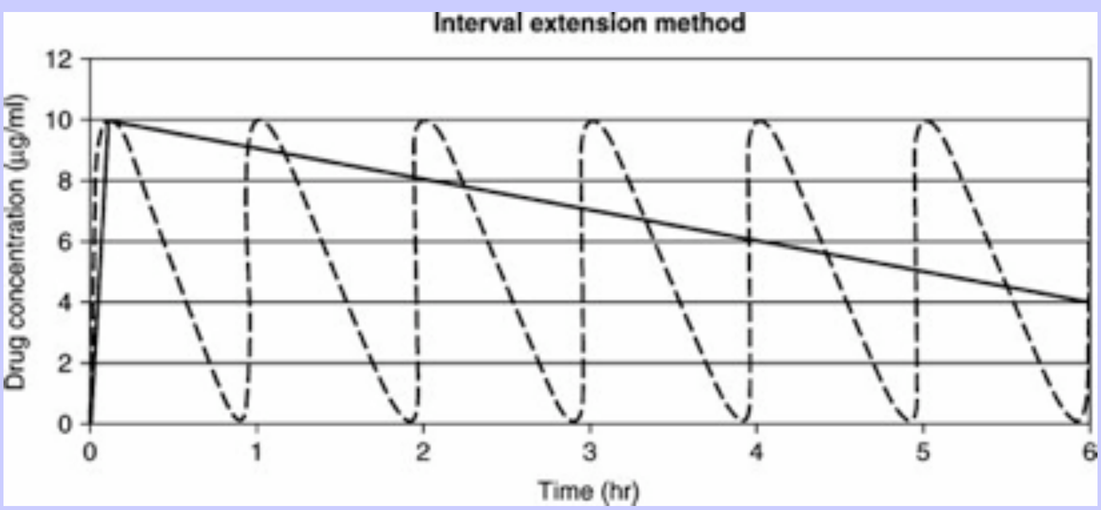
Figure 4.1-15 Comparison of an interval extension dosage regimen *(solid line)* in a renal failure patient with a normal dosage regimen in a healthy patient *(dotted line)*. Normal elimination half-life was 15 minutes; in the renal failure patient half-life increased to 8 hours.



With the interval extension method, one maintains the drug dose and extends the drug dosing interval:

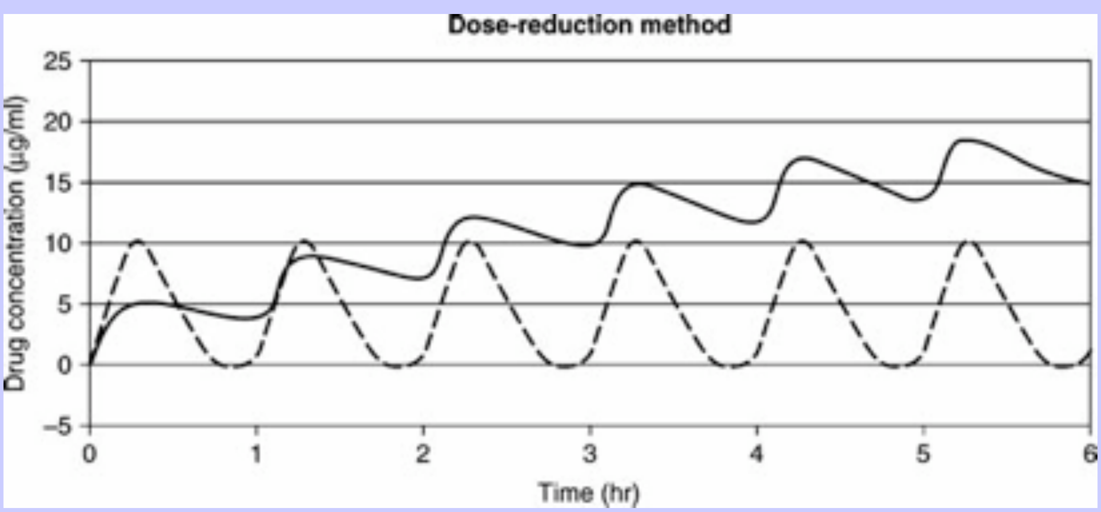
$$\text{Adjusted Interval} = \text{Normal Interval } [1/(\text{Patient's Cr Clearance}/\text{Normal Cr Clearance})]$$

or

$$\text{Adjusted Interval} = \text{Normal Interval } [1/(\text{Normal Serum Cr}/\text{Patient's Serum Cr})]$$

Both methods attempt to keep the average plasma drug concentrations constant. The interval extension method produces C_{max} and C_{min} values similar to that seen in healthy patients (Figure 4.1-15), but it does produce substantial periods of time during which drug concentrations may be subtherapeutic. Interval extension is the preferred method with aminoglycosides, which have a long postantibiotic effect and for which a low trough concentration is desirable to reduce the risk of nephrotoxicity. Depending on the relationship of the elimination $T_{1/2}$ to the dosage interval, significant drug accumulation may occur with the dose-reduction method (Figure 4.1-16), but at steady state no periods of time occur during which concentrations are subtherapeutic.[14] Dose reduction is the preferred method for the penicillin and cephalosporin antibiotics, for which maintaining the plasma concentration above the minimum inhibitory concentration of the pathogen correlates with efficacy and the drugs are relatively nontoxic even if accumulation occurs. To decide which method to use, the practitioner should determine if drug efficacy and toxicity are related to peak, trough, or average plasma concentrations and then should select the method that balances efficacy against potential 185
toxicity. The interval extension method is more client convenient, because one simply administers the normal 186
recommended dose less frequently. And if the drugs are available only in fixed dosage forms (e.g., capsules or unbreakable tablets), adjusting the dosage interval is easier.

Figure 4.1-16 Comparison of dose-reduction dosage regimen in renal failure patient *(solid line)* with normal dosage regimen in a healthy patient *(dotted line)*. Normal elimination half-life was 15 minutes; in the renal failure patient half-life increased to 8 hours.



Because the elimination half-life is prolonged in patients with renal disease and because six elimination half-lives always are required to reach 99% of steady state concentrations, a delay occurs in reaching steady state in renal failure patients compared with animals with normal renal function. Therefore the clinician may need to administer a loading dose to achieve therapeutic drug concentrations rapidly. For the dose-reduction method one achieves this by giving the usual dose initially, followed by the reduced dose the next time. For the interval extension method one achieves therapeutic drug concentrations by giving a double dose initially.

For renal failure patients in general one must consider the following:

1. One should avoid using any drugs at all unless definite therapeutic indications exist. If one must use a drug, one should try to select one that is metabolized hepatically and excreted in bile rather than eliminated by the kidneys (e.g., doxycycline).

2. If therapeutic drug monitoring is available, one should tailor the drug dosage regimen to that specific patient.

3. If therapeutic drug monitoring is unavailable, one should determine if clinically proven, adjusted dosage regimens are available for specific drugs. The package insert on human pharmaceuticals often gives guidelines for adjusting dosages.

4. If the drug has not been studied sufficiently to have dosage adjustment recommendations, one should determine whether sufficient information exists about its kinetics to estimate the proper drug dose in renal failure.

5. One should carefully monitor treated patients for signs of efficacy and toxicity.

4.1.14

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4.2

4.2—Antimicrobial Therapy

Patricia M. Dowling

In recent years many important changes have occurred in antimicrobial therapy. New antimicrobials are available and a greater database of species-specific pharmacokinetic information is available for antimicrobials used in equine medicine, which allows for more accurate drug dosing. Concerns over drug residues in food animals and the continued development of bacterial resistance has heightened the awareness of rational use of antimicrobials. 186

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4.2.1

Rational Use of Antimicrobial Agents

One must consider the following questions when developing an antimicrobial regimen:

1. *Does the diagnosis warrant antimicrobial therapy?* Using antimicrobials to treat minor infections or purely viral or inflammatory diseases is irrational and expensive, can be hazardous to the patient, and encourages antimicrobial resistance. Clients have come to expect antimicrobials for trivial infections or just in case an infection may develop. Equine practitioners must resist client pressure to use or prescribe drugs unnecessarily.

2. *What organism(s) are likely to be involved?* For many infections, the likely organism can be predicted successfully from the history and clinical signs.

3. *What is the in vitro antimicrobial susceptibility of the organism?* For many pathogens, the in vitro susceptibility can be predicted reliably. For example, *Streptococcus* species are typically susceptible to penicillin. However, many gram-negative bacteria have unpredictable susceptibilities, and susceptibility testing is essential for determining appropriate drug therapy.

4. *In what part of the body or tissue is the infection located? Will the antimicrobial penetrate to the infection?* Consideration of the pathophysiology of the infection aids in selecting effective therapy. Treating sequestered infections such as mastitis or meningitis requires antimicrobials that readily cross membrane barriers. Antimicrobials characterized by low values for volume of distribution (Vd) are unlikely to reach therapeutic concentrations in such sites.

5. *Will the antimicrobial be effective in the local environment of the organism?* For some antimicrobials the local infection environment reduces their efficacy. Sulfonamides are ineffective in purulent debris, because paraaminobenzoic acid (PABA) released from decaying neutrophils serves as a PABA source for bacteria and reduces the competitive effect of the sulfonamide. Aminoglycosides are ineffective in an abscess because of the acidic, anaerobic environment along with the presence of nucleic acid material from decaying cells that inactivates the aminoglycosides.

6. *What drug formulation and dose regimen will maintain the appropriate antimicrobial concentration for the proper duration of time?* Label doses only apply to label pathogens. When treating off-label, one must adjust the dosage regimen for the antimicrobial susceptibility of the specific pathogen.

7. *What adverse drug reactions or toxicities might be expected? Do the benefits outweigh the risks?* The risks of adverse reactions from antimicrobials often are underappreciated. A serious adverse reaction may complicate treatment of the original problem and even may be fatal. Failure to communicate the risks of adverse drug reactions to clients is a common cause of litigation.

8. *Can one choose an approved product? If using an antimicrobial in an extralabel manner, can one determine appropriate withdrawal times for food animals? Can one determine appropriate withdrawal times for performance horses?* The antimicrobials used in horses are not approved for horses intended for food and lack information on appropriate withdrawal times. Competitive horses are subject to the rules of the association governing the sport regarding drugs, which may vary between organization, state or province, and country. Procaine from procaine penicillin G is one of the most common drugs involved in violations. Understanding the principles of drug elimination allows the practitioner to determine appropriate withdrawal times for competitive or slaughter horses.

4.2.1.1

DOCUMENTING THE INFECTION

One must establish a diagnosis before administering any therapy. Culturing samples from all patients with infectious diseases is not always necessary to identify the organism involved. Often the practitioner can base a diagnosis on clinical experience from similar cases. The signs of some infectious diseases are so obvious that the need for microbiologic identification is minimal; but for those infectious diseases of unknown cause or for those attributable to organisms with irregular antimicrobial susceptibility, no substitute exists for isolation and identification of the causative agent. For these organisms, initial therapy while one waits for culture results must include an antimicrobial with a broad spectrum of activity. However, broad-spectrum drugs are usually more toxic and more expensive than narrow-spectrum drugs. Use of broad-spectrum antimicrobials for trivial infections encourages development of antimicrobial-resistant organisms. Without evidence of a susceptible pathogen, such use is irrational and exposes the patient to unnecessary risks.

Whenever possible, the practitioner should obtain a representative sample of material from the patient. One must beware of sampling grossly contaminated sites such as purulent nasal discharges. One can perform a gram-stain immediately from a direct smear and can direct initial therapy. One should submit samples for appropriate culture and identification. If the identified organism has unpredictable susceptibility patterns, one should request a susceptibility test such as Kirby-Bauer, E test, or minimum inhibitory concentration (MIC) method. In some clinical cases, identification of the pathogen may be made by serologic demonstration of antibodies (e.g., ehrlichiosis, leptospirosis, and brucellosis). 187

4.2.1.2 ANTIMICROBIAL DOSAGE REGIMEN DESIGN

Successful antimicrobial therapy relies on administering sufficient doses so that pathogens at the site of infection are killed or sufficiently suppressed that they can be eliminated by the host immune system. The relationship between the host, bacteria, and drug may be complex. High plasma antimicrobial concentrations are *assumed* to be advantageous in that a large concentration of drug will diffuse into various tissues and body fluids. Drug concentration at the infection site is assumed to be of major importance in determining drug efficacy. Drug movement from plasma to extravascular tissues depends on molecular size, lipid solubility, drug pK_a, local pH, specific cellular transport mechanisms, and degree of protein binding. The relationship between bacteria and drug in the laboratory is described by the following:

Minimum inhibitory concentration: The lowest drug concentration that inhibits bacterial growth.

Minimum bactericidal concentration (MBC): The lowest drug concentration that kills 99.9% of bacteria.

MIC is used to determine drug dose in an attempt to achieve blood and tissue concentrations that exceed the in vitro MIC for the pathogen.

4.2.1.3 INTERPRETING MINIMUM INHIBITORY CONCENTRATIONS

By the National Committee on Clinical Laboratory Standards (NCCLS) definition, MIC values are derived as serially doubling concentrations (in μg/ml).[1] The MIC of a particular pathogen is reported as one of the following numbers:

0.06	0.12	0.25	0.5	1	2	4
8	16	32	64	128	256	512

Susceptible (S), intermediate (I), and resistant (R) designations are derived from break points assigned by the laboratory based on safely achievable *plasma* concentrations and results of clinical trials.[2] When a pathogen is reported as *susceptible*, the recommended dosage of the antimicrobial will reach plasma or tissue concentrations that will inhibit bacterial growth in vivo. When a pathogen is reported as *resistant*, inhibitory antimicrobial concentrations are not safely attainable in the patient. If the pathogen is reported as *intermediate*, then administering the antimicrobial at higher than recommended doses may result in effective therapy.[3] The relationship between drug concentration and microbial inhibition is not a linear predication. As antimicrobial concentration increases in vitro, eventually all bacteria will be inhibited or killed. This fact should not be interpreted to mean that dosage regimens should target these concentrations. Susceptibility testing results predict which bacteria have intrinsic or acquired resistance mechanisms to a particular antimicrobial. In vitro tests do this because bacterial susceptibility usually clusters around a small range of MICs. In Figure 4.2-1 the bacterial inhibition of *Escherichia coli* by amoxicillin has a bimodal distribution. *E. coli* requiring an amoxicillin concentration of 16 μg/ml to be inhibited are likely to have intrinsic or acquired resistance mechanisms, and amoxicillin is unlikely to be a successful treatment for patients with this infection. The large cluster of *E. coli* inhibited by amoxicillin concentrations of 0.5 to 16 μg/ml are considered in the *normal*

range, and 16 μg/ml is considered the break point of susceptibility. As the MIC value increases within the normal range, the probability of successful therapy with amoxicillin decreases; meaning some susceptible *E. coli* are *more* susceptible than others. In vitro susceptibility tests predict treatment outcome fairly well, considering that many variables in the host-pathogen relationship are not taken into account.[2] Antimicrobial susceptibility data may not account for the following[4]:

1. *Host defenses*. The interactions between the host and the pathogen are complex and are not predicted by in vitro tests. Antimicrobial drug action takes place in concert with host defenses such as humoral and cell-mediated immunity, complement components, and nonspecific antibacterial factors such as lactoferrin, lactoperoxidase, and lysozyme.[5]

2. *Drug distribution in the body*. The S, I, and R designations assigned by the microbiology laboratory are based on safely achievable plasma concentrations and do not take into account extremely high concentrations of antimicrobials achieved in organs and fluids of excretion (kidney, urine, and bile) or with local administration of high drug concentrations (e.g., ophthalmic ointments). Pathophysiology may alter drug distribution, and some antimicrobials, such as the tetracyclines, accumulate in pneumonic lung tissues.[6]

3. *Growth rates and size of inoculum*. The incubator of the microbiology laboratory is an ideal world for bacterial growth. Conditions are managed to promote rapid growth, and rapidly dividing bacteria are more susceptible to antimicrobial drugs. Replication rates may be much slower at the infection site, and MICs are generally unreliable for slow-growing bacteria. Standardized inoculums used in the laboratory may over- or underrepresent pathogen numbers in infected tissues.[5]

4. *Mixed infections*. Separate susceptibility testing of pathogens in a mixed infection does not account for the pathologic synergism between bacteria. The by-products of one bacterial infection may facilitate the establishment and growth of another.[7]

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5. *Infection environment*. Many antimicrobials are inactive in purulent exudate, which is typically anaerobic, acidic, and hyperosmolar. Some antimicrobials have different activity in body fluids (plasma, milk, bile) than in nutrient-rich laboratory media. Deposition of fibrin may alter tissue penetration of antimicrobials. Many bacteria are capable of producing a polysaccharide slime capsule to protect them from host factors. Mastitis pathogens typically increase their replication rate when incubated in mastitic milk.[5]

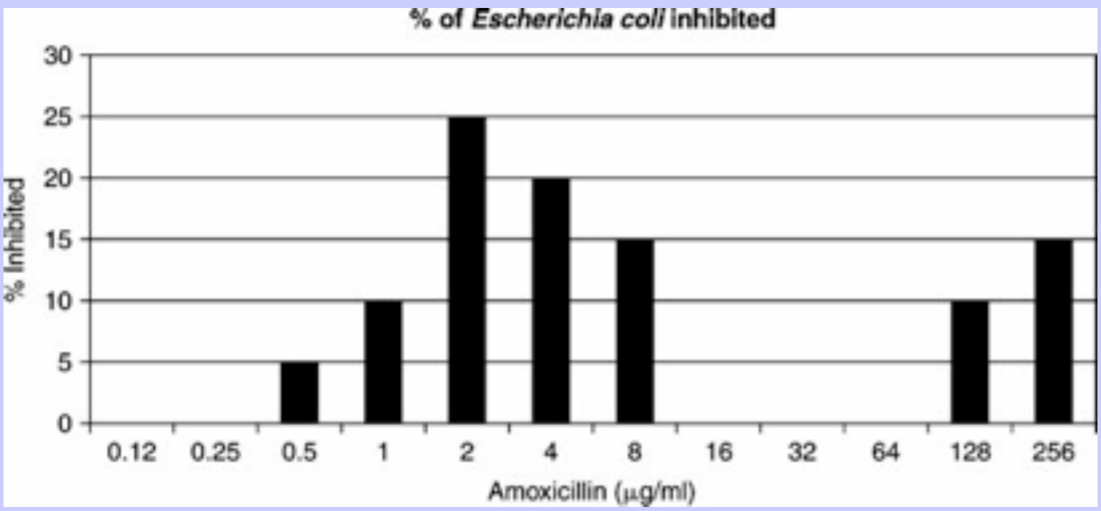
6. *Topically administered antimicrobials are not tested*. Veterinary microbiology laboratories may not do susceptibility testing routinely for antimicrobials that are used only topically. Polymyxin B is one of the most effective antimicrobials for superficial *Pseudomonas* infections,[8] but it causes neurotoxicity and nephrotoxicity if administered systemically and so rarely is included in susceptibility testing. Bacitracin and mupirocin are other examples of topical antimicrobials that rarely are tested in diagnostic laboratories.

7. *In vivo synergism may occur with antimicrobial combinations*. Despite predictions of resistance from susceptibility testing, therapy may be successful because of synergistic combinations of antimicrobials. Synergism between penicillins and aminoglycosides has been recognized for streptococcal, enterococcal, and staphylococcal infections.[9] The synergism is attributed to increased cellular uptake of the aminoglycoside after cell wall damage from the penicillin.

8. *The NCCLS break points may be inappropriate*. The NCCLS break points originally were established with bacterial isolates from human beings, using human pharmacokinetic data and clinical trials in human beings. A veterinary subcommittee was established only in 1993 and only recently has proposed veterinary-specific guidelines for susceptibility tests for some antimicrobials.[10]

Therefore the true relevance of any in vitro MIC in predicting the in vivo results of drug therapy is questionable. The MIC results are not a positive order to use a particular antimicrobial. In selecting the optimal antimicrobial one must take into consideration other factors, such as the site of infection, pharmacokinetics of the drugs, and effect of underlying diseases. Considering all of these factors, drug dosage regimens use a target plasma drug concentration based on some multiple of the in vitro MIC (usually 2 to 10).

Figure 4.2-1 The percentage of *Escherichia coli* inhibited by increasing concentrations of amoxicillin has a bimodal distribution. *E. coli* requiring more than 16 μg/ml of amoxicillin to be inhibited are likely to have intrinsic or acquired resistance mechanisms. Amoxicillin therapy in patients with this pathogen is unlikely to be effective.



4.2.1.4

BACTERICIDAL VERSUS BACTERIOSTATIC ANTIMICROBIALS

Antimicrobials commonly are classified as *bactericidal* or *bacteriostatic* (Box 4.2-1). If the ratio of the MBC to MIC is small (less than 4 to 6), a drug is considered bactericidal and obtaining drug concentrations that kill 99.9% of the organisms exposed is possible. If the ratio of MBC to MIC is large, safely administering dosages of the drug to kill 99.9% of the bacteria may not be possible, and the drug is considered bacteriostatic.[4]

For many drugs the distinction between bactericidal and bacteriostatic is not exact and depends on the drug concentration attained in the target tissue and the pathogen involved. Specific situations in which a

bactericidal drug is preferred over a bacteriostatic drug include immunocompromised patients such as neonates, life-threatening infections such as bacterial endocarditis and meningitis, and surgical prophylaxis. 189

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4.2.1.4.1 BOX 4.2-1 CLASSIFICATION OF ANTIMICROBIALS

4.2.1.4.1.1 Bactericidal

Aminoglycosides

β-Lactams

Fluoroquinolones

Trimethoprim/sulfonamides

4.2.1.4.1.2 Bacteriostatic

Chloramphenicol

Macrolides

Sulfonamides

Tetracyclines

4.2.1.5 POSTANTIBIOTIC EFFECT

For some bacterial-antimicrobial interactions, bacterial growth remains suppressed for a period after drug concentration has decreased below the MIC. This postantibiotic effect (PAE) may be the reason that dosage regimens that fail to maintain drug concentration above the MIC are still efficacious. The PAE depends on the antimicrobial and the bacterial pathogen (Table 4.2-1).[11]

4.2.1.6 PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIPS

The pharmacokinetic-pharmacodynamic relationship between an antimicrobial and a pathogen determines how one calculates the dosage regimen.[12,13] Antimicrobials are classified broadly as *concentration-dependent* or *time-dependent* for their antibacterial activity (Figures 4.2-2 and 4.2-3; Box 4.2-2).[4] For antimicrobials the efficacy of which is concentration-dependent, high plasma concentration levels relative to the MIC of the pathogen (also known as the inhibitory quotient) and the area under the plasma concentration-time curve that is above the bacterial MIC during the dosage interval (area under the inhibitory curve, or AUIC, which equals AUC/MIC) are the major determinants of clinical efficacy. These drugs also have prolonged PAEs, thereby allowing long dosing intervals with maximum clinical efficacy.[12,13]

TABLE 4.2-1 Length of Postantibiotic Effect for Selected Antimicrobials and Antibiotics

MICROBE	LONG PAE* (>3 HOURS)	INTERMEDIATE PAE	SHORT PAE (<1 HOUR)
Gram positive	Fluoroquinolones	Aminoglycosides	—
	Macrolides	Penicillins	—
	Chloramphenicol	Cephalosporins	—
	Tetracycline	—	—
Gram negative	Fluoroquinolones	—	Penicillins
	Aminoglycosides	—	Cephalosporins
	—	—	Trimethoprim/sulfanomides
Anaerobes	Metronidazole	—	—

* *PAE,* Postantibiotic effect.

For time-dependent antimicrobials, the time during which the antimicrobial concentration exceeds the MIC of the pathogen determines clinical efficacy. The time above the MIC should be at least 50% for most patients and should be closer to 100% for bacteriostatic drugs and for patients that are immunosuppressed. For these drugs, bactericidal activity does not increase with increasing plasma concentrations; once the drug exceeds the MIC of the bacteria, increasing the dosage frequency increases efficacy.[12,13]

4.2.2 Designing the Drug Dosage Regimen

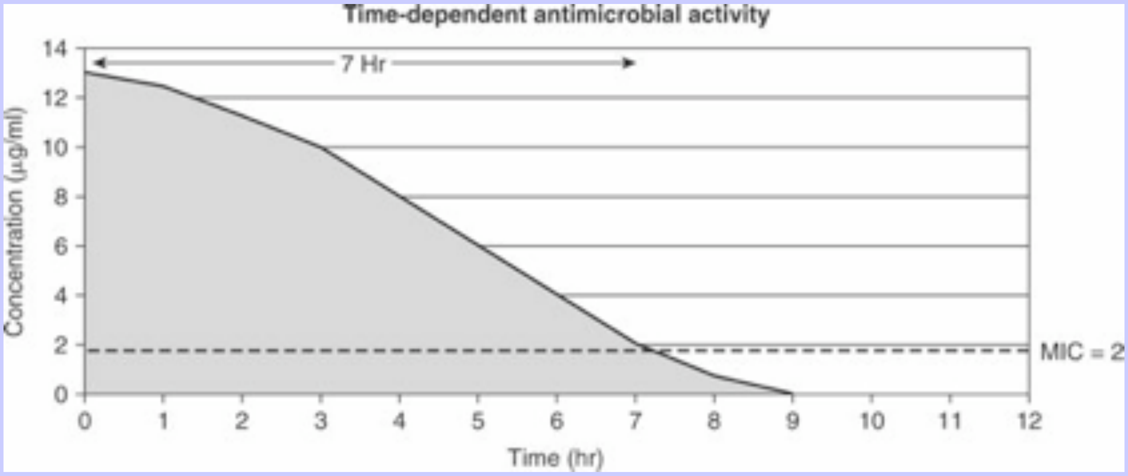
Using the previous information, one designs antimicrobial dosage regimens in one of two ways: to maximize plasma concentrations or to provide a plasma concentration above the bacterial MIC for most of the dosage interval. For concentration-dependent antimicrobials with a prolonged PAE the suggestion for peak plasma drug concentration is eight- to tenfold higher than the MIC of the pathogen.[13] If one knows the Vd of the antimicrobial and assumes once daily dosing, one can calculate a precise drug dosage regimen for the pathogen from the following equation (Box 4.2-3):

$$\text{Dose} = (\text{Vd})(\text{desired plasma concentration})$$

For time-dependent antimicrobials the objective is to keep the average plasma drug concentration above the MIC 190
of the pathogen for the duration of the dosage interval.[13] Using Vd and half-life ($T_{1/2}$) information, one can 191
calculate the dosing regimen from the following equation (Box 4.2-4):

$$\text{Dose} = \frac{(\text{desired average plasma concentration}) \times (\text{Vd})(\text{dosage interval})}{1.44\left(T_{1/2}\right)}$$

Figure 4.2-2 For time-dependent antimicrobials the time during which the antimicrobial concentration exceeds the minimum inhibitory concentration of the pathogen determines clinical efficacy.



4.2.3

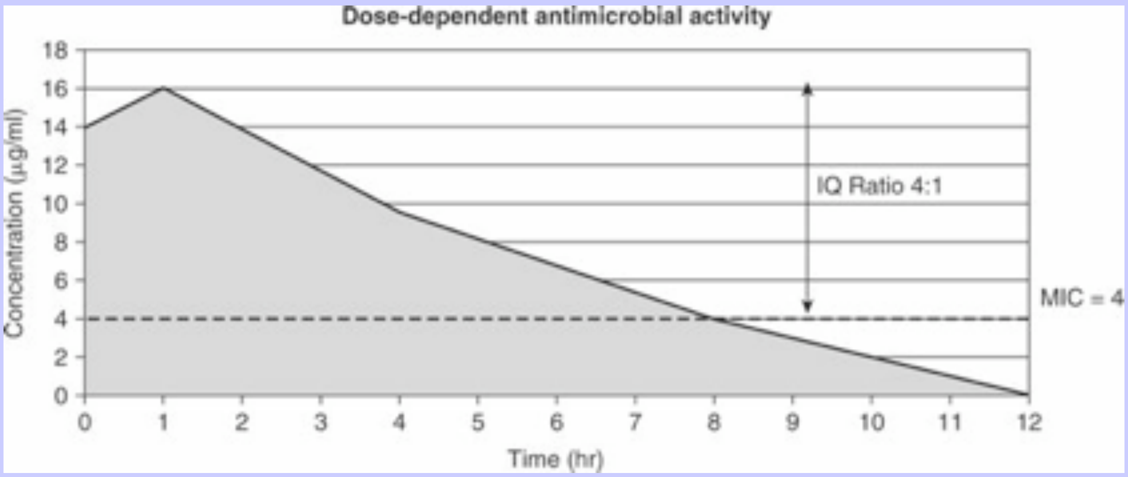
Concurrent Use of Additional Antimicrobials

Combination antimicrobial therapy is commonplace in veterinary medicine, but combination therapy rarely has been demonstrated as superior to single-drug therapy through clinical trials. Use of multiple antimicrobial drugs should be limited to the following situations:

1. Known synergism against specific organisms[9]
2. Prevention of rapid development of bacterial resistance[14]
3. Extension of antimicrobial spectrum in initial therapy of life-threatening conditions
4. Treatment of known mixed bacterial infections[15]

Multiple antimicrobial therapy is implicated as a cause of diarrhea in horses, likely from the additive antibacterial effects against normal gastrointestinal flora.[16] One should avoid antagonistic combinations such as penicillin and tetracyclines; penicillins require actively dividing bacteria to exert their bactericidal effects on the bacterial cell wall, whereas the bacteriostatic action of tetracyclines suppresses cell wall formation.[4]

Figure 4.2-3 For concentration-dependent antimicrobials, the inhibitory quotient *(IQ)* and the area under the inhibitory curve are the major determinants of clinical efficacy.



4.2.4

Prophylactic Use of Antimicrobials

Few reports have examined the efficacy of prophylactic antimicrobial use in veterinary medicine in general and 191
in horses specifically.[17] Because of the lack of controlled veterinary studies, information on prophylaxis is 192
extrapolated mostly from human studies. The risk of infection must warrant the use of prophylactic antimicrobials. The risks of adverse effects from the prophylactic drug administration must be less than the risk of developing disease and its consequences. In veterinary medicine, most of the risk of infection depends on the skill of the surgeon and handling practices in the hospital.[18] One should know or accurately predict the organism(s) likely to cause the infection and the antimicrobial susceptibility. The antimicrobial should be bactericidal and must be administered and must diffuse to the site of potential infection before the onset of infection. One should consider drugs that can be given intravenously and have a high Vd. Drugs used prophylactically should not be those that would be used therapeutically. The duration of antimicrobial prophylaxis should be as abbreviated as possible. Most of the time, a single preoperative dose is sufficient and cost-effective.[19]

4.2.4.1

BOX 4.2-2 CLASSIFICATION OF ANTIMICROBIALS BASED ON MEANS OF EFFECT

4.2.4.1.1

Concentration-Dependent

Aminoglycosides

Fluoroquinolones

Metronidazole

4.2.4.1.2

Time-Dependent

Cephalosporins

Chloramphenicol

Macrolides

Penicillins

Sulfonamides

Tetracyclines

4.2.4.2

BOX 4.2-3 DOSAGE REGIMEN FOR A CONCENTRATION-DEPENDENT ANTIMICROBIAL

You are treating a valuable neonatal foal with a *Klebsiella pneumoniae* infection with gentamicin. The minimum inhibitory concentration of gentamicin for *Klebsiella* is 2 μg/ml. The desired plasma concentration is 10 times the minimum inhibitory concentration at 20 μg/ml. The volume of distribution of gentamicin in the foal is 0.3 L/kg. The dose calculated is as follows:

$$\begin{aligned}\text{Dose} &= (300\ \text{ml/kg})(0.02\ \text{mg/ml})\\ &= 6\ \text{mg/kg}\end{aligned}$$

4.2.4.3

BOX 4.2-4 DOSAGE REGIMEN FOR A TIME-DEPENDENT ANTIMICROBIAL

You want to treat a foal with *Rhodococcus equi* pneumonia with erythromycin. The minimum inhibitory concentration for *R. equi* is 0.25 μg/ml. In foals the volume of distribution is 4 L/kg, and the elimination half-life 1 hour. You pick a dosage interval of 6 hours and an average desired plasma concentration of 10 times the minimum inhibitory concentration because *R. equi* is an abscess-forming, intracellular pathogen.

$$\text{Dose} = \frac{(\text{desired avg. plasma concn.})(\text{Vd})(\text{dosage interval})}{1.44\left(T_{1/2}\right)}$$

$$\text{Dose} = \frac{(0.0025\ \text{mg/ml})(400\ \text{ml/kg})(6\ \text{hours})}{1.44\ (1\ \text{hour})}$$

$$\text{Dose} = 4.2\ \text{mg/kg}$$

This agrees with published recommendations of 3 to 5 mg/kg for intravenously administered erythromycin for treating *R. equi*. Because of the poor bioavailability of the oral formulations, the dosage is increased 5 times for an oral dosage of 25 mg/kg every 6 hours.

4.2.5 β-Lactams: Penicillins and Cephalosporins

The β-lactam antibiotics are among the most commonly used antibiotics. Their use and popularity is due to their safety, efficacy, flexibility in dosage forms, and low expense. Penicillins and cephalosporins have a four-member β-lactam ring that is unstable and is a major target for bacterial resistance mechanisms.

4.2.5.1 MECHANISM OF ACTION

β-Lactam antibiotics act on enzymes called *penicillin-binding proteins (PBP)* responsible for building the bacterial cell wall.[20] Therefore they are only active against rapidly multiplying organisms when the binding of penicillin within the cell wall interferes with production of cell wall peptidoglycans and results in cell lysis in a hypo- or isoosmotic environment. Anywhere from two to eight PBPs may be in a bacterium. When β-lactam 192
antibiotics bind covalently and irreversibly to the PBPs, they disrupt the bacterial cell wall and lysis occurs. 193
Differences in the spectrum and action of β-lactam antibiotics are due to their relative affinity for different PBPs. To bind to the PBPs, the β-lactam antibiotic first must diffuse through the bacterial cell wall. Gram-negative organisms have an additional lipopolysaccharide layer that decreases antibiotic penetration. Therefore gram-positive bacteria are usually more susceptible to the action of β-lactams than gram-negative bacteria.[21] The penicillins penetrate mammalian cells poorly and so have little use in treating intracellular pathogens.

4.2.5.2 MECHANISMS OF RESISTANCE

Resistance mechanisms to the β-lactams include failure of the antibiotic to penetrate the outer bacterial cell layers and alteration of PBPs that decrease the affinity of the PBP for the antibiotic.[22] This resistance mechanism occurs with methicillin-resistant staphyloccoci. A third mechanism of resistance is from production of β-lactamase enzymes.[23] Bacteria produce as many as 50 β-lactamase enzymes (penicillinases, cephalosporinases).[24] These enzymes hydrolyze the cyclic amide bond of the β-lactam ring and inactivate the antibiotic. Coagulase-positive *Staphylococcus* spp. produce staphylococcal β-lactamases. The synthesis of these enzymes is plasmid-encoded, and the enzymes are exocellular. These enzymes typically do not inactivate cephalosporins and antistaphylococcal penicillins. Most of these β-lactamases can be inactivated by inhibitors such as clavulanic acid and sulbactam. The gram-negative β-lactamases are a diverse group of enzymes that may be encoded on chromosomes or phasmids.[24] *E. coli* lactamase is plasmid derived and hydrolyzes cephalosporins and penicillins. Chromosomal-mediated lactamases hydrolyze penicillin or cephalosporins or both.[23]

4.2.6 Benzylpenicillin (Penicillin G)

Penicillin G was the first antibiotic developed and remains one of the most effective antibiotics available. Penicillin G is still the initial drug of choice for many bacterial infections.

4.2.6.1 SPECTRUM OF ACTIVITY

Aerobic bacteria susceptible to penicillin G include most β-hemolytic streptococci, β-lactamase–negative staphylococci, *Actinomyces* species, some *Bacillus anthracis, Corynebacterium* spp., and *Erysipelothrix*

rhusiopathiae.[25] Most species of anaerobes are susceptible, excluding β-lactamase-producing *Bacteroides* spp.[26,27] Penicillin G is inactivated easily by β-lactamases and has little efficacy against organisms that can produce these enzymes. In addition, penicillin G is ineffective against those bacteria that are resistant by other mechanisms, such as having a relatively impermeable cell wall. Therefore penicillin G has little activity against many staphylococci and most gram-negative bacteria.[25]

4.2.6.2 PHARMACOKINETICS

4.2.6.2.1 Absorption

Penicillin is a weak acid with a pK_a of 2.7; therefore it is highly ionized in plasma. Gastric absorption of penicillin G is poor because it hydrolyzes rapidly in the acidic environment of the stomach. Phenoxymethyl penicillin (penicillin V) can be given orally to horses and has a $T_{½}$ of absorption of 0.2 hours.[28] The sodium and potassium salts of penicillin G are the only dosage forms that are suitable for intravenous administration, and they are absorbed quickly from intramuscular or subcutaneous sites of administration. Procaine penicillin G is absorbed more slowly from intramuscular administration than the sodium or potassium salts and so produces lower but more sustained plasma concentrations. Benzathine penicillin G is the least soluble of the dosage forms and is absorbed slowly, producing sustained but subtherapeutic plasma concentrations of penicillin G.[29] The rate of absorption from intramuscular injections of procaine penicillin G varies depending on the injection site, with injections into the neck muscle producing more rapid absorption and higher plasma concentrations than with injections into the hindquarters.[30]

4.2.6.2.2 Distribution

The Vd of sodium penicillin G is 0.7 L/kg,[31] and a moderate degree of protein binding occurs (52% to 54%).[32] After absorption, penicillin diffuses mainly in extracellular fluid and may not reach therapeutic concentrations in sequestered infections.[33]

4.2.6.2.3 Elimination

Elimination of penicillin is primarily renal, as unchanged drug, by glomerular filtration and active renal tubular secretion. The elimination $T_{½}$ of penicillin G is 1 hour,[32,33] and total clearance is 8.5 ± 1.33 ml/min/kg.[31] Orally administered penicillin V has an elimination $T_{½}$ of 3.7 hours.[28]

4.2.6.3 ADVERSE EFFECTS AND DRUG INTERACTIONS

4.2.6.3.1 Immune-Mediated Reactions

Penicillin is associated with autoimmune hemolytic anemia (type II hypersensitivity),[34,35] and anaphylaxis (type I hypersensitivity) in horses.[36] The immune-mediated anemia usually resolves with discontinuation of penicillin therapy. Anaphylaxis usually occurs after previous exposure to penicillin and can be fatal. Intravenously administered epinephrine, oxygen administration, and respiratory support are indicated.

Although penicillin and cephalosporins are assumed widely to be cross-reactive in sensitive individuals, the actual incidence in human beings is low.[37] 193

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4.2.6.3.2

Procaine Reactions and Use in Performance Horses

When procaine penicillin G products are administered accidentally intravascularly, they cause extreme central nervous system (CNS) stimulation.[38,39] Most horses survive unless they fatally traumatize themselves during the reaction. Diazepam attenuates the reaction if given before the procaine but has no effect if given after procaine administration. Veterinary formulations contain higher concentrations of procaine than human formulations, and high temperatures increase the solubility of procaine. Therefore procaine penicillin G should be refrigerated and administered by careful intramuscular injection.[39] Procaine is eliminated slowly and commonly causes violative residues in racehorses and performance horses.[38]

4.2.6.3.3

Electrolyte Imbalances

The sodium or potassium content of intravenous formulations of penicillin G can contribute to electrolyte imbalances associated with congestive heart failure and renal function impairment. A million units of potassium penicillin contains 1.7 mEq of potassium and so should be administered by slow intravenous injection.

4.2.6.3.4

Phenylbutazone

Concurrent administration of phenylbutazone in horses increases plasma concentrations of penicillin G but lowers tissue concentrations. The effect is likely caused by a lower peripheral distribution.[31]

4.2.6.4

FORMULATIONS

Sodium and potassium penicillin (also called crystalline penicillin) are water-soluble formulations and may be injected intravenously, intramuscularly, or subcutaneously. They achieve rapid plasma concentrations but have short elimination half-lives and so must be administered frequently. Potassium penicillin is usually less expensive than sodium penicillin but must be administered more carefully, because rapid intravenous administration can cause cardiac arrhythmias. Procaine penicillin G is a poorly soluble salt that is absorbed slowly following intramuscular or subcutaneous injection. Procaine is the most commonly used formulation of penicillin. Benzathine penicillin G is an insoluble salt used in long-acting penicillin preparations that contain a 50:50 mixture of procaine penicillin G and benzathine penicillin G.

4.2.6.5

CLINICAL USE

Penicillin is still the antimicrobial of choice for many diseases in equine practice such as streptococcal and anaerobic infections. The intravenous formulations are used when high doses are necessary to get adequate concentrations at the site of infection in life-threatening situations. The dosage of penicillin G varies greatly depending on the formulation, species, and disease being treated. Penicillin often is used irrationally to treat traumatic wounds infected with staphylococci, because most equine isolates are β-lactamase–producing *Staphylococcus aureus*.[40]

4.2.7 Aminopenicillins

4.2.7.1 SPECTRUM OF ACTIVITY

The aminopenicillins are able to penetrate the outer layer of gram-negative bacteria better than penicillin G; therefore they have activity against many of the gram-negative bacteria (*Escherichia coli, Salmonella*, and *Pasteurella* spp.) and gram-positive bacteria. However, gram-negative bacteria easily acquire resistance to the aminopenicillins, so the antibiotics are not usually effective against *Klebsiella, Proteus, Pseudomonas*, and *Staphylococcus aureus*. Most anaerobes are sensitive, except β-lactamase–producing strains of *Bacteroides*.[41] Amoxicillin penetrates the gram-negative cell more easily than does ampicillin; therefore it has greater activity against gram-negative bacteria.[25]

4.2.7.2 PHARMACOKINETICS

4.2.7.2.1 Absorption

In horses, ampicillin sodium is absorbed well following intramuscular or subcutaneous administration; however, adult horses absorb oral dosage forms poorly.[42] Intramuscularly administered ampicillin trihydrate produces lower ampicillin blood concentrations that extend over a longer period than does intramuscularly administered ampicillin sodium.[43] Oral absorption of amoxicillin is between 5.3% and 10.4% in adult horses,[44,45] but 36% to 42% in foals.[46] The ampicillin prodrugs are converted to ampicillin as they are absorbed from the gastrointestinal tract. Compared with the bioavailability of oral ampicillin (2%), the ampicillin esters have improved oral bioavailabilities in adult horses: pivampicillin (31%), bacampicillin (39%), and talampicillin (23%).[47] The low oral bioavailability of ampicillin esters is caused by chemical hydrolysis in the high pH of equine ileal contents.

4.2.7.2.2 Distribution

The aminopenicillins diffuse rapidly and widely into most body fluids; distribution into cerebrospinal fluid (CSF) is low unless the meninges are inflamed.[41] Penetration into synovial fluid is high.[42,45,48,49] The Vd of amoxicillin is 0.19 in adult horses[45] and 0.27 L/kg in neonatal foals.[46] The Vd of ampicillin in horses is 0.18 L/kg.[43] Peak serum concentrations of ampicillin are 6.2 to 9.7 mg/ml 16 minutes after an intramuscular dose of 10 mg/kg of ampicillin sodium.[42] In subcutaneous tissue chambers in ponies, concentrations of ampicillin sodium (intravenous), pivampicillin (oral), and procaine penicillin G (intramuscular) remained 194
above the MIC of *Streptococcus zooepidemicus* for 8, 12, and 24 hours, respectively.[50] The protein binding 195
of amoxicillin is moderate (37% to 38%),[51] whereas protein binding is low for ampicillin (6% to 8%).[32]

4.2.7.2.3 Elimination

Amoxicillin and ampicillin are excreted unchanged primarily in the urine. Ten percent to 25% of the administered dose of amoxicillin is excreted in the form of penicilloic acid.[41] The elimination $T_{1/2}$ of

amoxicillin is approximately 1 hour in horses and foals.[44,46,51] The elimination $T_{½}$ of ampicillin ranges from 0.5 hour to 1.5 hours.[42,43,48,52]

4.2.7.3 ADVERSE EFFECTS AND DRUG INTERACTIONS

Amoxicillin and ampicillin have the same adverse effects as penicillin G. A beneficial interaction is their synergism when combined with β-lactamase inhibitors such as clavulanic acid and sulbactam.

4.2.7.4 FORMULATIONS

Sodium ampicillin is available as an aqueous formulation for intravenous, intramuscular, and subcutaneous injection. The reconstituted aqueous formulations are unstable after more than a few hours. Ampicillin trihydrate is a poorly soluble, slow-release aqueous suspension; absorption is erratic and the drug produces prolonged but low plasma concentrations. Ampicillin trihydrate is approved for use in large animals.

4.2.7.5 CLINICAL USE

Indications for ampicillin or amoxicillin are few, because they offer little advantage over benzylpenicillins because of acquired resistance in gram-negative bacteria. Sodium ampicillin may be substituted for sodium or potassium penicillin as a choice for surgical prophylaxis.

4.2.8 Antipseudomonal Penicillins

4.2.8.1 SPECTRUM OF ACTIVITY

Carbenicillin, ticarcillin, and piperacillin are the antipseudomonal penicillins. This group of penicillins can penetrate the outer cell wall of *Pseudomonas* spp. and other gram-negative bacteria. They are susceptible to β-lactamase inactivation by *Klebsiella*. This group has activity against gram-negative bacteria at the expense of activity against gram-positive bacteria. They retain activity against anaerobic bacteria and are synergistic when administered with aminoglycosides.[41] Ticarcillin has been administered systemically to horses.[53,54]

4.2.8.2 PHARMACOKINETICS

Absorption of intramuscularly administered ticarcillin is 65%, and the elimination $T_{½}$ is less than 1 hour in horses.[53,54] Peak endometrial concentrations after intravenous administration are 12.9 μg/g but are greater than 150 μg/g when 6 g of ticarcillin is diluted in 250 ml of saline and infused into the uterus.[54]

4.2.9 β-Lactamase Inhibitors

4.2.9.1 SPECTRUM OF ACTIVITY

The β-lactamase inhibitors are a specific class of drugs with little antibacterial action of their own, but they inhibit bacterial β-lactamase. β-lactamase inhibitors only assist in the destruction of bacteria that produce β-lactamase enzymes; other forms of resistance, such as alteration of penicillin-binding proteins, are not

affected. β-lactamase inhibitors are always combined with β-lactam antibiotics. The primary drugs of this class are clavulanic acid and sulbactam. They extend the spectrum of amoxicillin and ampicillin to include β-lactamase producing *E. coli, Klebsiella, Proteus*, and *Staphylococcus*. Most anaerobes, including *Bacteroides fragilis*, are susceptible. These drugs combine with the β-lactamase that is produced by gram-negative and some gram-positive bacteria. An inactive enzyme complex forms, and the co-administered antibiotic then is able to exert its effect.[41] Ampicillin-sulbactam[55,56] and ticarcillin–clavulanic acid have been used in foals.[57,58]

4.2.9.2 PHARMACOKINETICS

4.2.9.2.1 Absorption

Absorption of ticarcillin and clavulanic acid in foals depends on age, for neonatal foals have a higher systemic bioavailability than older foals (100% and 88% versus 54% and 68%, respectively).[58] When administered intramuscularly with clavulanic acid, ticarcillin demonstrates flip-flop kinetics in which the elimination half-life is longer after intramuscular than intravenous injection because of slow absorption from the injection site.[57]

4.2.9.2.2 Distribution

The Vd of ticarcillin in older foals was 0.24 L/kg, and the Vd of clavulanic acid was 0.48 L/kg.[57] In neonatal foals the Vd of ticarcillin was higher at 0.69 L/kg, as would be expected for a low-Vd drug in a neonate with its increased extracellular fluid volume.[58]

4.2.9.2.3 Elimination

Ticarcillin demonstrates age-dependent elimination in foals. In neonatal foals the Vd and clearance of ticarcillin were approximately double those reported for older foals and mares.[58] The renal elimination mechanism of ticarcillin appears immature in a 3-day-old foal but is near normal adult function by 28 days of age.

4.2.9.3 ADVERSE EFFECTS AND DRUG INTERACTIONS

When administered intramuscularly into the hindquarters at a concentration of 400 mg/ml ticarcillin plus 13.2 195
mg/ml clavulanic acid, foals showed signs of significant local discomfort.[57] A lower concentration of drug did 196
not cause signs of discomfort in neonatal foals.[58]

The penicillin–β-lactam inhibitor drugs can be used with aminoglycosides for a synergistic effect against the pathogens commonly encountered in septicemic foals.

4.2.9.4 FORMULATIONS

Amoxicillin–clavulanic acid is only available as oral tablets or suspension for human beings and small animals. Ampicillin-sulbactam is available as a trihydrate formulation labeled for cattle in Canada. Sodium

ampicillin-sulbactam and ticarcillin disodium–clavulanic acid formulations are available for human use in the United States.

4.2.9.5 CLINICAL USE

The ampicillin–sulbactam trihydrate formulation frequently is used in horses in Canada as a treatment option for respiratory infections.[55,56] Ticarcillin–clavulanic acid is a treatment option for gram-negative bacterial infections in valuable foals.

4.2.10 Cephalosporins

In 1945 a fungus, *Cephalosporium acremonium*, was isolated from a seawater sample near a sewage outlet in Sardinia. The fungus was found to produce an antibiotic compound that inhibited the growth of many gram-positive and gram-negative bacteria. In addition, the compound resisted the action of staphylococcal β-lactamases. This resistance to β-lactamases and the widespread emergence of penicillin-resistant staphylococci in the 1950s provided the impetus for the development of the cephalosporin antibiotics.

4.2.10.1 SPECTRUM OF ACTIVITY

In human medicine, cephalosporins are used widely; however, cephalosporins are said to be drugs in search of diseases to treat, meaning that precise therapeutic indications for these drugs are difficult to define and a tendency exists to use these drugs when older or cheaper drugs would suffice.[59] The use of cephalosporins in equine medicine is not so widespread because of their expense and the fact that equine clinical experience includes only a few specific agents. Cephalosporins usually are grouped into three generations based primarily on their antibacterial activity, but some of the newer cephalosporins do not easily fit into this scheme.[20]

First-generation cephalosporins are effective against most gram-positive cocci, including β-lactamase–producing staphylococci, by virtue of higher affinity for the PBPs. They also have greater activity against members of the Enterobacteriaceae than other β-lactam antibiotics but may be degraded by β-lactamases (except staphylococcal β-lactamase), which makes them ineffective against some gram-negative bacteria.[21] The first-generation cephalosporins are active against staphylococci and streptococci with the exception of the methicillin-resistant staphylococci. Although most corynebacteria are susceptible, *Corynebacterium* (now *Rhodococcus*) *equi* is usually resistant.[20] Among the commonly encountered gram-negative bacteria, *E. coli, Klebsiella, Haemophilus, Proteus, Actinobacillus, Pasteurella*, and *Salmonella* species are regularly susceptible to first-generation cephalosporins. Indole-positive *Proteus, Enterobacter, Serratia*, and *Pseudomonas* species are resistant. Most anaerobic bacteria are susceptible, except β-lactamase–producing *Bacteroides*.[59] None of the first-generation cephalosporins is approved for use in horses, but cefazolin,[60] cefadroxil,[61,62] cephalothin,[63] cephapirin,[64,65] and cephradine[66] have been used in horses.

Second-generation cephalosporins in general have greater activity against gram-negative bacteria that are resistant to the first-generation cephalosporins (e.g., *Escherichia coli, Klebsiella, Proteus*, and *Enterobacter*) but are no more active against the gram-positive bacteria than the first-generation drugs.[20] The increased activity of these cephalosporins is caused by increased resistance to gram-negative β-lactamases.[21] Cefoxitin has been used in mares.[67]

Third-generation cephalosporins have increased activity against gram-negative bacteria because of their resistance to β-lactamases, and most achieve therapeutic concentrations in the CNS.[20] In the past, these cephalosporins were considered to be unreliable and erratic against gram-positive organisms. However, recent evidence indicates they actually have good activity against gram-positive organisms, except for enterococci and *Listeria*.[59] Some of the third-generation products have good activity against *Pseudomonas* spp.[21] Cefoperazone, cefotaxime, and ceftriaxone have been investigated in horses,[68–70] and cefotaxime has been used successfully to treat septicemia and meningitis in neonatal foals.[71]

Fourth-generation cephalosporins have broad-spectrum activity against gram-positive and gram-negative bacteria by increased resistance to β-lactamases. Cefepime is active against many gram-negative bacteria that are resistant to third-generation cephalosporins and has antipseudomonal activity similar to ceftazidime. However, cefepime lacks activity against methicillin-resistant staphylococci and enterococci and has variable activity against anaerobes.[59] Cefepime has been evaluated in neonatal foals and adult horses.[72,73]

Ceftiofur is marketed as a *new*-generation cephalosporin because it does not fall clearly into the previous classification scheme. Ceftiofur is active against respiratory pathogens such as streptococci, *Pasteurella* spp., 196
and *Haemophilus* spp. and most anaerobes but has less activity against *Staphylococcus aureus* and 197
Enterobacteriaceae.[74] *Bacteroides* spp. and *Pseudomonas* spp. are resistant.[75] Ceftiofur is approved only in horses for treating respiratory tract infections caused by *Streptococcus zooepidemicus*. When administered, ceftiofur is metabolized rapidly to the active metabolite desfuroylceftiofur.[76] Desfuroylceftiofur is less active than ceftiofur against *Staphylococcus aureus* and *Proteus* spp.[77] Diagnostic laboratories use a ceftiofur disk for susceptibility testing because of the instability of desfuroylceftiofur, so susceptibility testing results for staphylococci and *Proteus* sp. may not be reliable for predicting the efficacy of ceftiofur therapy.

4.2.10.2 PHARMACOKINETICS

4.2.10.2.1 Absorption, Distribution, and Elimination

Table 4.2-2 gives the pharmacokinetic parameters for the cephalosporins in horses. Intramuscular and subcutaneous administration of cephalosporins results in rapid drug absorption, but the extent varies with the drug and the species. Oral bioavailability is acceptable in neonates but rapidly becomes too low to be practical for older foals or adults.[61,62] The values for Vd in horses for the cephalosporins are typically low (<0.3 L/kg), indicating distribution primarily to extracellular fluid, and indeed have good distribution into the extracellular fluid of most tissues, including pleural, pericardial, and synovial fluid.[21] Penetration into cortical and cancellous bone is usually adequate.[20] Cephalosporins penetrate poorly into the ocular humor and, except for some third-generation drugs, do not achieve therapeutic concentrations in the CNS.[21] In general the third-generation cephalosporins have an increased ability to penetrate the CNS.[59] Higher Vd values for foals reflect the increased extracellular fluid compartment of the neonate.[61,78] Most cephalosporins are eliminated rapidly as unchanged drug in the urine. The liver deacetylates cephalothin, cephapirin, cefotaxime, and ceftiofur.[20] Their metabolites have significant antibacterial activity. For ceftiofur, most of the antibacterial activity is attributed to its metabolite, desfuroylceftiofur.[76] Renal excretion of cephalosporins occurs through a combination of glomerular filtration and active tubular secretion. Therefore the dosage regimen for most cephalosporins must be modified for patients in renal

failure.[20] For the cephalosporins that undergo hepatic metabolism, hepatic insufficiency may result in decreased metabolism and increased drug accumulation.[20] Dose-dependent kinetics occurred in foals
administered cefadroxil, as evidenced by increasing mean residence time, suggesting saturation of immature 197
renal tubular secretion at high doses.[61] For some cephalosporins, delayed absorption from the intramuscular 198
injection site results in flip-flop kinetics.[68]

TABLE 4.2-2 Pharmacokinetics of Cephalosporins in Horses

DRUG	VOLUME OF DISTRIBUTION (L/kg)	HALF-LIFE OR MRT[*] (HOUR)	PROTEIN BINDING (%)	CLEARANCE (ml/min/kg)	BIOAVAILABILITY (%)	DOSE[†] (mg/kg)
FIRST GENERATION						
Cefadroxil						
Adults[62]	0.46	0.8	—	7	—	IV: 25
Foals[61]	0.52	1.4	—	—	37–100	IV: 5; PO: 5–20
Cefazolin[60]	0.19	0.6–0.8	8	5.51	—	IV: 11
Cephalothin[63]	0.15	0.25	18	13.6	—	IV: 11
Cephapirin[64]	0.17	0.9	—	10	95	IV, IM: 20
Cephradine[66]	0.4	1.6	—	6.7	—	IV, PO: 25
SECOND GENERATION						
Cefoxitin[67]	0.12	0.8	—	4.32	77	IV, IM: 20
THIRD GENERATION						
Cefoperazone[68]	0.68	IV: 0.77	—	12	42	IV, IM: 30
		IM: 1.52				
Ceftriaxone[69]	0.15	0.81	—	2.81	—	IV: 14
Cefotaxime[70]	0.29	0.6	—	5.2	—	IV: 40
FOURTH GENERATION						
Cefepime						
Adults[73]	0.23	2.1	—	—	100	IV, M: 2.2
Foals[72]	0.18	1.65	—	1.33	—	IV: 14
NEW GENERATION						
Ceftiofur						
Adults[76]	0.43	5.11	99	—	42	IM: 2.2
Foals[78]	0.76	—	—	3	—	—

* *MRT,* Mean residence Time.

† *IV,* Intravenous; *PO,* oral; *IM,* intramuscular.

4.2.10.2.2

Adverse Effects and Drug Interactions

The adverse effects of the cephalosporins are similar to those reported for the penicillins. In general, cephalosporins have a high therapeutic index. Bleeding disorders are reported in human beings but have not been reported in animals.[20] The reaction appears to be related to vitamin K antagonism.[20] Ceftriaxone and cefepime cause gastrointestinal disturbances after administration to foals and horses.[69,72,73] Ceftiofur is associated with injection site inflammation and diarrhea from altered gastrointestinal flora in horses.[79–81] The currently available cephalosporins are considered to be potentially nephrotoxic via deposition of immune complexes in the glomerular basement membrane or from a direct toxic effect leading to acute tubular necrosis.[20] In human medicine, cephalosporins are recommended not to be used with aminoglycosides; however, animal studies demonstrate a protective effect of the cephalosporins against nephrotoxicity.[20] Like the penicillins, cephalosporins can be synergistic with aminoglycosides.

4.2.10.3

FORMULATIONS

Cefadroxil is available as veterinary formulated tablets or suspension for small animals. The parenteral formulations of cephalosporins are usually sodium salts. Cefepime is available as a hydrochloride formulation.

4.2.10.4

CLINICAL USE

Ceftiofur is the only cephalosporin routinely used in equine practice and is a good alternative to penicillin or trimethoprim/sulfonamides for treating streptococcal infections. First-generation cephalosporins are an effective alternative to penicillins in treating staphylococcal and streptococcal infections. All of the first-generation cephalosporins have essentially the same spectrum of activity, so the choice of an individual drug is based on pharmacokinetic and economic factors. No second-generation cephalosporins are approved for use in veterinary medicine. Because the cost of these drugs is roughly twice that of the first-generation cephalosporins, their use should be limited to situations in which the infection is resistant to a first-generation drug and susceptible to a second-generation drug and the only other therapy options are unacceptably toxic. The third- and fourth-generation cephalosporins usually are restricted to patients with bacterial infections caused by strains with multiple-drug resistance. All of the third- and fourth-generation cephalosporins are expensive and are used rarely in equine practice. These drugs are most likely to be useful in valuable patients with nosocomial pneumonia, postoperative wound infections, and urinary tract infections related to catheterization and in patients with reduced renal function so that aminoglycosides are contraindicated.

4.2.11

Aminoglycosides

The aminoglycoside antibiotics include *streptomycin, neomycin, gentamicin, amikacin, tobramycin*, and *kanamycin*. They have a chemical structure of amino sugars joined by a glycoside linkage. This group is important in veterinary medicine for treating gram-negative infections caused by enteric pathogens such as *Escherichia coli*.[82] Gentamicin and amikacin are the most commonly used aminoglycosides in horses. Tobramycin is related structurally to kanamycin and has 4 times the activity of gentamicin against *Pseudomonas* spp. Because of the expense, kanamycin use in horses usually is limited to treating melting corneal ulcers caused

by gentamicin-resistant *Pseudomonas*. Streptomycin is no longer available for use, and neomycin is administered only topically because of systemic toxicity.

4.2.11.1
MECHANISM OF ACTION

The aminoglycosides are large molecules with numerous amino acid groups, making them basic polycations that are highly ionized at physiologic pHs. Aminoglycosides must penetrate the bacteria to assert their effect. Susceptible, aerobic gram-negative bacteria actively pump the aminoglycoside into the cell, an action initiated by an oxygen-dependent interaction between the antibiotic cations and the negatively charged ions of the bacterial membrane lipopolysaccharides. This interaction displaces divalent cations (Ca^{2+}, Mg^{2+}), which affects membrane permeability. Inside the bacterial cell, aminoglycosides bind to the 30-S ribosomal subunit and cause a misreading of the genetic code, interrupting normal bacterial protein synthesis. The interruption leads to changes in the cell membrane permeability, additional antibiotic uptake, further cell disruption, and ultimately cell death.[83]

Aminoglycoside action is bactericidal and dose (concentration) dependent. For example, concentrations of gentamicin in the range of 0.5 to 5.0 μg/ml are bactericidal for gram-positive and some gram-negative bacteria. At 10 to 15 μg/ml, gentamicin is effective against the more resistant bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Proteus mirabilis*. The clinical implication is that high initial doses of aminoglycosides increase ionic bonding, enhancing the initial concentration-dependent phase of rapid antibiotic internalization, leading to greater immediate bactericidal activity. Human clinical studies demonstrate that proper initial therapeutic doses of aminoglycosides are critical in reducing mortality from gram-negative septicemia.[84]

The aminoglycosides have a significant *postantibiotic effect*, the time in which antimicrobial concentrations 198
are below the bacterial MIC, but the antimicrobial-damaged bacteria are more susceptible to host defenses. 199
The duration of the PAE tends to increase as the initial aminoglycoside concentration increases.[84]

An alkaline environment (pH 6 to 8) enhances antimicrobial activity of aminoglycosides. They bind to and are inactivated by the nucleic acid material released by decaying white blood cells. Therefore they are usually ineffective in the acidic, hyperosmolar, anaerobic environment of abscesses.[82]

4.2.11.2
SPECTRUM OF ACTIVITY

The aminoglycosides are effective against most gram-negative bacteria, including *Pseudomonas*. They are somewhat effective against staphylococci, although resistance can occur. They are often effective against enterococci, but therapy against streptococci is more effective when combined with a β-lactam antibiotic. *Salmonella* and *Brucella* spp. are intracellular pathogens and are often resistant. Aminoglycosides are ineffective against anaerobic bacteria because aminoglycoside penetration into the bacteria requires an oxygen-dependent transport mechanism. Gentamicin and amikacin are the most commonly used aminoglycosides in horses. Gentamicin has been used widely for treating gram-negative organisms and some gram-positive organisms in horses. Its use is limited primarily by risk of toxicity. Amikacin was developed from kanamycin and has the broadest spectrum of activity of the aminoglycosides. Amikacin is effective against strains not susceptible to other aminoglycosides because it is more resistant to bacterial enzymatic inactivation and is considered the least nephrotoxic.[82]

4.2.11.3

MECHANISMS OF RESISTANCE

Aminoglycoside resistance is caused primarily by enzymes encoded by genes located on bacterial plasmids.[82] These phosphotransferases, acetyltransferases, and adenylyltransferases act internally to alter the aminoglycoside and prevent it from binding to ribosomes. Amikacin is least susceptible to enzyme inactivation. Plasmid-mediated resistance to the aminoglycosides is transferable between bacteria. A single type of plasmid may confer cross-resistance to multiple aminoglycosides and to other unrelated antimicrobials. A single bacterial isolate may have any one of a variety of combinations of resistance to different antibiotics conferred by the particular plasmid it carries. As an example, an *E. coli* strain may be resistant to ampicillin, apramycin, chloramphenicol, gentamicin, kanamycin, sulfonamide, streptomycin, tetracycline, and trimethoprim.[85] The nature of resistance in organisms such as *E. coli* and *Salmonella* species has been a focus of international research because of concerns about potential transference of antimicrobial resistance from animal to human pathogens.[86] Bacteria also may use other methods of reducing the efficacy of aminoglycosides. Some strains of bacteria are less permeable to aminoglycosides, requiring much higher concentrations of aminoglycosides to kill them, and therefore can be selected during treatment.[82] Resistance developed by chromosomal means is minimal and develops slowly for most of the aminoglycosides, with the exception of streptomycin or dihydrostreptomycin; resistance to streptomycin can occur from a single-step mutation.[82]

Subinhibitory and inhibitory aminoglycoside concentrations produce resistance in bacterial cells surviving the initial ionic binding.[87] This adaptive resistance is caused by decreased aminoglycoside transport into the bacteria.[82] Exposure to one dose of an aminoglycoside is sufficient to produce resistant variants of an organism with altered metabolism and impaired aminoglycoside uptake. In vitro, animal and clinical studies show that the resistance occurs within 1 to 2 hours of the first dose. The duration of adaptive resistance relates directly to the elimination $T_{1/2}$ of the aminoglycoside. With normal aminoglycoside pharmacokinetics, the resistance may be maximal for up to 16 hours after a single dose of aminoglycoside, followed by partial return of bacterial susceptibility at 24 hours and complete recovery at around 40 hours.[88] If the aminoglycoside is dosed multiple times a day or the drug concentration remains constant, as with a continuous infusion, adaptive resistance persists and increases. Adaptive resistance is likely to persist in peripheral compartments, which are often the site of infection, because of the persistence of aminoglycosides at these sites. Dose administration at 24-hour intervals or longer may increase efficacy by allowing time for adaptive resistance to reverse.[87,89,90] Some clinicians have expressed reservations about once-daily dosing when intestinal damage allows continued exposure to bacteria that may replicate during the prolonged periods of subtherapeutic aminoglycoside concentrations, but this has not been documented clinically.

4.2.11.4

PHARMACOKINETICS

4.2.11.4.1

Absorption

Table 4.2-3 gives the pharmacokinetics of gentamicin and amikacin in horses. Amikacin and gentamicin are absorbed well and rapidly from intramuscular and subcutaneous routes of administration but are not absorbed orally.[83]

4.2.11.4.2 Distribution

The aminoglycosides are polar antibiotics; therefore distribution is limited to the extracellular fluid space. The Vd in most species ranges from 0.15 to 0.3 L/kg but is higher in neonates.[91,92] Following parenteral administration, effective concentrations are obtained in synovial, perilymph, pleural, peritoneal, and 199
pericardial fluid.[93] Therapeutic concentrations are not achieved in bile, CSF, respiratory secretions, or 200
prostatic and ocular fluids.[83,94] Gentamicin does not cross the placenta of late-term mares.[95] The predominant site of drug accumulation is the renal cortex in most species. The following general gentamicin concentrations are reached over time with repeated doses, from highest to lowest concentrations: renal cortex to renal medulla to liver/lung/spleen to skeletal muscle.[83] Gentamicin is distributed into jejunal and colonic tissue with a peak gentamicin concentration of 4.13 μg/ml in the large colon and 2.26 μg/ml in the jejunum.[96]

TABLE 4.2-3 Pharmacokinetics of Aminoglycosides in Horses

DRUG	VOLUME OF DISTRIBUTION (L/kg)	HALF-LIFE OR MRT* (HOUR)	CLEARANCE (ml/min/kg)	DOSE† (mg/kg)
AMIKACIN				
Foals, 3-day-old[187]	0.42	2.7	1.92	7
Foals, premature, hypoxic[188]	0.60	5.4	1.9	7
Neonatal, high sepsis score[92]	0.34	4.10	1.17	IV: 6.6
Horses[189]	0.14–0.2	1.14–1.57	1.28–1.49	IV: 4.4, 6.6, 11
Horses[190]	0.21	2.8	0.75	IV: 6
GENTAMICIN				
Foals, 1-day-old[91]	0.31	2.2	1.75	4
Foals, 1-month-old[191]	0.24	3.07	0.9	IV: 4
Mares, late pregnancy[95]	0.15	2.26	1.06	—
Horses[97]	0.17	1.66	1.41	IV: 3
Horses, endotoxic[97]	0.14	1.54	1.17	IV: 3
Horses[94]	0.12	0.78	—	IV: 6.6
Horses[93]	0.27	2.17	1.56	IV: 2.2
Horses[126]	0.14	3.0	—	IV, IM: 6.6
Ponies[101]	0.19	1.82	1.27	IV, IM: 5
Horses, intravenous fluids[98]	0.15	1.96	1.04	IV: 2.2
Horses, postoperative[99]	0.17	1.47	1.27	IV: 6.6

* *MRT,* Mean residence time.

† *IV,* Intravenous; *IM,* intramuscular.

In endotoxemia, gentamicin concentrations increase in serum from a fever-induced decrease in the volume of the extracelluar fluid compartment.[97] The administration of therapeutic fluids, similar to those that are used to treat colic, does not change the pharmacokinetics of concurrently administered gentamicin significantly.[98] The pharmacokinetics of gentamicin are unchanged in horses undergoing colic surgery.[99]

Peritoneal lavage had no effect on pharmacokinetics of gentamicin in healthy horses after abdominal surgery, in which localized nonseptic peritonitis was induced.[100]

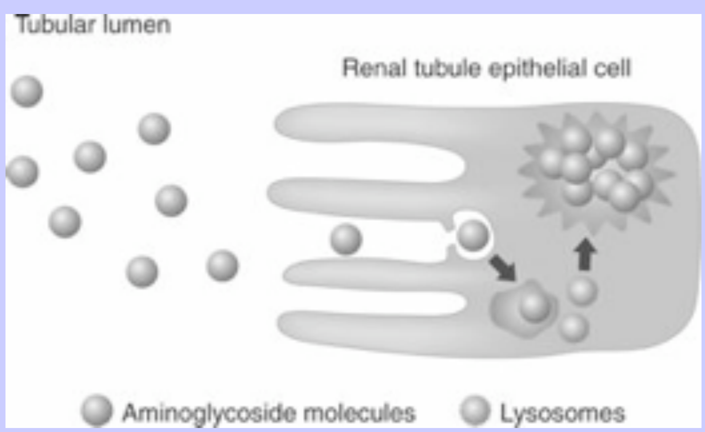
Endometrial tissue concentrations of gentamicin are higher than plasma concentrations after 7 days of intramuscular therapy with a dose of 5 mg/kg every 8 hours.[101] Intrauterine administration of 2 g of amikacin produces a peak of greater than 40 μg/g of endometrial tissue within 1 hour after infusion. Twenty-four hours after infusion, 2 to 4 μg of amikacin per gram of endometrial tissue is still present.[102] Intrauterine administration of 2.5 g of gentamicin once daily for 5 days resulted in endometrial tissue concentrations of 41.65 μg/g 24 hours after the last dose.[103]

Gentamicin diffuses into synovial fluid in normal horses and reaches a peak of 6.4 μg/ml at 2 hours with a single 4.4-mg/kg intravenous dose.[104] However, local inflammation may increase drug concentrations in the joint, and concentrations may increase with repeated doses. Intraarticular administration of 150 mg of gentamicin results in a peak synovial concentration of 1828 μg/ml 15 minutes after injection, and synovial concentrations remain greater than 10 μg/ml for at least 24 hours.[105] Regional perfusion techniques are excellent methods of local delivery of aminoglycosides and avoid the adverse effects of systemic therapy.[106–109] When intraosseous perfusion was compared with intravenous perfusion, each technique produced mean peak concentrations of amikacin ranging from 5 to 50 times that of recommended peak serum concentrations for therapeutic efficacy.[110] Gentamicin-impregnated polymethylmethacrylate beads also may be used to achieve high local concentrations of drug while avoiding systemic toxicity.[111–114]

4.2.11.4.3 Elimination

The aminoglycosides are eliminated almost exclusively by glomerular filtration.[83] The practical elimination 200
half-lives range from 1 to 3 hours in adult animals but increase in neonates or animals with renal 201
dysfunction. Renal accumulation results in extended withdrawal times for food animals.

Figure 4.2-4 Nephrotoxicity occurs from ionic binding of aminoglycoside molecules to polysaccharide cations on the proximal tubular epithelium, followed by pinocytosis and accumulation within lysosomes.



4.2.11.5 ADVERSE EFFECTS AND DRUG INTERACTIONS

4.2.11.5.1 Nephrotoxicity

The aminoglycosides enter the renal tubule after filtration through the glomerulus (Figure 4.2-4). From the luminal fluid the cationic aminoglycoside molecules bind to anionic phospholipids on the proximal tubular cells.[115] The aminoglycoside is taken into the cell via carrier-mediated pinocytosis and is translocated into cytoplasmic vacuoles, which fuse with lysosomes.[116] The drug is sequestered unchanged in the lysosomes. With additional pinocytosis, drug continues to accumulate within the lysosomes. The accumulated aminoglycoside interferes with normal lysosomal function, and eventually the overloaded lysosomes swell and rupture. Lysosomal rupture releases lysosomal enzymes, phospholipids, and the aminoglycoside into the cytosol of the proximal tubular cell, disrupting other organelles and causing cell death.[117]

The risk factors for aminoglycoside toxicity include prolonged therapy (greater than 7 to 10 days), acidosis and electrolyte disturbances (hypokalemia, hyponatremia), volume depletion (shock, endotoxemia), concurrent nephrotoxic drug therapy, preexisting renal disease, and elevated plasma trough concentrations.[83,118–122] Calcium supplementation can reduce the risk of nephrotoxicity.[123] Feeding the patient a high-protein, high-calcium diet such as alfalfa also can decrease the risk of nephrotoxicity, for protein and calcium cations compete with aminoglycoside cations for binding to renal tubular epithelial cells.[124] High dietary protein also increases glomerular filtration rate and renal blood flow, reducing aminoglycoside accumulation.[125]

Uptake and accumulation of aminoglycosides into renal tubular epithelium demonstrates saturable kinetics. Because nephrotoxicity is related to aminoglycoside accumulation in the renal proximal tubular cells, logically peak concentrations are not related to toxicity and longer dose intervals result in less total drug exposure to the renal brush border membrane. High-dose, once-daily dosing of aminoglycosides has now become common in human and veterinary medicine to take advantage of the concentration-dependent killing and long PAE of these drugs and avoid first exposure adaptive resistance and toxicity.[84,94,99,126]

4.2.11.5.2 Ototoxicity

Aminoglycoside ototoxicity occurs from the same mechanisms as nephrotoxicity.[127] Gentamicin damages the cochlear division of the eighth cranial nerve resulting in vertigo, whereas amikacin damages the auditory division of the eighth cranial nerve resulting in permanent deafness. This drug-specific toxicity may be caused by the distribution characteristics of each drug and concentration achieved in each sensory organ.[128]

4.2.11.5.3 Therapeutic Drug Monitoring

Individual horses can differ widely in the serum concentrations produced from the same aminoglycoside dosage regimen. When this unpredictability is combined with the often small difference between therapeutic and toxic serum concentrations, determining serum concentrations in a particular patient becomes valuable. The tendency is to underdose neonatal patients, especially those that are receiving aggressive fluid therapy.[129] To maximize efficacy and minimize toxicity, therapeutic drug monitoring of gentamicin or amikacin is

recommended. Peak concentrations are targeted to achieve an inhibitory quotient ratio of 10.[99] Because the trough concentration is associated with nephrotoxicity, trough concentrations are recommended to be less than 2 μg/ml for gentamicin and less than 5 μg/ml for amikacin before the next dose is administered.[83,88] To allow for the distribution phase, one should take the blood sample for the peak concentration at a half hour to 1 hour after intravenous administration and the trough sample before the next dose. One then can use the peak and trough concentrations to estimate the elimination $T_{1/2}$ for the individual patient. An increase in the elimination $T_{1/2}$ during therapy is a sensitive indicator of early tubular insult.[130] If one uses a once-daily regimen, a blood sample taken just before the next dose will be well below the recommended trough concentrations and may even be below the limit of detection of the assay. For these patients, an 8-hour postdose sample provides a more accurate estimate of the elimination $T_{1/2}$. 201

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If therapeutic drug monitoring is unavailable, then once-daily high-dose therapy is recommended, and the practitioner can detect development of nephrotoxicity by an increase in urine γ-glutamyltransferase enzyme and an increase in the ratio of γ-glutamyltransferase to creatinine in the urine. The ratio may increase to 2 to 3 times baseline within 3 days of a nephrotoxic dose.[94,131] If these tests are not available, the development of proteinuria is the next best indicator of nephrotoxicity and is determined easily in a practice setting.[130] Elevations in serum urea nitrogen and creatinine confirm nephrotoxicity but are not seen for 7 days after significant renal damage has occurred.[92] Elimination half-lives of 24 to 45 hours have been reported in horses with renal toxicity, further prolonging the toxic exposure to the drug.[118] Although peritoneal dialysis is useful in lowering creatinine and serum urea nitrates, it may not be effective in significantly increasing the elimination of the accumulating aminoglycoside.[118] The ability of the horse to recover most likely depends on the type of medication exposure and the amount of healthy renal tissue remaining to compensate.

4.2.11.5.4 Neuromuscular Blockade

Neuromuscular blockade is a rare effect, related to blockade of acetylcholine at the nicotinic cholinergic receptor.[132] The effect occurs most often when anaesthetic agents are administered concurrently with aminoglycosides.[133] The clinician should treat affected patients promptly with parenterally administered calcium.[132]

4.2.11.5.5 Drug Interactions

Aminoglycosides are inactivated if combined in vitro with other drugs because of pH incompatibilities. The aminoglycosides are synergistic against streptococci, *Pseudomonas*, and other gram-negative bacteria if combined with β-lactam antibiotics because the antibiotic disrupts the bacterial cell wall.[82] Halothane anesthesia causes significant changes in the pharmacokinetics of gentamicin; total body clearance and Vd decrease, and the elimination $T_{1/2}$ increases.[134] A longer gentamicin dosing interval after anesthesia may help correct for the changes, but one seriously should consider choosing another antimicrobial. One should not use neuromuscular blocking agents or drugs with neuromuscular blocking activity concurrently with aminoglycosides because they can increase the risk of neuromuscular blockade, particularly during anesthesia.[82] One should avoid other nephrotoxic drugs when possible during aminoglycoside therapy. Concurrent administration of phenylbutazone with gentamicin decreases the elimination $T_{1/2}$ of gentamicin by 23% and decreases the Vd by 26% but does not affect the pharmacokinetics of phenylbutazone.[135]

Flunixin has no effect on the pharmacokinetics of gentamicin when administered concurrently to adult horses.[99]

4.2.11.6 FORMULATIONS

Gentamicin and amikacin are available as brand name and generic solutions for intrauterine infusion in mares. Although not labeled for other routes of administration, they commonly are administered to horses by intravenous, intramuscular, subcutaneous, intraarticular, and intraosseous routes. Gentamicin is also available in ophthalmic formulations for treating gram-negative keratitis.

4.2.11.7 CLINICAL USE

Gentamicin and amikacin are used commonly to treat serious gram-negative infections in horses. Amikacin is used in circumstances in which antimicrobial resistance to gentamicin has developed in gram-negative pathogens, but gentamicin usually retains greater activity against streptococci than amikacin. The aminoglycosides usually are administered concurrently with β-lactam antimicrobials for a possible synergistic effect. However, using aminoglycosides to treat infection in horses has been tempered by toxicity considerations. Use of the aminoglycosides also is limited by their poor penetration of cellular membranes and inactivation in the local environment at the therapeutic site.

4.2.12 Chloramphenicol and Florfenicol

Chloramphenicol was isolated in 1947 from a soil actinomycete from Venezuela. Florfenicol is a fluorinated derivative of chloramphenicol.

4.2.12.1 MECHANISM OF ACTION

Chloramphenicol and florfenicol are bacteriostatic antibiotics that inhibit protein synthesis by binding to ribosomal subunits of susceptible bacteria, leading to the inhibition of peptidyl transferase and thereby preventing the transfer of amino acids to growing peptide chains and subsequent protein formation.[136] These antibiotics have a wide spectrum of activity, including streptococci, staphylococci, anaerobes, *Haemophilus, Salmonella, Pasteurella, Mycoplasma*, and *Brucella* spp. Florfenicol has activity against chloramphenicol-resistant strains of *E. coli, Klebsiella, Proteus, Salmonella*, and *Staphylococcus aureus*. These antibiotics are also active against rickettsia, chlamydia, and *Hemobartonella*.[137]

4.2.12.2 MECHANISMS OF RESISTANCE

Bacterial resistance to chloramphenicol occurs from plasmid-mediated bacterial production of acetylase enzymes. Acetylation of hydroxyl groups prevents drug binding to the 50-S ribosomal subunit. Florfenicol has a fluorine atom instead of the hydroxyl group located at C-3 in the structure of chloramphenicol and 202
thiamphenicol. Initially, florfenicol was less susceptible to deactivation by bacteria with plasmid-transmissible 203
resistance to chloramphenicol.[138] Recently, new resistances to chloramphenicol and florfenicol have been identified in cattle pathogens.[139,140]

4.2.12.3 PHARMACOKINETICS

4.2.12.3.1 Absorption

Chloramphenicol and florfenicol are absorbed rapidly and extensively after oral administration. The oral bioavailability of chloramphenicol in foals is 83%[141] but is only 40% after a single administration in mares, and bioavailability declines to 20% after five doses.[142] The oral bioavailability of florfenicol in horses is 81%.[143] The commercially available formulation of florfenicol is a long-acting product for cattle and is characterized by delayed absorption and low plasma concentrations.[144]

4.2.12.3.2 Distribution

Because of high lipid solubility and low protein binding, chloramphenicol and florfenicol diffuse throughout the body. The highest drug levels occur in the liver and kidney, but therapeutic drug concentrations occur in most tissues and fluids, including ocular humor and synovial fluid.[137] Chloramphenicol may achieve concentrations up to 50% of plasma concentrations in the CSF when the meninges are normal and more if inflammation is present.[145] Florfenicol does not penetrate the blood-brain barrier as readily as chloramphenicol.[146] The Vd of chloramphenicol is 2.83 L/kg in horses[147] and 1.6 L/kg in neonatal foals.[141] The Vd of florfenicol is 0.72 L/kg in horses.[143] The degree of protein binding of chloramphenicol in horses is 30%,[148] and florfenicol has a low degree of protein binding in cattle.[149]

4.2.12.3.3 Elimination

In most species, these drugs are eliminated by renal excretion of parent drug and by hepatic glucuronide conjugation and elimination in feces. The elimination $T_{1/2}$ of chloramphenicol in foals older than 7 days and adult horses is less than 1 hour.[141,147,148,150,151] In 1-day-old foals the elimination $T_{1/2}$ is 5.3 hours, indicating the immaturity of the hepatic metabolism capacity of the foal.[150] Florfenicol has an elimination $T_{1/2}$ of 1.8 hours in horses after intravenous administration.[143] The long-acting formulation for cattle is eliminated slowly because of slow absorption from the intramuscular injection site (flip-flop kinetics).[144]

4.2.12.4 ADVERSE EFFECTS AND DRUG INTERACTIONS

Because these drugs are protein synthesis inhibitors, a dose-related anemia and pancytopenia are associated with chronic therapy (>14 days), causing a decrease in protein synthesis in the bone marrow. Florfenicol is more likely than chloramphenicol to cause reversible bone marrow suppression with chronic dosing.[146] In human beings an idiosyncratic aplastic anemia occurs with exposure to chloramphenicol.[152] The reaction is rare (1 in 30,000) and not dose-related. Toxic effects are related to the presence of the paranitro group on the chloramphenicol molecule. This reaction does not occur with florfenicol because it lacks the paranitro group. Chloramphenicol is a hepatic microsomal enzyme inhibitor that decreases the clearance of other drugs metabolized by the same cytochrome P-450 enzymes including phenytoin, phenobarbital, pentobarbital, phenylbutazone, xylazine, and cyclophosphamide.[137,153,154] Whether florfenicol affects the metabolism of other drugs is not known.

Although chloramphenicol is well tolerated by horses, florfenicol alters fecal consistency with single doses administered intravenously, orally, or intramuscularly.[143] In a chronic dosing study with the cattle intramuscular formulation, all horses remained clinically normal but had dramatic alterations in enteric flora. *Salmonella* spp., *E. coli*, and *Clostridium perfringens* in these horses rapidly developed resistance to florfenicol.[144]

Chloramphenicol may suppress antibody production if given before an antigenic stimulus and may affect vaccination response.[137] This effect has not been reported for florfenicol. One should not administer chloramphenicol or florfenicol concurrently with penicillins, macrolides, aminoglycosides, or fluoroquinolones. Chloramphenicol or florfenicol may antagonize the activity of penicillins or aminoglycosides, and they act on the same ribosomal site as the macrolides. Inhibition of protein synthesis by chloramphenicol or florfenicol interferes with the production of autolysins necessary for cell lysis after fluoroquinolones interfere with DNA supercoiling.

4.2.12.5 FORMULATIONS

Chloramphenicol sodium succinate is a water soluble formulation for intravenous use and is hydrolyzed to chloramphenicol in the liver. Chloramphenicol free base and chloramphenicol palmitate are available for oral administration. The chloramphenicol palmitate is hydrolyzed in the gastrointestinal tract to chloramphenicol. Ophthalmic formulations of chloramphenicol are also available. Florfenicol is only available as a cattle product in three carriers (2-pyrrolidone, propylene glycol, and polyethylene glycol) to give it a long-acting effect.

4.2.12.6 CLINICAL USE

Chloramphenicol is banned for use in any type of food animal because it causes idiosyncratic aplastic anemia in human beings. Chloramphenicol is used in small animals and horses for a variety of bacterial infections, especially where penetration into the CNS is desired, but one should take appropriate precautions in handling the product to prevent human exposure. One should not use florfenicol routinely in horses because of its 203
effects on gastrointestinal flora and the clinical limitations of the long-acting cattle formulation. 204

4.2.13 Potentiated Sulfonamides

The sulfonamides are a group of organic compounds with chemotherapeutic activity (hence, they are antimicrobials, not antibiotics). They have a common chemical nucleus that is related closely to *p*-aminobenzoic acid, an essential component in the folic acid pathway of nucleic acid synthesis. Sulfonamides are combined with diaminopyrimidines such as trimethoprim and pyrimethamine, which inhibit an essential step further along the folate pathway. The potentiated sulfonamides are remarkably synergistic and nontoxic and so are used commonly in equine medicine. Their use is complicated by differences in pharmacokinetics between trimethoprim/pyrimethamine and the various sulfonamides used in the combinations.

4.2.13.1 MECHANISM OF ACTION

The sulfonamides inhibit the bacterial enzyme dihydropteroate synthetase in the folic acid pathway, thereby blocking bacterial nucleic acid synthesis. Sulfonamides substitute for PABA, preventing its conversion to

dihydrofolic acid. Alone, this action is considered bacteriostatic. Because the activity is by competitive substitution, the sulfonamide tissue concentration must be kept high enough to prevent bacterial access to PABA. Therefore the sulfonamides are ineffective in pus and necrotic tissue, which provide additional sources of PABA to the bacteria. The sulfonamides are nontoxic to mammalian cells because they use dietary folate for synthesis of dihydrofolic acid and do not require PABA. The addition of trimethoprim or pyrimethamine to a sulfonamide creates a bactericidal combination. Trimethoprim inhibits bacterial folic acid synthesis at the next step in the folic acid sequence, inhibiting the conversion of dihydrofolic acid to tetrahydrofolic acid by inhibiting dihydrofolate reductase. This enzyme is present in bacterial and mammalian cells, but the bacterial enzyme is inhibited at much lower concentrations than necessary to inhibit the mammalian enzyme.[155] The minimum inhibitory concentrations against specific susceptible bacteria for each drug generally decrease when the antimicrobials are administered in the potentiated sulfonamide combination. The resistance developed to the potentiated sulfonamides is lower than that to each individual drug; this is an important benefit because resistance to sulfonamides is common and resistance develops rapidly to diaminopyrimidines when used alone.[156]

The potentiated sulfonamides have a broad spectrum of activity. Bacteria that are usually susceptible include *Streptococcus, Proteus, E. coli, Pasteurella, Haemophilus*, and *Salmonella* spp. Staphylococci, anaerobes, *Nocardia, Corynebacterium, Klebsiella*, and *Enterobacter* are susceptible but may become resistant. *Pseudomonas* spp., *Bacteriodes* spp., and enterococci are usually resistant.[40,157,158] Other significant organisms that are susceptible to potentiated sulfonamides include protozoa *(Toxoplasma gondii, Sarcocystis neurona)* and coccidia. Pyrimethamine is more effective against protozoa than trimethoprim.[156] The potentiated sulfonamides are formulated at a fixed ratio of 1:5 of trimethoprim to sulfonamide. The optimum concentration for bactericidal action is 1:20.[155] When and where this optimum concentration is achieved is difficult to predict in vivo, but a 1:20 ratio is used in susceptibility testing.[159]

4.2.13.2

MECHANISMS OF RESISTANCE

Bacterial resistance to the sulfonamides is common and may be from chromosomal mutations or may be mediated by plasmids. Chromosomal mutations can lead to bacterial hyperproduction of PABA, which overcomes the competitive substitution of the sulfonamides. Plasmid-encoded resistance results in a bypass of the drug-sensitive step by production of altered forms of the dihydropteroate synthetase enzyme with a lower affinity for sulfonamides. Resistance to trimethoprim usually occurs by plasmid-encoded production of trimethoprim-resistant dihydrofolate reductase. Other resistance mechanisms include excessive bacterial production of dihydrofolate reductase and reduction in ability of drug to penetrate the bacterial cell wall.[155] Cross-resistance between sulfonamides is considered complete and often occurs between pyrimidines. Resistance to the trimethoprim-sulfonamide combinations develops slowly but is now common among equine bacterial isolates.[160,161]

4.2.13.3

PHARMACOKINETICS

Pharmacokinetics of the potentiated sulfonamides are complicated by the distinct differences between disposition of trimethoprim/pyrimethamine and the various sulfonamides. When sulfonamides and diaminopyrimidines are administered concurrently to horses, the pharmacokinetics of each drug appear to be unaffected by the presence of the other. Table 4.2-4 gives the pharmacokinetics of specific potentiated sulfonamide combinations in horses. Although the potentiated sulfonamides frequently are used interchangeably, pharmacokinetics studies show that they are not bioequivalent in horses.

4.2.13.3.1

Absorption

In general, potentiated sulfonamides are absorbed readily from the gastrointestinal tract of horses but may be affected by feeding.[162–164] For trimethoprim and sulfachlorpyridazine, peak plasma concentrations and bioavailabilities are reduced significantly when the drug is mixed with concentrate compared with nasogastric administration.[165] Both drugs also demonstrate a biphasic absorption pattern, which appears to be caused by a portion of the trimethoprim and sulfachlorpyridazine dose binding to feed, with a second absorption phase occurring in the large intestine.[166] Bioavailability after intrauterine administration is 23% to 43% for trimethoprim and 29% to 34% for sulfadoxine. After intrauterine administration, trimethoprim and sulfadiazine are detected in the milk.[167] The oral bioavailability of pyrimethamine is 56% in horses.[168]

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TABLE 4.2-4 Pharmacokinetics of Trimethoprim, Pyrimethamine, and Sulfonamides in Horses

DRUG	VOLUME OF DISTRIBUTION (L/kg)	HALF-LIFE OR MRT[*] (HOUR)	PROTEIN BINDING (%)	CLEARANCE (ml/min/kg)	DOSE[†] (mg/kg)
Trimethoprim	—	2.4	—	—	PO: 5
Sulfadiazine[163]		7.4			PO: 25
Trimethoprim	2.0	2.8	35	8.8	IV: 2.5
Sulfadiazine[185]	0.5	4.6	20	1.5	IV: 12.5
Trimethoprim	1.5	3	50	—	IV: 8
Sulfadoxine[173]	0.39	14	14–72		IV: 40
Trimethoprim	2.8	3.4	—	11	IV: 7.5
Sulfamethoxazole[192]	0.5	4.8	—	1.4	IV: 36.5
Trimethoprim	1.6	1.9	—	13	IV: 2.5
Sulfamethoxazole[170]	0.33	3.5	—	1.3	IV: 12.5
Trimethoprim	1.5	2.6	—	7.7	IV: 5
Sulfachlorpyridazine[165]	0.26	3.8	—	2.6	IV: 25
Pyrimethamine[168]	1.5	12	—	1.6	IV, PO: 1
Sulfadiazine[174]	0.4	3.8	43	2.3	IV: 20
Sulfamerazine[174]	0.49	3.2	44	1.8	IV: 20
Sulfamethazine[162]	0.63	11.4	—	0.8	IV: 160
Sulfamethazine[174]	0.33	5.4	69	0.9	IV: 20

* *MRT,* Mean residence time.

† *PO,* Oral; *IV,* intravenous.

4.2.13.3.2 Distribution

The sulfonamides are weak acids and are hydrophilic; therefore they distribute well in extracellular and interstitial fluids and typically have values for Vd of 0.3 to 0.7 L/kg. Initial concentrations of sulfonamides in tissues are generally lower than those in plasma. The diaminopyrimidines are lipophilic weak bases and penetrate tissues better than sulfonamides, resulting in values for Vd of 1.5 to 2.7 L/kg and higher tissue concentrations than plasma concentrations.[155] Distribution of potentiated sulfonamides has been investigated broadly in the horse. Sulfadiazine and trimethoprim[169] and sulfamethoxazole and trimethoprim[170,171] are well distributed into peritoneal fluid, CSF, synovial fluid, and urine. Inflammation in the meninges or synovium does not affect distribution into the respective fluids significantly. After repeated doses, sulfamethoxazole, unlike trimethoprim, accumulates in the CSF.[170] CSF concentrations of pyrimethamine reach 25% to 50% of serum concentrations but do not appear to accumulate in horses with daily dosing.[172]

Sulfonamides can be highly bound to plasma proteins, but the extent of binding depends on the species, drug, and concentration. In the horse, degree of protein binding varies from 33% for sulfaphenazole to 93% for sulfamethoxine.[155] Approximately 50% of trimethoprim is protein bound, and binding is independent of plasma concentration.[173] Although only free drug is available for antimicrobial action, protein-bound drug serves as a reservoir and extends the duration of action of these drugs.

4.2.13.3.3 Metabolism

The liver metabolizes diaminopyrimidines and sulfonamides, usually by acetylation, aromatic hydroxylation, and glucuronidation.[155] The acetylated, hydroxylated, and conjugated forms of the sulfonamides are significantly less microbiologically active than their parent compounds. The precise metabolic pathways for trimethoprim or pyrimethamine have not been elucidated.[171] Metabolites may compete with the parent drug for involvement in folic acid synthesis. They have little detrimental effect on bacteria, so their presence can decrease the activity of the remaining parent drug.[174]

4.2.13.3.4 Elimination

Sulfonamides are excreted primarily in urine, but excretion in feces, bile, milk, sweat, and tears also occurs. Renal excretion of unchanged drug and metabolites occurs by glomerular filtration and active tubular secretion.[155] Reabsorption occurs in the distal tubule by passive diffusion. Because most sulfonamides are weak acids, alkaline urine increases their ionization and elimination. Renal excretion of trimethoprim 205
occurs by glomerular filtration, active tubular secretion, and reabsorption. In horses a large percentage of 206
trimethoprim appears to be metabolized before elimination in urine (46%) and feces (52%).[173] Urine pH, plasma concentration, and extent of diuresis affect the clearance of the diaminopyrimidines. In contrast to the sulfonamides, alkaline urine increases the reabsorption of the basic trimethoprim.[175]

4.2.13.4

ADVERSE EFFECTS AND DRUG INTERACTIONS

The potentiated sulfonamides are noted for their widely varying adverse effects. Crystalluria, hematuria, and renal tubular obstruction can result from poorly soluble sulfonamides, especially in dehydrated patients with acidic urine.[155] However, the lower doses of sulfonamide used in the potentiated sulfonamide combinations makes crystallization less likely to occur than with sulfonamides administered alone. Local infusion of potentiated sulfonamides into the uterus of mares causes irritation of the endometrium and a decreased pregnancy rate.[167] Intramuscular administration causes tissue irritation from the organic solvents, high concentration, and high pH of the formulations and should be avoided. Intravenous administration must be slowly and carefully done. Rapid administration is associated with thrombophlebitis and anaphylaxis.[155,176] The concurrent use of potentiated sulfonamides with detomidine is contraindicated, because the potentiated sulfonamide appears to sensitize the myocardium and results in cardiac dysrhythmias and hypotension that may be fatal.[177,178] The procaine in procaine penicillin G is a PABA analog and may reduce efficacy if used concurrently with potentiated sulfonamides.[41]

4.2.13.4.1

Folate Antagonism Effects

The nonregenerative anemias seen in response to long-term administration of potentiated sulfonamides are believed to be related to folate reduction with long-term, high-dose administration, such as for treatment of equine protozoal encephalomyelitis. Concurrent therapy with trimethoprim and pyrimethamine does not increase the efficacy against protozoa and is suspected to increase the incidence of side effects because of folate reduction. Supplementation with oral folic acid often is recommended for horses for long-term potentiated sulfonamide therapy.[179] The oral administration of folic acid to pregnant mares being treated for equine protozoal encephalomyelitis may not protect the fetus from the effects of folate deficiency. Mares have delivered foals with congenital defects after oral administration of potentiated sulfonamides. These mares also had been given oral folic acid and vitamin E during the period of antibiotic treatment. Each of three mares on this dosage regimen produced a foal with renal hypoplasia or nephrosis and bone marrow aplasia or hypoplasia. In mares and foals, serum folate concentrations were below the laboratory reference range, and in two foals, folate was less than 30% of the minimum reference range.[180] One should consider the risk of congenital defects when treating pregnant mares with pyrimethamine and sulfonamide. Treatment with trimethoprim-sulfamethoxazole and pyrimethamine does not affect semen quality, testicular volume, sperm production efficiency, erection, or libido of healthy stallions. However, treatment may induce changes in copulatory form and agility and alter the pattern and strength of ejaculation.[181] Stallions that develop neurologic signs during treatment should be used with caution for breeding. Trimethoprim-sulfamethoxazole has been associated with immune-mediated hemolytic anemia in a horse.[182]

4.2.13.4.2

Effects on Gastrointestinal Flora

The effects of potentiated sulfonamides on normal gastrointestinal flora are controversial. In some studies, potentiated sulfonamides alone[183] or with concurrent penicillin therapy[16] are associated with diarrhea in horses. Other studies show little effect on fecal flora.[184,185] The likelihood of any antimicrobial therapy causing diarrhea in a horse depends on several factors, including the antibacterial spectrum of the drug and the drug concentrations achieved in the gastrointestinal tract. The presence or absence of potential pathogens in the composition of the microflora of the horse and the presence of antimicrobial-resistant

pathogens in the hospital or clinic environment are also important factors in the incidence of gastrointestinal disturbances.

4.2.13.5
FORMULATIONS

Trimethoprim-sulfadiazine is available as a 48% injectable solution for intravenous administration in horses and is also available in oral paste and powder formulations for horses. Human generic tablets of trimethoprim-sulfamethoxazole commonly are administered to horses. Pyrimethamine-sulfadiazine formulations are routinely available through compounding pharmacies.

4.2.13.6
CLINICAL USE

Applying pharmacokinetic principles to determine drug dosage regimens for the potentiated sulfonamides is difficult. Different pathogens have varying MIC values, and the optimal ratio of trimethoprim or pyrimethamine to sulfonamide also varies between bacteria and protozoa. The most important component of the formulation for efficacy appears to be the diaminopyrimidine, and the choice of sulfonamide may not be nearly as important. Therefore considerable controversy exists regarding the dosage regimen of these combinations. The veterinary products are labeled for once-daily administration, but studies indicate that twice-daily dosing is better for attaining therapeutic plasma concentrations.[185,186] 206

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4.2.14
Tetracyclines

Tetracycline was discovered after a team of workers examined 100,000 soil samples from around the world. Tetracycline derivatives include *oxytetracycline, chlortetracycline, doxycyline*, and *minocycline*. Oxytetracycline and doxycycline are used in horses.

4.2.14.1
MECHANISM OF ACTION

The tetracyclines bind to the 30-S ribosomal subunit and interfere with bacterial protein synthesis. They are bacteriostatic at usual therapeutic concentrations, but bactericidal at high concentrations. Drug entry into the bacteria is by an energy-dependent mechanism. Mammalian cells do not possess the tetracycline transport mechanism. Tetracyclines are most active at an acidic pH. The tetracyclines are broad spectrum in activity; they are effective against gram-positive and gram-negative bacteria, as well as *Chlamydia, Mycoplasma, Rickettsia*, and some protozoa *(Haemobartonella, Anaplasma)*.[187] Their activity against staphylococci is limited, and they are not active against group D streptococci (enterococci). *Pseudomonas, Escherichia coli, Klebsiella*, and *Proteus* are usually resistant.[188,189] Most anaerobes are susceptible to doxycycline.[187] In vitro concentrations of oxytetracycline greather than 0.01 μg/ml effectively suppress growth of *Ehrlichia risticii*.[190]

4.2.14.2
MECHANISMS OF RESISTANCE

Widespread acquired resistance in many pathogens limits the clinical usefulness of the tetracyclines. Resistance occurs from plasmid-mediated failure in the active transport of the drug into the bacterial cell and increased efflux from the cell. Another major mechanism of resistance involves cytoplasmic production of a protein that protects the ribosome from tetracycline action.[187]

4.2.14.3 PHARMACOKINETICS

4.2.14.3.1 Absorption

Oral absorption of oxytetracycline is erratic and is not recommended in horses because of adverse effects on gastrointestinal flora.[184] At a dose of 10 mg/kg, doxycycline produced serum, synovial fluid, peritoneal fluid, and endometrial tissue concentrations greater than 0.25 μg/ml, suggesting effective therapy for gram-positive infections.[191] A precise bioavailability cannot be determined, because intravenous administration of doxycycline causes cardiac toxicity.[192] The long-acting formulation of oxytetracycline in polyethylene glycol has a bioavailability of 83% after intramuscular injection in horses.[193]

4.2.14.3.2 Distribution

The tetracyclines are well distributed to most tissues, except the CNS. Therapeutic levels may be achieved, however, when the meninges are inflamed. Tetracyclines readily diffuse into milk.[187] Oxytetracycline reaches 50% of plasma concentrations in synovial fluid and peritoneal fluid. Oxytetracycline concentrations in urine are high, with peak concentrations greater than 1500 μg/ml.[194] The Vd of oxytetracycline in neonatal foals is 2 L/kg[195] and in adult horses is 0.34 to 0.95 L/kg.[193,196] The apparent Vd of doxycycline in horses is 25 L/kg, indicating a high lipid solubility and tissue penetration.[191] Synovial and peritoneal fluids achieve the same doxycycline concentrations as plasma, and endometrial tissue concentrations are more than twice plasma concentrations. Doxycycline is not detectable in CSF after oral administration.[191] Oxytetracycline is 50% protein bound in horses.[197] Although not reported for horses, doxycycline is highly protein bound (90%) in other species.[187]

4.2.14.3.3 Elimination

The tetracyclines are not known to be biotransformed to any significant extent before elimination. Oxytetracycline is eliminated unchanged in urine primarily by glomerular filtration. Unmetabolized drug also is eliminated with bile into the gastrointestinal tract and may undergo enterohepatic recirculation, prolonging its effects.[187] Doxycycline is excreted primarily into the feces via nonbiliary routes in an inactive form. Therefore doxycycline does not accumulate in patients with renal insufficiency.[198] The clearance of oxytetracycline is 3.3 ml/min/kg in foals[195] and 2.2 ml/min/kg in adult horses.[193] When administered intravenously, the elimination $T_{1/2}$ of oxytetracycline is 7 hours in foals[195] and 6 hours in horses.[193] Because of flip-flop kinetics, the elimination $T_{1/2}$ is 22 hours after intramuscular administration of oxytetracycline in polyethylene glycol.[193]

4.2.14.4 ADVERSE EFFECTS AND DRUG INTERACTIONS

4.2.14.4.1 Gastrointestinal Effects and Interactions

Calcium-containing products (milk, antacids) or other divalent cations chelate with tetracyclines and interfere with gastrointestinal absorption.[187] Doxycycline is less likely than the older tetracyclines to form chelation complexes with divalent and trivalent metals, so less interference occurs with oral absorption by calcium or other substances.[198]

The clinical use of oxytetracycline in horses is controversial because of reports of adverse gastrointestinal effects. However, adverse effects also were associated with excessive dosage,[199] concomitant use of other antimicrobials, and stressors such as surgery and transport.[200–203] Anecdotally, oxytetracycline therapy has been used successfully in equine practice, and the recognition of the equine ehrlichial diseases has increased oxytetracycline use in horses.[204,205] In a chronic dosing study using a long-acting formulation of 207
oxytetracycline, no deleterious effects on fecal flora were detected and treated horses remained clinically 208
normal.[206] Doxycycline is less likely to cause adverse gastrointestinal effects because it is bound in an inactive form in the intestines.[187]

4.2.14.4.2 Renal Effects

Renal tubular necrosis from oxytetracycline is associated with high doses, outdated parenteral products, endotoxemia, dehydration and hypovolemia, and concurrent pigment nephropathy.[187,207] In normal foals, high-dose intravenous oxytetracycline administration for the correction of flexural deformities does not cause renal toxicity.[208] Oliguric renal failure developed in a foal given 70 mg/kg of oxytetracycline intravenously for a flexural deformity with concurrent neonatal isoerythrolysis.[207]

4.2.14.4.3 Cardiovascular Effects

Rapid intravenous administration of oxytetracycline results in hypotension and collapse, which is attributed to intravascular chelation of calcium and/or decreased blood pressure from the drug vehicle (propylene glycol). Pretreatment with calcium borogluconate intravenous prevents collapse.[209,210] Rapid intravenous administration of doxycycline to horses causes tachycardia, systemic arterial hypertension, collapse, and death.[192,211] One suggestion is that this reaction is caused by chelation of intracellular calcium, resulting in neuromuscular blockade of the myocardium.

4.2.14.4.4 Musculoskeletal Effects

Intramuscular injection of long-acting oxytetracycline formulations cause localized pain and swelling at the injection site.[187,206] Oxytetracycline causes flexor tendon relaxation; this effect has been used to treat foals with flexural deformities.[212,213] The mechanism of oxytetracycline-induced relaxation is unknown but may be related to calcium-chelation or neuromuscular blockade.

4.2.14.5 FORMULATIONS

Injectable oxytetracycline products are formulated as short- or long-acting products. The short-acting solutions are in propylene glycol and have concentrations of 50 or 100 mg/ml. The long-acting solutions are in 2-pyrrolidone or polyethylene glycol and have a concentration of 200 mg/ml. The polyethylene glycol formulation is less irritating than the 2-pyrrolidone formulation. The long-acting formulations may be administered by slow intravenous injection but the long-acting effect is lost.[193]

4.2.14.6 CLINICAL USE

Oxytetracycline is the drug of choice for Potomac horse fever *(Ehrlichia risticii)* and equine ehrlichiosis *(E. equi)*.[204,205,214,215] Oxytetracycline also is used to treat contracted flexor tendons in foals and calves.[212,213] Its use for other microbial infections in horses is controversial because of concerns of adverse gastrointestinal effects and widespread antimicrobial resistance. Doxycycline also is indicated for rickettsial diseases in horses and may be a suitable oral alternative to oxytetracycline.

4.2.15 Macrolides and Azalides

The macrolide antibiotics include *erythromycin, clarithromycin, tylosin, tilmicosin*, and *tiamulin*. Azalides, such as *azithromycin*, have a similar mechanism of action but have a methylated nitrogen in the macrocyclic ring. Because of adverse gastrointestinal effects, one should avoid using these drugs for horses, except for erythromycin and azithromycin in foals.

4.2.15.1 MECHANISM OF ACTION

The macrolides and azalides bind to the 50-S ribosomal subunit in a manner similar to chloramphenicol and florfenicol and interfere with protein synthesis. They usually are considered bacteriostatic but may be bactericidal at high concentrations. Macrolides are not effective against gram-negative bacteria, except some strains of *Pasteurella* and *Haemophilus* in cattle.[216] Azithromycin is more active than the macrolides against gram-negative bacteria and anaerobes.[217] Susceptible bacteria include staphylococci, streptococci, *Campylobacter jejunii, Clostridium, Rhodococcus equi, Mycoplasma* spp., and *Chlamydia* spp.[40,189,218] Optimum antimicrobial activity of these weak bases is at alkaline pH; therefore they have reduced activity in acidic environments (pus, abscesses) but may be clinically effective in high concentrations because of ion trapping. Erythromycin also has nonantimicrobial effects on host cell metabolism, inflammatory mediators, and gastrointestinal motility.[219,220]

4.2.15.2 MECHANISMS OF RESISTANCE

The routine use of the macrolides is limited because bacterial resistance develops quickly from repeated exposure.[221] Mechanisms of resistance include decreased drug entry into bacteria, inability to bind to the bacterial 50-S ribosomal subunit, and plasmid-mediated production of esterases.[24,221] Recently, 13% of *R. equi* isolates were found to be resistant to erythromycin.[222] Extensive cross-resistance occurs between the macrolides.[216]

4.2.15.3 PHARMACOKINETICS

4.2.15.3.1 Absorption

Erythromycin is available for oral administration as enteric coated erythromycin base, erythromycin esters (ethylsuccinate or estolate) or erythromycin salts (phosphate or stearate). Because of expense, many practitioners administer the drug as crushed enteric coated tablets of erythromycin base. However, 208
erythromycin base is degraded in the stomach because of the gastric acid. The esterified formulations are 209
absorbed intact and must be hydrolyzed to the active erythromycin A. The erythromycin salts are absorbed unchanged.[223] The oral bioavailability of erythromycin base is 17% in fasted foals, with most of the drug being degraded and absorbed as the microbiologically inactive anhydroerythromycin A.[224] Microencapsulation of the base improves the oral bioavailability of erythromycin base to 26% in fasted foals but remains only 7.7% in fed foals.[225] The oral bioavailability of erythromycin estolate in fasted foals is 36% but only 16% for erythromycin phosphate. The estolate formulation appears to have the best pharmacokinetic profile in foals.[226] Because of injection site irritation, intramuscular administration of erythromycin is not recommended in horses. The oral bioavailability of azithromycin in foals is approximately 50%.[227]

4.2.15.3.2 Distribution

The macrolides are well distributed into most tissues. Erythromycin and azithromycin concentrate in leukocytes, making them effective against intracellular pathogens such as *R. equi*.[228,229] Because macrolides are weak bases, they are ion trapped in milk, CSF, and gastric fluids. The Vd for erythromycin is 2.7 L/kg in foals.[224] Azithromycin is known for its high degree of lipid solubility, and the Vd of azithromycin in foals is 18.6 L/kg. Peritoneal and synovial fluid concentrations of azithromycin parallel serum concentrations. Bronchoalveolar cell and pulmonary epithelial lining fluid concentrations are fifteen- to 170-fold and one- to sixteenfold higher than concurrent serum concentrations, respectively.[227]

4.2.15.3.3 Elimination

Erythromycin is metabolized extensively, with much of the parent drug and active metabolite excreted into the bile, resulting in an elimination half-life of 1 to 2 hours.[224–226,230] Erythromycin inhibits the metabolism of a number of other drugs by interfering with cytochrome P-450 enzymes.[216] The degradation product anhydroerythromycin A also is a potent inhibitor of cytochrome P-450–mediated metabolism.[219] Erythromycin undergoes enterohepatic cycling.[216] Azithromycin is not highly metabolized and is primarily eliminated in bile.[216] The elimination $T_{1/2}$ is 20.3 hours in foals.[227]

4.2.15.4 ADVERSE EFFECTS AND DRUG INTERACTIONS

The use of erythromycin in horses is associated with a number of adverse effects. After erythromycin came into clinical use in the 1950s, the gastrointestinal side effects that frequently accompany therapy became apparent. Macrolide antibiotics, including erythromycin and clarithromycin, are motilin receptor agonists.

They also appear to stimulate motility via cholinergic and noncholinergic neuronal pathways. At microbially ineffective doses, they stimulate migrating motility complexes and antegrade peristalsis in the gastrointestinal tract.[220,231,232] When used at antimicrobial doses, erythromycin is associated with potentially fatal colitis from *Clostridium* spp.[233–235]

Erythromycin also is associated with acute respiratory distress syndrome, hyperthermia, gastroenteritis, and hepatotoxicity in foals.[219,236,237] The mechanism of hyperthermia in foals treated with erythromycin has not been elucidated but likely results from derangement of the hypothalamic temperature set-point.[236] One should take extreme care when administering erythromycin to foals with respiratory disease during periods of hot weather. Foals should not be left outside on hot, sunny days while receiving erythromycin therapy. Erythromycin also interferes with host cell metabolism and decreases inflammatory responses in airways, but the clinical significance of this has not been determined.[219]

Erythromycin is administered commonly with rifampin to take advantage of antimicrobial synergism and reduce the chance of resistance development.[14] Erythromycin may interact with other drugs metabolized by the same cytochrome P-450 enzyme system. Concurrent administration of erythromycin with theophylline results in doubling of plasma theophylline concentrations and can result in seizures in foals.[216]

Azithromycin is associated with fewer adverse effects than erythromycin in human beings.[217] No adverse reactions were detected during or after repeated intragastric administration of azithromycin in foals.[227]

4.2.15.5 FORMULATIONS

The base of erythromycin is unstable in gastric acid; therefore the base is formulated as enteric coated erythromycin tablets. Erythromycin stearate and erythromycin phosphate are insoluble salt formulations that disassociate in the intestine, allowing absorption of the free erythromycin base. Erythromycin ethylsuccinate and erythromycin estolate are ester formulations that are absorbed intact from the intestine; then plasma esterases release active drug. Erythromycin is available in an intramuscular formulation approved for cattle. Because of its highly irritating nature, intramuscular use is not recommended in horses and intravenous injection can be fatal. A human-labeled formulation of erythromycin lactobionate is available for intravenous use.

4.2.15.6 CLINICAL USE

Because of its association with potentially fatal adverse effects, erythromycin usually is limited to treatment of
R. equi infections in foals.[14,218,238–240] Erythromycin also has been shown to be an effective therapy of 209
Potomac horse fever[190,241] and equine proliferative enteropathy caused by *Lawsonia intracellulare*.[242] Its 210
motilin-like activity is exploited to treat adynamic ileus in horses.[220] Azithromycin recently has become an attractive alternative to erythromycin for treating *R. equi* infections in foals because of its pharmacokinetic profile, which allows once a day or every other day dosing, and an apparent reduced incidence of adverse effects.

4.2.16 Fluoroquinolones

The quinolones are a group of synthetic antimicrobials. The first was *nalidixic acid*, which was introduced in 1964. Nalidixic acid had good activity against gram-negative bacteria but had a low Vd and numerous adverse effects and was limited to treatment of urinary tract infections. Further chemical manipulation resulted in development of the fluorinated quinolones, which had extended antimicrobial activity with improved safety. Included in this group are *ciprofloxacin, enrofloxacin, danofloxacin, difloxacin, sarafloxacin, norfloxacin, orbifloxacin, marbofloxacin*, and *ofloxacin*. No fluoroquinolones are approved for use in horses, but because of their pharmacokinetics and antimicrobial activity, they are used commonly for serious gram-negative infections.[243]

4.2.16.1 MECHANISM OF ACTION

The fluoroquinolones have a unique mechanism of action for bacterial killing. The fluoroquinolones inhibit bacterial topoisomerase DNA gyrase (also known as topisomerase II). Bacteria have a single chromosome consisting of double-stranded DNA. Within the bacterial cell the chromosome is folded around an RNA core, and each fold is supercoiled. DNA gyrase, which has been found in every organism examined, is responsible for supercoiling the strand of bacterial DNA. The DNA gyrase structure has four subunits: two A monomers and two B monomers. The enzyme forms a heart-shaped molecule, with the A monomers forming atria and the B monomers forming ventricles. The bacterial DNA binds to the gyrase in the cleft between the A and B subunits. The DNA gyrase nicks double-stranded DNA, introduces negative supercoils, and seals the nicked DNA. The fluoroquinolones bind to the DNA-DNA gyrase complex and inhibit the DNA resealing, resulting in abnormal spatial DNA configuration, which leads to DNA degradation by exonucleases.[244]

Fluoroquinolone activity is concentration dependent. All of the fluoroquinolones are bactericidal; however, these drugs have an optimal concentration that is most bactericidal. Higher or lower drug concentrations result in reduced bactericidal activity. The DNA-DNA gyrase complex is thought to have two binding sites for fluoroquinolones. At low drug concentrations only one binding site is occupied, resulting in single-stranded nicks in the DNA. Reduced killing at high concentrations is thought to be caused by dose-dependent inhibition of RNA or protein synthesis. RNA and protein synthesis are required for production of bacterial autolysins, which are responsible for the fluoroquinolone-induced cell lysis.[244,245]

The fluoroquinolones are broad spectrum in activity against most gram-negative bacteria, some gram-positive bacteria, and *Mycoplasma, Chlamydia*, and *Rickettsia* spp. They are particularly effective against the enteric gram-negative pathogens, including some strains resistant to aminoglycosides and cephalosporins. Reported MICs are low, and MBCs are 1 to 2 times the MIC for most pathogens. They are usually active against staphylococci but have variable activity against streptococci and enterococci.[245] Most diagnostic laboratories use ciprofloxacin or enrofloxacin to determine pathogen susceptibility; however, MIC values vary among the fluoroquinolones. Ciprofloxacin has the greatest activity against *Pseudomonas* spp. Orbifloxacin has lower MIC values than enrofloxacin for the gram-negative bacteria *Actinobacillus equuli*, *Escherichia coli*, *Pasteurella* spp., and *Salmonella* spp. Enrofloxacin has lower MIC values for the gram-positive bacteria and *Pseudomonas* spp.[243]

Most fluoroquinolones are not active against anaerobic bacteria.[245] This susceptibility pattern may be a therapeutic advantage in treating enteric infections in horses, because gastrointestinal anaerobes rarely cause disease and usually are protective by competitively inhibiting colonization by pathogenic aerobic organisms.

The fluoroquinolones are concentrated within phagocytic cells. Uptake occurs by simple diffusion, and intracellular concentrations may be several times greater than plasma concentrations. Intracellular drug is microbiologically active against intracellular pathogens such as *Brucella* spp., *Mycoplasma* spp., and *Mycobacterium* spp.[245] Exposure of gram-negative bacteria to fluoroquinolones at concentrations several times the MIC for 1 to 2 hours results in a PAE with a recovery period of 1 to 6 hours. This effect suggests that fluoroquinolone dosage regimens can tolerate plasma concentrations below the MIC of the pathogen for extended periods of time without a reduction in efficacy.[244]

4.2.16.2 MECHANISMS OF RESISTANCE

Microbial resistance to the fluoroquinolones is caused by chromosomal mutations that alter bacterial DNA gyrase, decrease cell wall permeability, or increase fluoroquinolone efflux from the cell. Plasmid-mediated resistance has not been documented for the fluoroquinolones. The most common mechanism of resistance is from alteration of the bacterial DNA gyrase, resulting in decreased fluoroquinolone binding to the DNA-DNA gyrase complex. This occurs from point mutations in the genes coding for the enzyme structure, resulting in 210
physical obstruction for the fluoroquinolones as they bind to the DNA-DNA gyrase complex.[246] Gyrase 211
mutations confer high-level cross-resistance to all fluoroquinolones but are not associated with resistance to other unrelated antimicrobials.[247]

The fluoroquinolones must penetrate bacteria to reach the target DNA gyrase. The second mechanism of fluoroquinolone resistance is from decreased cell wall permeability.[245,246] The fluoroquinolones diffuse through porin channels in the outer membrane of gram-negative bacteria. The identified mutations result in a decrease in porin channel proteins, resulting in decreased uptake of the fluoroquinolones into the cell. *Pseudomonas* spp. resistance is associated with alterations in a wide range of outer membrane proteins. From these mutations the increase in the MICs of the fluoroquinolones is low (two- to thirty-twofold). However, cross-resistance with unrelated antibiotics occurs, most frequently with trimethoprim, tetracycline, chloramphenicol, and cefoxitin.[246]

The third mechanism for fluoroquinolone resistance is from increased fluoroquinolone efflux.[246] Efflux is the energy-dependent process of the inner bacterial membrane that exports drug into the periplasm or outside of the cell. This type of resistance occurs in staphylococci and streptococci and is associated with the presence of a resistance gene, *nor*A.[247] The degree of resistance conferred by increased fluoroquinolone efflux is less than the resistance caused by the DNA gyrase mutations, but the increase in MICs is sufficient for resistant mutants to arise during fluoroquinolone therapy.[246]

Because the fluoroquinolones have been used intensively in human medicine in the last 2 decades, high-level resistance has emerged in some pathogens. Because resistance is chromosomally mediated, chronic fluoroquinolone use encourages the development of resistance. In high-level resistant bacterial strains, one resistance mechanism alone is usually not responsible, rather two or three mechanisms of resistance operate together. In resistant *Staphylococcus aureus*, increased efflux often is coupled with a gyrase mutation.[247] In resistant *E. coli*, gyrase mutations usually are associated with changes in the outer membrane proteins.[248]

4.2.16.3 PHARMACOKINETICS

4.2.16.3.1 Absorption

The fluoroquinolones are absorbed well and rapidly from the gastrointestinal tract of monogastrics and preruminant calves. Enrofloxacin is more lipid soluble than ciprofloxacin and has a higher oral bioavailability than ciprofloxacin in horses and small animals. The oral bioavailability of ciprofloxacin is only 6.8% in adult ponies. The oral bioavailability of enrofloxacin is approximately 60% in adult horses and 42% in foals.[249,250] Antacids containing divalent cations (calcium, magnesium) chelate fluoroquinolones and reduce oral bioavailability.[245] Bioavailability from parenteral injection is nearly 100% for all fluoroquinolones, but intramuscular injections of enrofloxacin irritate tissues.[251]

4.2.16.3.2 Distribution

The fluoroquinolones are lipid soluble and well distributed to most tissues. The Vd of enrofloxacin is 2.3 L/kg in mares and 2.5 L/kg in foals.[249,251] The Vd of orbifloxacin in mares is 4 L/kg.[243] Tissue concentrations typically exceed plasma concentrations during therapy. High concentrations occur in the kidney, urine, liver, and bile. Therapeutic concentrations for gram-negative bacteria may be achieved in the CSF and ocular fluids.[243,252–254]

4.2.16.3.3 Elimination

The fluoroquinolones are excreted predominantly as unchanged drug in the urine by glomerular filtration and active tubular secretion.[244] Ciprofloxacin undergoes some sulfoxidation, and its metabolites also have antimicrobial activity. Enrofloxacin is metabolized (deethylated) to ciprofloxacin in horses, with serum ciprofloxacin concentrations reaching 20% to 35% of enrofloxacin concentrations.[251] The elimination $T_{1/2}$ of ciprofloxacin in ponies is 2.5 hours and in horses is 5 hours.[255] Enrofloxacin has an elimination $T_{1/2}$ of 4.4 hours after intravenous administration and 10 hours after intramuscular administration, indicating flip-flop kinetics.[251] With oral administration the elimination $T_{1/2}$ of enrofloxacin is 8 hours.[254] After oral administration the elimination $T_{1/2}$ of orbifloxacin is 9 hours and the clearance is 6.3 ml/min/kg in adult mares.[243] Difloxacin has prolonged elimination in the horse, with a $T_{1/2}$ of 18 hours (J. Bertone, unpublished data, 2001).

4.2.16.4 ADVERSE EFFECTS AND DRUG INTERACTIONS

Toxicity of fluoroquinolones is mild in most species and gastrointestinal irritation is the most common side effect.[244] The most clinically concerning effects are arthropathies. Transient arthropathies occur when fluoroquinolones are used to treat *Pseudomonas* pneumonia in children with cystic fibrosis, but the benefits are considered to outweigh the risks.[256] Chronic, high-dose fluoroquinolone therapy causes articular cartilage lesions in juvenile dogs, particularly in weight-bearing joints.[257] No documented arthropathies have been reported for calves, swine, or poultry. An in vitro study using equine cartilage explants did not demonstrate cartilage damage from enrofloxacin, although high doses reduced proteoglycan synthesis.[258] Arthropathies

have been documented in 2-week-old foals after receiving 10 mg/kg of enrofloxacin orally. Damage was characterized by synovial joint effusion and lameness, erosion, and cleft formation in articular cartilage.[259] Arthropathies were not seen in adult horses that were given up to 25 mg/kg of enrofloxacin intravenously 211
daily for 3 weeks or 15 mg/kg orally every 12 hours for 3 weeks.[253,260] Although not recommended for use in 212
pregnant women or animals, the fluoroquinolones appear to have little effect on the developing fetus.[261] Enrofloxacin was used successfully to treat chronic pleuritis in a pregnant mare with no apparent detrimental effects on the foal.[262]

Ciprofloxacin and enrofloxacin interfere with the cytochrome P-450 system metabolism of methylxanthines such as theophylline. Serum theophylline concentrations may double and result in CNS and cardiac toxicity, so one must monitor concentrations during therapy.[263]

The fluoroquinolones may cause adverse CNS effects in human beings and animals because of a γ-aminobutyric acid receptor antagonism, which has been associated with an increase in seizure incidence in human beings and dogs.[244] Administration of enrofloxacin to human beings results in hallucinations. Rapid intravenous administration of high doses of enrofloxacin to horses causes transient neurologic signs, including excitability and seizurelike activity.[260] These signs are avoidable with slow injection or dilution of the dose. Photosensitivity reactions and Achilles' tendon rupture have been associated with fluoroquinolone use in human beings but have not been reported in animals.[244]

The fluoroquinolones have been used with other antimicrobial agents to expand the therapeutic spectrum, to suppress emergence of drug-resistant bacterial populations, or to exploit inhibitory or bactericidal synergism against drug-resistant populations. Minimal synergy occurs between fluoroquinolones and β-lactams or aminoglycosides against gram-negative enteric bacteria because of the already high susceptibility of these organisms. Combinations with aminoglycosides, β-lactams, or vancomycin are additive or indifferent against staphylococci. Antagonism between fluoroquinolones and chloramphenicol or rifampin appears to be caused by the inhibition of bacterial autolysin synthesis from concurrent administration of bacterial protein synthesis inhibitors.[245]

4.2.16.5 FORMULATIONS

Ciprofloxacin is available as human labeled tablets, as a diluted solution for intravenous administration, and as a solution for ophthalmic use. Enrofloxacin is available as oral tablets and a 50 mg/ml injectable solution for intramuscular injection in dogs. In the United States, enrofloxacin is available as 100 mg/ml injectable solution for the subcutaneous treatment of cattle. Both injectable solutions can be administered intravenously to horses. Orbifloxacin and danofloxacin are available as oral tablets for small animals.

4.2.16.6 CLINICAL USE

The use of fluoroquinolones in horses has been limited by fear of inducing arthropathies; however, enrofloxacin has been used successfully in clinical cases, and fluoroquinolones may be the only viable option for treating some infections.[262,264–266] Because the fluoroquinolones are concentration-dependent killers with a long PAE, the ideal dosage regimen is once-daily, high-dose therapy.

4.2.17 Rifampin

4.2.17.1 MECHANISM OF ACTION

The rifamycins are antibiotics produced from *Streptomyces mediterranei*. Rifampin inhibits DNA-dependent RNA polymerase in susceptible organisms, suppressing RNA synthesis. Rifampin has no effect on the mammalian enzyme. Its action is bacteriostatic or bactericidal depending on the susceptibility of the bacteria and the concentration of the drug. Rifampin is effective against a variety of mycobacterium species and *Staphylococcus aureus, Haemophilus*, and *Rhodococcus equi*.[267] Rifampin is considered especially active in staphylococcal and rhodococcal infections and in the eradication of pathogens located in difficult-to-reach target areas, such as inside phagocytic cells. Rifampin is active at an acid pH, making it a rational choice for treating septic foci and granulomatous infections. Rifampin has moderate activity against *Actinobacillus suis, Actinobacillus equuli, Bordetella bronchiseptica*, and *Pasteurella* spp.[267,268] Equine isolates of *Pseudomonas aeruginosa, Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Proteus* spp., and *Salmonella* spp. are resistant.[267] The ability of rifampin to reach intracellular bacteria can make predicting in vivo therapy results difficult based on in vitro sensitivity tests. Bacterial resistance to rifampin develops rapidly; therefore it usually is administered with another antimicrobial.[269] Although rifampin commonly is combined with erythromycin to treat *R. equi*,[239] resistance to the combination has been reported.[221,270] The combination is also effective for treating equine monocytic ehrlichiosis (Potomac horse fever).[241]

4.2.17.2 PHARMACOKINETICS

4.2.17.2.1 Absorption

The oral bioavailability of rifampin varies from 70% in fasted horses to 26% when administered with feed.[30,268,271] Because rifampin is administered most often with feed, recommended dosages compensate for the decreased bioavailability. Bioavailability after intramuscular injection is 60%.[271]

4.2.17.2.2 Distribution

Rifampin is lipophilic and penetrates most tissues, including milk, bone, abscesses, and the CNS. Feces,
saliva, sweat, tears, and urine may be discolored red-orange by rifampin and its metabolites.[272] The Vd of 212
rifampin in horses is 0.6 to 0.9 L/kg.[268,271] Rifampin is 78% bound to plasma proteins.[268] 213

4.2.17.2.3 Elimination

In other species, rifampin is deacetylated hepatically to a metabolite that also has antibacterial activity. Desacetylrifampin was not detected in serum samples after an intravenous dose of 10 mg/kg or oral doses of 10 mg/kg every 12 hours for seven doses.[268] Desacetylrifampin was measured in urine, but rifampin was much more predominant. However, only 6.82% of the total dose was recovered in the urine as rifampin or desacetylrifampin.[268] The elimination $T_{1/2}$ of rifampin is 6 to 8 hours after intravenous administration and 12 to 13 hours after oral administration.[267,268] Because of immature hepatic metabolism, elimination of

rifampin is delayed in very young foals.[273,274] Plasma clearance ranges from 1.14 to 1.34 ml/min/kg.[268,271] As a hepatic enzyme inducer, rifampin induces its own metabolism so that multiple oral dosing significantly decreases the elimination $T_{1/2}$.[268] Enzyme induction typically has not been observed with less than 5 days of therapy, but once it occurs, the increase in enzyme activity may last for more than 2 weeks after discontinuation of treatment.[275]

4.2.17.3 ADVERSE EFFECTS AND DRUG INTERACTIONS

Rifampin stains everything it contacts, and treated animals may produce red urine, tears, sweat and saliva. Discoloration causes no harmful consequences. Most horses object to the taste of rifampin, so one must take care to deposit a dose well back on the tongue and rinse the mouth of the horse afterward. Microsomal enzyme induction from rifampin may shorten the elimination $T_{1/2}$ and decrease plasma drug concentrations of chloramphenicol, corticosteroids, theophylline, itraconazole, ketoconazole, warfarin, and barbiturates.[272]

4.2.17.4 FORMULATIONS

Rifampin is available as human labeled capsules or suspension for oral administration or as a diluted solution for intravenous use.

4.2.17.5 CLINICAL USE

Rifampin is used primarily to treat pulmonary abscess from *R. equi* in foals in combination with erythromycin.

4.2.18 Metronidazole

4.2.18.1 MECHANISM OF ACTION

Bacteria readily take up metronidazole and metabolize it by a reduction process to cytotoxic derivatives (short-lived free radical compounds). These cytotoxic compounds damage DNA and other critical intracellular macromolecules. Aerobic bacteria lack the reductive pathway necessary to produce the radical compounds.[276] Metronidazole is highly effective against anaerobic bacteria, including *Bacteroides fragilis* (penicillin-resistant), *Fusobacterium*, and *Clostridium* spp. Metronidazole also has good activity against protozoa, including *Giardia* and *Trichomonas* spp.[277]

4.2.18.2 PHARMACOKINETICS

4.2.18.2.1 Absorption

Metronidazole is absorbed well and rapidly after oral administration in horses, with an oral bioavailability of 75% to 85%.[277,278] In horses with gastrointestinal ileus, metronidazole may be administered per rectum and is absorbed rapidly; however, the bioavailability is only 30%.[279]

4.2.18.2.2
Distribution

Metronidazole is lipophilic and widely distributed in tissues. Metronidazole penetrates bone, abscesses, and the CNS. The Vd in mares is 0.7 to 1.7L/kg.[277,278]

4.2.18.2.3
Elimination

Metronidazole is metabolized primarily in the liver. Metabolites and unchanged drug are eliminated in urine and feces. Plasma clearance is 2.8 ml/min/kg, and elimination $T_{1/2}$ is 3 to 4 hours in horses.[277–280]

4.2.18.3
ADVERSE EFFECTS AND DRUG INTERACTIONS

In clinical use, metronidazole is associated only with anorexia with oral administration.[281] Metronidazole produces mutations in bacteria, and carcinogenicity occurs in laboratory mice with prolonged exposure. Therefore metronidazole is banned for use in food animals. Metronidazole has been implicated as a teratogen in laboratory animals, so one should avoid using it in pregnant animals.[282]

Because of its limited antimicrobial spectrum, metronidazole is merely additive to aminoglycosides and β-lactams for treating polymicrobial infections.

4.2.18.4
FORMULATIONS

Metronidazole is available only as human labeled formulations and most commonly is administered orally as tablets and capsules. Because metronidazole is poorly soluble, the intravenous formulation must be diluted in a large volume for administration and is expensive in horses.

4.2.18.5
CLINICAL USE

Metronidazole is used to treat anaerobic infections, especially pleuropneumonia and lung abscesses caused by
penicillin-resistant *Bacteroides fragilis* and clostridial enterocolitis.[281,283,284] Although rectal absorption is 213
inferior to oral absorption, rectal administration is a viable option for treatment when oral administration is not 214
feasible.

4.2.19
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4.3

4.3—Nonsteroidal Antiinflammatory Drugs

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The most commonly used drugs for pain and inflammation in horses are the nonsteroidal antiinflammatory drugs (NSAIDs). The NSAIDs inhibit the enzyme cyclooxygenase, which converts arachidonic acid to the prostaglandins, thromboxane, and prostacyclin (Figure 4.3-1). Blocking these ecosanoids results in antiinflammatory, analgesic, antipyretic, antiendotoxic, and antithrombotic effects.[1]

4.3.1

Mechanism of Action

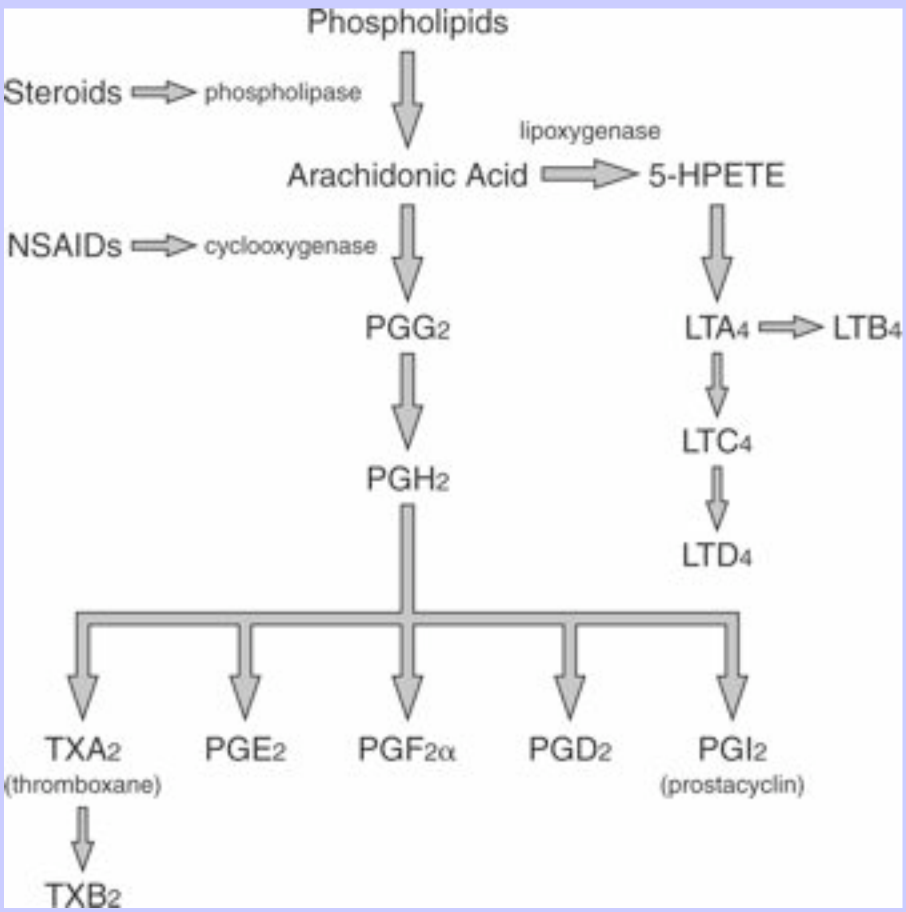
4.3.1.1

CYCLOOXYGENASE INHIBITION

Two different, distinct forms of cyclooxygenase (COX) have been identified. The constitutively expressed form is considered normal for homeostasis and is referred to as *COX-1*, whereas the inducible form in response to injury is considered detrimental and is referred to as *COX-2*.[2] COX-1 is found in platelets, the kidneys, and the gastrointestinal tract. COX-2 is identified in fibroblasts, chondrocytes, endothelial cells, macrophages, and mesangial cells. COX-2 is induced by exposure to various cytokines, mitogens, and endotoxin and is upregulated at inflammation sites.[3] Unfortunately, this classification of “good” versus “bad”

COX is too simplistic to explain the roles of its different forms.[4] COX-2 now appears to be be produced constitutively in the brain, spinal cord, kidney, ovary, uterus, placenta, thymus, bone, cartilage, synovia, endothelia, prostate, and lung. Hormones, nitric oxide, cytokines, and lipoxygenase products also can induce COX-2. COX-2 also is involved in cellular processes including gene expression, differentiation, mitogenesis, apoptosis, bone modelling, wound healing, and neoplasia.[5]

Figure 4.3-1 In the arachidonic acid cascade, cyclooxygenase works on arachidonic acid to produce prostaglandins *(PG)*, thromboxanes *(TX)*, and prostacycline, whereas lipoxygenase works on arachidonic acid to produce leukotrienes *(LT)*. *NSAIDS*, Nonsteroidal antiinflammatory drugs; *HPETE*, hydroperoxyeicosatetraenoic acid.



The prostaglandins produced in the gastrointestinal tract and the kidney that maintain mucosal integrity in the upper gastrointestinal tract and renal perfusion appear to be derived from COX-1.[6] Therefore suppressing COX-1 activity by NSAIDs is believed to be critical to the development of toxicity. One suggestion is that COX-2–selective NSAIDs would suppress prostaglandin synthesis at sites of inflammation but would spare

constitutive prostaglandin synthesis in the gastrointestinal tract and kidney.[3] The currently available NSAIDs vary in their potency as inhibitors of COX-1, but virtually all are far more potent inhibitors of COX-2 than of COX-1.[6] The pharmaceutical companies raced to develop COX-2– selective NSAIDs, but this now appears not to be a perfect solution. If COX-2 is primarily responsible for the prostaglandins that mediate pain, inflammation, and fever, then COX-2–selective drugs are not necessarily more therapeutically effective, because the nonselective NSAIDs are already effective inhibitors of COX-2.[5] Moreover, COX-1–derived prostaglandins now have been shown to contribute to pain, inflammation, and fever, so COX-2–selective NSAIDs actually may be less effective.[5,7] Studies are published now showing that some COX-2– selective drugs are only therapeutically effective at doses high enough to inhibit COX-1.[7] Also, COX-2 may produce beneficial prostaglandins; therefore highly selective COX-2 inhibitors may produce adverse reactions not seen with existing NSAIDs.[6] Most gastrointestinal ulceration also is associated with significant mucosal inflammation. In these circumstances, COX-2 likely is expressed and the derived prostaglandins are responsible for promoting healing.[8] NSAIDs are well known to retard the healing of ulcers and exacerbate inflammatory bowel disease.[6]

4.3.1.2

ANTIINFLAMMATORY EFFECTS

The NSAIDs are primarily antiinflammatory because of their inhibition of prostaglandin production. Therefore NSAIDs do not resolve inflammation but prevent its ongoing occurrence. So while prostaglandin production rapidly diminishes, any previously present prostaglandin must be removed before inflammation subsides. From tissue cage work, phenylbutazone, ketoprofen, and carprofen have been shown to have delayed peak concentrations at the site of inflammation and persist in inflammatory exudates for long periods after plasma concentrations are negligible.[9–11] This action explains the delayed onset and prolonged duration of antiinflammatory action that does not correlate with plasma pharmacokinetics of the NSAIDs.

The NSAIDs commonly are used in horses to attenuate the prostaglandin-mediated effects of endotoxin.[12,13] 221
Low-dose flunixin has antiendotoxin effects without obscuring the signs of colic pain or causing toxicity but 222
does not alter endotoxin-induced leukopenia.[14–19] A combination of flunixin and pentoxifylline may offset the deleterious hemodynamic effects of endotoxin more effectively than either drug alone.[20] Flunixin and phenylbutazone significantly inhibit movement of polymorphonuclear and mononuclear cells and antagonize the effects of endotoxin on bowel motility.[12,21]

4.3.1.3

OTHER ANTIINFLAMMATORY EFFECTS

COX inhibition does not explain all of the antiinflammatory activity of NSAIDs. NSAIDs are more lipophilic at a low pH, such as is found in inflamed tissues. Some antiinflammatory action appears to be related to their ability to insert into the lipid bilayer of cells and disrupt normal signals and protein-protein interactions in cell membranes. In the cell membrane of neutrophils, NSAIDs inhibit neutrophil aggregation, decrease enzyme release and superoxide generation, and inhibit lipoxygenase.[22]

4.3.1.4

ANALGESIC EFFECTS

The NSAIDs act as analgesics by inhibiting COX and preventing the production of prostaglandins that sensitize the afferent nociceptors at peripheral sites of inflammation.[23] However, increasing evidence indicates

that some NSAIDs have a central mechanism of action at the level of the spinal cord for analgesia unrelated to COX inhibition.[24] This action is synergistic with opioids and α_2-adrenergic drugs.[24,25] Work with the specific enantiomers of some NSAIDs have shown the S enantiomers to have good COX inhibitory effects, whereas the R forms can have weak activity against COX yet still produce analgesia.[26,27]

4.3.1.5 CLINICAL IMPLICATIONS

Therefore for managing pain and inflammation in horses, NSAIDs are more effective as analgesics when inflammation is a part of the pain process and when they are given before the onset of the inflammatory process or insult. The time to onset and duration of analgesia of NSAIDs does not correlate well with their antiinflammatory properties. The analgesic effect has a more rapid onset and shorter duration of action than the antiinflammatory action, so that dosage regimens for effective analgesia may need to be different from those for antiinflammatory effects.[23]

4.3.1.6 EFFECTS ON CARTILAGE

One should consider the effects of NSAIDs on proteoglycan synthesis in their clinical use for equine joint disease. Many NSAIDs affect cartilage anabolism in addition to their antiinflammatory actions.[28] Salicylates produce profound suppression of proteoglycan synthesis in osteoarthritic cartilage compared with normal cartilage.[29] A few NSAIDs, such as carprofen and meloxicam, have been shown to increase proteoglycan synthesis.[26,29,30] Phenylbutazone does not affect proteoglycan synthesis or chondrocyte viability but is protective against chondrocyte-mediated catabolism.[28]

4.3.2 Chirality

Many of the NSAIDs are *stereoisomers*, which consist of enantiomers with the same molecular formula, but because of asymmetrically oriented chemical groups on a central carbon, they form three-dimensional, nonsuperimposable mirror images and are known as *chiral* compounds.[31] This means that they are like one's hands: superimposable palm to palm, but not palm to back. For the NSAIDs the S (sinister) and R (rectus) designations for each of a pair of enantiomers commonly are used.[32] Although each member of a pair of enantiomers differs in three-dimensional orientation, its physical properties (e.g., melting and boiling points, refractive index, and solubility) are identical.[31] Biologic systems are highly chiral environments, and the pharmacokinetics and pharmacodynamic effects of each of a pair of enantiomers may be different. Stereospecificity may occur in the pharmacokinetic processes of absorption, distribution, metabolism, and excretion, especially if the process involves a carrier protein. If the fit of a drug molecule into the binding site on a protein, enzyme, or receptor involves the chiral center, then the affinity for attachment will be different for each of a pair of enantiomers.[33] Therapeutic efficacy and toxicity may be related specifically to one enantiomer. For the chiral NSAIDs, the S enantiomer typically is associated with COX inhibition, and the R enantiomer is associated with analgesic effects. Most chiral drugs are formulated as racemic mixtures, containing equal amounts of each enantiomer, for pure enantiomeric compounds are difficult and expensive to manufacture. All of the propionic acid NSAIDs (ketoprofen, carprofen, vedaprofen, and naproxen) are chiral compounds, and except for naproxen they are formulated as racemic mixtures. After administration, some enantiomers undergo *chiral inversion* as hepatic enzymes convert one form of the enantiomer to the other form. The chiral inversion of propionic acid derivatives is almost invariably unidirectional from R to S.[31] The degree of chiral inversion varies

between species and cannot be predicted from one species to another, making extrapolating dosages for NSAIDs from one species to another hazardous.

4.3.3 Physical Properties

Almost all NSAIDs are weak acids and highly bound to plasma proteins such as albumin. Therefore they are 222
well absorbed from the stomach, and most of the drug in the plasma is protein bound. Because of protein 223
binding, they are distributed predominantly in the extracellular fluid, and only low concentrations of NSAIDs are found in normal tissues and joint fluid. In damaged tissues and joints, however, NSAID concentrations increase to therapeutic levels because of increased blood flow, vascular permeability, and acute phase protein penetration into sites of inflammation. Most NSAIDs undergo hepatic metabolism through oxidation or glucuronide conjugation before being eliminated in the urine.[1]

4.3.4 Drug Interactions

One must consider the occurrence and potential hazards of drug interactions with therapeutic use of the NSAIDs. In general, any two NSAIDs administered together are additive in their effect.[34,35] Because most NSAIDs act by similar mechanisms of COX inhibition, a higher dose of a single NSAID should produce the same response. Because all of the NSAID drugs are highly bound to plasma proteins, one must use caution when administering other highly protein-bound drugs such as warfarin (coumarin).[34,36,37] Competition for protein binding sites can result in dramatic increases in free drug available for pharmacologic action. Antacids, mucoprotective agents, and adsorbent antidiarrheal drugs can interfere with NSAID absorption.[34] Concurrent use of corticosteroids potentiates gastrointestinal ulceration.[38] Chloramphenicol decreases the elimination rate, and rifampin significantly increases the elimination rate of phenylbutazone.[39] Concurrent administration of phenylbutazone and procaine penicillin G results in higher serum concentrations of penicillin because of decreased tissue distribution.[40] Concurrent administration of gentamicin and phenylbutazone results in increased distribution and delayed elimination of gentamicin.[41] Phenylbutazone decreases urinary excretion of furosemide and attenuates furosemide-induced increases in urinary excretion of sodium and chloride.[42]

4.3.5 Adverse Effects of NSAIDs

The adverse effects of the NSAIDs are related to COX inhibition in tissues where prostaglandins are beneficial and protective. Platelet aggregation classically is inhibited by NSAIDs by preventing thromboxane production via the COX-1 pathway.[1] Recovery of platelet function depends on the pharmacokinetics of the NSAID and the mechanism of COX inhibition.[43–46] Aspirin permanently modifies COX, so platelet function is restored only by the production of new platelets.[43,47] Reducing the protective prostaglandins results in blood vessel constriction and tissue necrosis in the kidney and reduction in blood flow and protective mucus production in the gastrointestinal tract.[6] Renal papillary necrosis (medullary crest necrosis), oral and gastrointestinal ulceration, and right dorsal colitis have been associated with NSAID use in the horse.[48–56] The NSAIDs have a higher incidence of toxicity in neonates because kidney and liver function is not developed fully.[55,57–59] When indicated in neonates, one should administer NSAIDs at the lowest possible doses and at extended dosing intervals. One should administer NSAIDs cautiously to dehydrated animals.[48,49] Because NSAIDs predominately distribute in extracellular water, plasma concentrations are greater than normal in the dehydrated animal and are more likely to cause toxicity.

Treatment of NSAID gastrointestinal toxicity is intensive and mainly symptomatic.[50] One can correct the hypoproteinemia that results from loss of plasma proteins into the ulcerated gastrointestinal tract with intravenous infusions of plasma. One can manage the fluid and electrolyte losses that accompany the diarrhea with commercially available intravenous fluids. Broad-spectrum antibiotics are indicated for bacterial septicemia. One must manage pain with opioid analgesics. Antiulcer medications may be beneficial and speed recovery. Surgical removal of damaged sections of stomach or colon may be necessary in some cases.[50] Recovery is usually slow, and in severe cases the prognosis is always guarded.

The renal toxicity of NSAIDs is a major concern, particularly in the perioperative period. NSAIDs typically have little effect on renal function in normal adult animals.[60] However, NSAIDs decrease renal blood flow and glomerular filtration rate in patients that have congestive heart failure, are hypotensive or hypovolemic (especially during anesthesia and surgery), or have chronic renal disease.[22] Under these circumstances, NSAIDs may precipitate acute renal failure as they block the ability of renal prostaglandins to mitigate the vasoconstrictive effects of norepinephrine and angiotensin II on glomerular arteries. COX-2–selective drugs do not seem to avoid this problem.[61] A more severe dose-dependent toxicity associated most commonly with phenylbutazone is renal papillary necrosis.[48,55,56] Although attributed to impaired renal blood flow, other mechanisms such as direct nephrotoxicity of the drug or its metabolites also may be involved.

4.3.6 Aspirin

Aspirin (sodium salicylate) is only available in oral forms. Because aspirin is a weak acid, it is best absorbed in the acidic environment of the upper gastrointestinal tract. During absorption, aspirin is hydrolyzed partially to salicylic acid and distributed throughout the body. The highest concentrations are attained in the liver, heart,
lungs, renal cortex, and plasma. The extent of protein binding is moderate (about 60%) and depends on species 223
and drug and albumin concentrations. Aspirin is metabolized hepatically by glycine and glucuronide 224
conjugation. Salicylates and their metabolites are excreted rapidly in urine via glomerular filtration and active tubular excretion, with an elimination half-life in the horse of approximately 1 hour.[1] In the horse, salicylic acid is the primary salicyl compound found in urine.[62] Significant tubular reabsorption occurs, which depends greatly on pH.[1]

Aspirin is the most effective NSAID for antiplatelet therapy.[43,63,64] Aspirin irreversibly acetylates the COX present in platelets, which inhibits the formation of thromboxane A_2, which is responsible for vasoconstriction and platelet aggregation.[43,46,47,64,65] Antiplatelet therapy may be beneficial for managing equine laminitis, disseminated intravascular coagulation, and equine verminous arteritis. A precise antiplatelet dose has not been established, but a dose of 12 mg/kg prolongs bleeding time for 48 hours.[43]

4.3.7 Carprofen

Carprofen is a propionic acid derivative formulated as a racemic mixture. Carprofen is currently available for use in horses in Europe, and its approval is being sought for use in North America. The volume of distribution (Vd) is 0.1 L/kg for the R enantiomer and 0.29 L/kg for the S enantiomer. At the recommended dose of 0.7 mg/kg, carprofen has a longer elimination half-life in horses than most other NSAIDs. After intravenous administration the plasma elimination half-life is 21 hours for the R enantiomer and 17 hours for the S enantiomer.[9] The R enantiomer predominates in plasma and exudates because of hepatic stereospecificity for glucuronidation of the

S enantiomer, leading to its more rapid clearance. Chiral inversion of carprofen does not occur in the horse.[66] Like other NSAIDs, carprofen accumulates in inflammatory exudate but produces only modest reductions in the concentrations of ecosanoids compared with flunixin or phenylbutazone.[9,67] Despite this, carprofen produces significant analgesia, likely because of central actions of the R enantiomer.[30]

4.3.8 Flunixin Meglumine

Flunixin meglumine is a potent inhibitor of COX that is approved for use in horses and is available in injectable and oral formulations. Flunixin is absorbed rapidly following oral administration, with a bioavailability of 86% and peak serum levels within 30 minutes.[68] Feeding delays absorption.[69] The Vd is 0.1 to 0.3 L/kg in horses, and the plasma elimination half-life is 1 to 2 hours.[68,70,71] In newborn foals the elimination half-life is prolonged at 13.4 hours. The Vd also increases in the newborn foal, as is expected with a low-Vd drug in the neonate.[72] Flunixin is highly protein bound (86%) but appears to partition into tissues readily, hence the high volume of distribution.[73,74] The elimination half-life in inflammatory exudate is 16 hours.[75] The onset of antiinflammatory action is within 2 hours, peak response occurs between 12 and 16 hours, and duration of action is 36 hours. Analgesic effects have a more rapid onset and shorter duration. Only 14% of a dose is excreted in urine, but otherwise little is known about the metabolism of flunixin.[1]

Flunixin is used in horses for a variety of inflammatory and painful conditions including colic, colitis, exertional rhabdomyolysis, endotoxic shock, respiratory disease, ocular disease, general surgery, and laminitis.[1] Flunixin is more effective at preventing the clinical signs of endotoxemia than phenylbutazone but appears equivalent to ketoprofen.[76] Flunixin may prevent abortion in endotoxic mares.[77] The label dose is 1.1 mg/kg every 12 hours, whereas low-dose therapy at 0.25 mg/kg every 8 hours inhibits the cardiovascular effects of endotoxemia. Extremely high doses of flunixin may mask signs of surgical colic pain and interfere with treatment decisions.

Flunixin has a good safety profile, but high doses or chronic dosing can cause anorexia, depression, and gastrointestinal ulcers.[53,78] In normal foals the label dose of flunixin administered for 5 days did not produce adverse effects, but 6 times the label dose resulted in gastrointestinal ulcers.[59] In another study, foals were administered flunixin at the label dose for 30 days and all treated foals developed gastric ulcers.[57] Intramuscular injections of flunixin greatly irritate muscle and have been incriminated in cases of clostridial myositis in horses and so should be avoided when possible despite the label directions.[79,80]

4.3.9 Ketoprofen

Ketoprofen is a chiral propionic acid derivative approved for horses as a racemic solution for intravenous or intramuscular injection. Oral and rectal bioavailability is too poor for these routes to be used clinically.[81–83] Ketoprofen is 92.8% protein bound in horses.[84] Ketoprofen has a moderate Vd for both enantiomers of approximately 0.5 L/kg and short plasma elimination half-lives of 1 to 1.5 hours.[11,27,75,83,84] Ketoprofen is metabolized hepatically by conjugation reactions, with only 25% of a dose eliminated as unchanged drug in urine.[84] The S enantiomer is associated with antiprostaglandin activity and toxicity, whereas the R enantiomer is associated with analgesia and does not produce gastrointestinal ulceration.[11,85] Because of chiral inversion the S enantiomer predominates in horses.[11] Ketoprofen accumulates in inflammatory exudates in the horse, where the elimination half-life of the S enantiomer is 23 hours and of the R enantiomer is 20 hours. The maximum 224

antiinflammatory effects of ketoprofen occur at 4 hours after a dose and last for 24 hours, illustrating that the antiinflammatory effects are not related to plasma concentrations.[75]

In studies of noninfectious arthritis, endotoxemia, and colic, ketoprofen is clinically similar to flunixin meglumine in efficacy.[75,76,85] In an experimentally induced synovitis model, phenylbutazone was more effective in reducing lameness and synovial fluid prostaglandin concentrations.[86] In horses with chronic laminitis, ketoprofen was more effective than phenylbutazone at relieving pain, but only at a higher-than-label dose.[87] In a comparative toxicity study, ketoprofen at the label dose had less potential for toxicity than flunixin meglumine or phenylbutazone.[53] In drug tolerance studies using 25 times the label dose for 5 days, horses developed depression, icterus, nephritis, hepatitis, and hemorrhagic necrosis of the adrenal glands.[88]

4.3.10 Meclofenamic Acid

Meclofenamic acid is an oral granule used in horses for treating musculoskeletal conditions. The drug is older and has not been researched extensively in veterinary medicine. The plasma elimination half-life in horses ranges from 1 to 1.4 hours after intravenous administration but increases to 2.6 hours with oral administration because of flip-flop kinetics.[89,90] Meclofenamic acid is highly protein bound (98.9%).[74] Therapeutic efficacy does not correlate well with plasma concentrations because the onset of clinical action is 36 to 96 hours after administration, and significant efficacy can be seen for days following a dose.[91] The dose is 2.2 mg/kg orally every 24 hours. Feeding before dosing may delay absorption of meclofenamic acid.[92] Repeated daily dosing does not result in drug accumulation; therefore drug is useful for chronic inflammatory conditions.[91]

Many horses can be maintained comfortably with twice weekly dosing without side effects. In clinical studies, researchers found clinical improvement in the lameness of two thirds of treated horses but found predicting which horses would respond to meclofenamic acid to be difficult.[91] At normal doses, some decrease in plasma protein concentration may occur. Doses of 6 to 8 times the label dose result in toxicity, including mouth ulcers, anorexia, depression, edema, and weight loss. Chronic administration at the label dose to stallions and pregnant mares caused no toxic effects.[93]

4.3.11 Phenylbutazone

Phenylbutazone is the most widely used NSAID in the horse and is available in many generic intravenous and oral formulations. Following oral administration, phenylbutazone is well absorbed; however, feeding may delay time to peak concentration.[92,94–96] The Vd is 0.15 L/kg, with highest concentrations in the liver, heart, kidney, lungs, and plasma.[96] The elimination half-life is 3.5 to 7 hours.[97] In neonatal foals the Vd is higher (0.27 L/kg) and the elimination half-life is longer (6.4 to 22.1 hours) than in adult horses.[98] Plasma protein binding in horses is greater than 99%.[97] The liver metabolizes phenylbutazone to oxyphenbutazone, an active metabolite that is eliminated more slowly from the body than phenylbutazone. Oxyphenbutazone inhibits the metabolism of phenylbutazone.[99] Phenylbutazone and its metabolite cross the placenta and are excreted in milk. Less than 2% is excreted in the urine as unchanged drug. The capacity of the liver to metabolize phenylbutazone becomes overwhelmed at low drug doses, resulting in dose-dependent kinetics.[97] The elimination half-life increases with increasing dose rates and increasing age.[93,97,100] The elimination half-life from exudate is 24 hours.[10] Therapeutic efficacy lasts for more than 24 hours because of irreversible binding of phenylbutazone to COX, slow elimination from inflamed tissues, and long elimination half-life of oxyphenbutazone.[97] Therefore high or

frequent doses of phenylbutazone result in disproportionately increasing plasma concentrations, which easily result in toxicity.

Phenylbutazone is used extensively in horses for a variety of musculoskeletal disorders.[25] Phenylbutazone may be useful for acute colic cases, antagonizing the disruptive effects of endotoxin on bowel motility without entirely blocking the cardiovascular effects that indicate a surgical lesion.[12] Phenylbutazone appears to inhibit prostaglandin synthesis at low plasma concentrations in the horse (5 to 15 μg/ml), whereas in human beings inhibition requires much higher drug concentrations (50 to 150 μg/ml).[97] This effect probably is due to a species difference in the structure of COX. An initial dose of 4.4 mg/kg every 12 hours the first day of therapy is followed by a decreased dose and increased dosing interval for subsequent therapy. Because of accumulation from the long elimination half-life of phenylbutazone and oxyphenbutazone, chronic therapy should be at least every other day.

Phenylbutazone has a narrow safety margin, especially in foals, ponies, and dehydrated horses.[48,58] Phenylbutazone toxicity most commonly results in gastrointestinal effects, including oral, esophageal, gastric, cecal, and right dorsal colonic ulcerations and accompanying protein-losing enteropathy, hypoproteinemia, leukopenia, and anemia.[48–52,101] Renal papillary necrosis (renal medullary crest necrosis) occurs because of inhibition of prostaglandins that maintain renal blood flow and direct toxicity of phenylbutazone and metabolites.[56] Because phenylbutazone can mask signs of lameness in horses for several days following therapy, 225
one may use it to disguise lameness for the purpose of soundness examinations or for competition.[97] 226
Phenylbutazone may interact with other highly protein bound drugs such as warfarin (coumarin).[97] Extravascular administration results in severe tissue necrosis. Phenylbutazone may decrease bone healing in fractures.[102] Phenylbutazone significantly suppresses total thyroxine and free thyroxine concentrations in horses for 10 days.[103]

4.3.12 Vedaprofen

Vedaprofen is related structurally to ketoprofen and carprofen and also is formulated as a racemic mixture of S and R enantiomers. Vedaprofen is available as a palatable gel for oral administration with a loading dose of 2 mg/kg followed by 1 mg/kg every 12 hours. Oral bioavailability is approximately 100%, and vedaprofen is highly protein bound (99%). Within 2 hours after intravenous administration, the mean R:S plasma concentration ratio is 95:5, which results from significant distribution and elimination differences between the enantiomers and not from chiral inversion. The R enantiomer has an elimination half-life of 2.2 hours and a Vd of 0.23 L/kg, whereas the S enantiomer has an elimination half-life of 0.76 hours and a Vd of 0.5 L/kg. Both enantiomers accumulate in inflammatory exudate and are cleared more slowly from exudate than from plasma. In an equine acute nonimmune inflammation model, vedaprofen produced significant inhibition of inflammatory swelling and partially inhibited leukocyte migration into the exudate.[104] Inhibition of leukocyte migration was not seen in this model with other NSAIDs.

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4.4 4.4—Respiratory Pharmacology

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Dogs, cats, horses, and human beings develop spontaneous bronchoconstriction associated with airway inflammation and characterized by chronic cough and wheeze. Effective therapy of inflammatory airway disease is species-dependent because of the inflammatory mediators involved in bronchoconstriction. In the horse, two clinical syndromes are described. Recurrent airway obstruction (RAO) or heaves is an inflammatory, obstructive airway disease clinically evident in middle-aged horses. Exposure of susceptible animals to antigens (typically hay dust, molds, and pollens) induces attacks of airway obstruction. Inflammatory airway disease (IAD) is a low-grade 228
inflammation of the small airways that is a common cause of poor performance in young to middle-aged athletic 229
horses. The goals of therapy for RAO and IAD are to prevent recurrent exacerbations of bronchoconstriction and dyspnea and to avoid emergency veterinary visits; to provide optimal chronic antiinflammatory therapy with minimal or no adverse effects; to maintain (near) normal pulmonary function; and to meet the clients' expectations of quality of life for their horses. Traditional therapy includes the systemic administration of bronchodilators and antiinflammatory drugs. Recently, methods of local delivery of aerosol medications have been developed for horses. Nebulizers, metered-dose inhalers (MDIs), and dry powder inhalers have been adapted for use in horses, with MDIs being the most versatile method of drug delivery. Inhalation therapy delivers high drug concentrations directly to the lungs, avoiding or minimizing systemic side effects. The onset of action for inhaled bronchodilators and antiinflammatory drugs is substantially shorter than that of oral or parenteral formulations. In the United States, MDIs are labeled according to the amount of drug delivered at the valve, whereas in Canada, they are labeled according to the amount of drug delivered to the mouthpiece of the actuator. Despite being ideal for therapy of RAO and IAD, the use of inhaled medications in performance horses is currently controversial, and little is known regarding their potential for causing drug rule violations.

4.4.1 Bronchodilators

The primary innervation of the equine respiratory tract is from the parasympathetic system, which reaches the lung via the vagus nerve. When the parasympathetic nerves release acetylcholine, it binds to M3-muscarinic receptors on airway smooth muscle, releasing calcium from intracellular stores to cause smooth muscle contraction and bronchospasm. α_2-Inhibitory and β_2-excitatory adrenoceptors modulate cholinergic nerves in the airways.[1] In acute bouts of RAO or IAD, histamine, leukotriene D_4, and serotonin contribute to increased

cholinergic airway tone.[2] Stimulation of the β_2-adrenergic receptors leads to increased activity of the enzyme adenylate cyclase, increased cyclic adenosine monophosphate, and relaxation of bronchial smooth muscle.[3]

4.4.1.1 β_2-ADRENERGIC AGONISTS

The β_2-adrenergic agonists have beneficial effects in the treatment of bronchoconstrictive respiratory tract diseases but always should be used with antiinflammatory drugs to treat inflammatory respiratory disease.[4] The β_2-agonists currently used as bronchodilators are racemic mixtures of R and S enantiomers, and their action is stereospecific.[5] In the horse the R enantiomers augment acetylcholine release yet still potently inhibit tracheal smooth muscle contraction. In contrast, the S enantiomers facilitate acetylcholine release but do not inhibit tracheal smooth muscle contraction and therefore do not contribute to the observed bronchodilatory effect. Stimulation of β-receptors on mast cells decreases the release of inflammatory mediators from mast cells but does not suppress other inflammatory cells.[6,7] The β_2-adrenergic receptor agonists also increase mucociliary clearance in the respiratory tract.[8–10] Chronic use of β_2-adrenergic agonists may lead to down regulation of β_2-adrenergic receptors and decreased clinical efficacy.[11]

Epinephrine (adrenaline) is a nonspecific adrenergic agonist; it stimulates α- and β-receptors, producing pronounced vasopressive and cardiac effects in addition to bronchodilation.[1,12,13] Epinephrine is reserved for emergency treatment of life-threatening bronchoconstriction (anaphylaxis). The nonspecific stimulation of other receptors and short duration of action make it unsuitable for long-term use. A 1 mg/ml solution is available, and the dose is 0.005 to 0.01 mg/kg intravenously or 0.02 mg/kg intramuscularly or subcutaneously.

Isoproterenol is a potent β-receptor agonist that causes bronchodilation in horses.[14] Isoproterenol is selective for β-receptors, but the cardiac (β_1) effects make it unsuitable for long-term use. The drug has been administered by inhalation or injection and also has a short duration of action (<1 hour). For emergency relief in horses, isoproterenol is given by slow intravenous injection at a dilution of 0.2 mg/50 ml of saline. Administration is discontinued when the heart rate doubles.[15]

Terbutaline is similar to isoproterenol in its β_2 activity.[16,17] Oral bioavailability in horses is less than 1%, and a short plasma elimination half-life indicates terbulatine should be given as an intravenous infusion to maintain therapeutic concentrations, but adverse cardiac and central nervous system effects may occur.[18]

Albuterol (salbutamol) is similar to isoproterenol.[14] Albuterol is administered to horses most commonly via MDI and is now available as a specific equine device for inhalation therapy (Torpex, 3M and Boehringer Ingelheim Vetmedica).[19] When dosed at 360 or 720 μg in horses with RAO, the onset of action is rapid (5 minutes), and bronchodilation lasts from 30 minutes to 3 hours.[20] The equine product is dosed at 360 μg (3 actuations) every 6 hours. Transient sweating, muscle tremors, or excitement may occur following dosing. *Pirbuterol* and *fenoterol* also have been administered to horses with RAO via metered dose inhalers with similar efficacy.[21–23]

Salmeterol is a long-acting β_2-agonist available in an MDI. Its onset of action is slow (15 to 30 minutes), but 229
its duration of action is long (6 hours).[24] Salmeterol is not recommended for use in an acute attack, but it 230

improves symptom control when used daily in addition to corticosteroids more than just increasing the corticosteroid dose.[25] Salmeterol is dosed at 125 µg (5 actuations) every 12 hours.[26]

Clenbuterol commonly is used orally to treat RAO or IAD in horses. Clenbuterol is available in injectable and oral formulations in Canada, whereas only the oral formulation is available in the United States. Clenbuterol has a lower binding affinity to the β_2-adrenoceptors compared with salbutamol and terbutaline, and efficacy studies have produced conflicting results.[9,17,27,28] Clenbuterol has a significant effect on increasing mucociliary transport in horses with RAO.[8,10] The initial oral dose is 0.8 µg/kg orally every 12 hours. If no response is seen after 3 days, one should increase the dose by 0.8 µg/kg every 3 days. If no response is seen at 3.2 µg/kg, the horse can be considered to have irreversible bronchospasm. Despite being fairly specific for β_2-receptors, the most common adverse effects are tachycardia and muscle tremors.[27] At therapeutic doses in horses, chronic clenbuterol administration is a repartitioning agent, increasing muscle mass while decreasing body fat,[29] and may have a negative effect on cardiac function.[30] Human intoxication has occurred after ingestion of products from food animals treated with clenbuterol, so the drug is banned for use in any animal destined for human consumption.[31]

4.4.1.2 METHYLXANTHINES

The methylxanthines include theophylline, theobromine, and caffeine. Theophylline and the theophylline salt aminophylline have been used as bronchodilators in horses.[32,33] Once the mainstay of human asthma therapy, theophylline has a high incidence of side effects, and its use has diminished with the development of other drugs. The methylxanthines inhibit phosphodiesterase, which increases intracellular cyclic adenosine monophosphate.[34] However, antagonism of adenosine currently is thought to be the most important action for bronchodilation. Adenosine induces bronchoconstriction in asthmatic patients and antagonizes adenylate cyclase. Adenylate cyclase is responsible for the synthesis of cyclic adenosine monophosphate, which is important for bronchial smooth muscle relaxation and inhibition of the release of inflammatory mediators from mast cells.[28,35] Methylxanthines also inhibit prostaglandins, augment the release of catecholamines from storage granules, increase calcium availability to contractile proteins of the heart and diaphragm, and interfere with mobilization of calcium in smooth muscle cells.[36]

Theophylline is available in several formulations, including injectable, aqueous solutions; elixirs; tablets; capsules; and sustained-release formulations. Theophylline is poorly soluble in water and often produces gastrointestinal irritation when administered orally. Aminophylline is a theophylline salt that is 78% to 86% theophylline, is more water soluble, and produces less gastrointestinal irritation. Other theophylline salts are available, and one must consider their theophylline content when developing a drug dosage regimen.

The pharmacokinetics of theophylline and aminophylline have been described in horses.[32,37–41] These drugs are absorbed almost completely after oral administration. Aminophylline is recommended to be dosed orally at 5 mg/kg every 12 hours.[37] An oral loading dose of 20 mg/kg of a sustained-release formulation followed by maintenance doses of 15 mg/kg every 24 hours achieves trough-peak theophylline plasma concentrations between 6 and 17 µg/ml.[39] Ideal therapy requires therapeutic drug monitoring; otherwise, adverse effects such as agitation and tachycardia can occur. These effects and the need for clients to administer numerous tablets or capsules twice daily make chronic therapy with theophylline impractical.

4.4.1.3 ANTICHOLINERGIC DRUGS

The anticholinergic (parasympatholytic) drugs are effective bronchodilators by inhibiting vagally mediated cholinergic smooth muscle tone in the respiratory tract. Asthmatic individuals appear to have excessive stimulation of cholinergic receptors.[42]

Atropine is used for acute bronchodilation in horses, in which a low intravenous dose (0.01 mg/kg) is more effective and less toxic than intravenous aminophylline.[43] A test dose of 5 to 7 mg has been advocated to determine prognosis in horses affected by RAO, with the implication that horses that fail to improve are unlikely to be managed successfully with bronchodilators.[3] Recently, the response to atropine of horses affected by RAO was shown to underestimate improvement in respiratory tract function that results from maintenance on pasture.[44] Atropine is not used for chronic bronchodilation because low doses and even ocular application may cause tachycardia, ileus, neurologic derangement, and blurred vision in horses.[45–48]

Glycopyrrolate is twice as potent as atropine in human beings and does not cross the blood-brain barrier. Information about the use of this drug in horses is sparse, but doses of 2 to 3 mg have been suggested for intramuscular administration.[49]

Ipratropium bromide is a quaternary derivative of atropine that lacks the adverse side effects of atropine and is available in an MDI alone and in combination with albuterol (salbutamol). In asthmatic human beings, ipratropium bromide is used as an additional medication to reverse bronchoconstriction when inhaled short acting β_2-agonists do not give enough relief.[50] The anticholinergic action of ipratropium bromide also 230
decreases mucous secretions. Ipratropium has shown efficacy in horses with RAO.[51–54] The suggested dose is 231
270 μg (15 actuations) every 6 hours.[26]

4.4.2 Glucocorticoids

Glucocorticoids are the mainstay of therapy for inflammatory airway diseases in human beings and animals. They attenuate the inflammatory response, suppressing the generation of cytokines and recruitment of airway eosinophils and release of inflammatory mediators. Glucocorticoids reduce severity of symptoms, improve peak expiratory flow, diminish airway hyperresponsiveness, prevent exacerbations, and possibly prevent airway wall remodelling.[55]

For acute exacerbations of RAO or IAD, parenterally administered glucocorticoids traditionally have been recommended.[3] The most commonly administered drugs are dexamethasone (20 mg intravenously or intramuscularly), isoflupredone acetate (5 to 20 mg intramuscularly), flumethasone (0.04 to 0.15 mg/kg intravenously or intramuscularly), and triamcinolone acetonide (0.022 to 0.044 mg/kg intramuscularly or subcutaneously).[3,49,56]

Based on efficacy in treating human asthma, availability, and low cost, oral prednisone was used commonly for chronic treatment of RAO and IAD.[3,15] Prednisone is a prodrug and in most species is metabolized hepatically to the active drug prednisolone. The efficacy of oral prednisone was questioned,[57–59] and a recent study demonstrated that oral absorption of prednisone is poor and hepatic metabolism to the active drug prednisolone does not appear to occur in horses. Oral prednisolone is effective for treating RAO and IAD,[60] but convenient-

sized tablets are currently unavailable. Oral dexamethasone is available as an equine formulation and is efficacious for chronic therapy in horses.[49]

Systemic administration of glucocorticoids to horses is associated with potentially severe adverse effects. Glucocorticoid administration always has the potential to suppress endogenous production of cortisol. The horse appears to be sensitive to suppression of endogenous cortisol production.[61] The likelihood and degree of suppression increases with duration of action of the corticosteroid.[62] The immunosuppressive effects of corticosteroids are related to dose, steroid potency, and dose interval and can predispose treated horse to infectious disease. Laminitis is associated with dexamethasone and triamcinolone administration in horses.[62,63]

Inhaled corticosteroids are the most potent inhaled antiinflammatory drugs currently available. In human beings, early intervention with inhaled corticosteroids improves asthma control and normalizes lung function and may prevent irreversible airway damage. The potential but small risk of adverse side effects is well balanced by their efficacy. Oral candidiasis (thrush), dysphonia, and reflex cough and bronchospasm are the most common adverse effects in human beings; the use of a spacer reduces all of these effects.[55] Glucocorticoid formulations in MDIs include fluticasone, beclomethasone, budesonide, flunisolide, and triamcinolone. Fluticasone and beclomethasone have shown efficacy in horses with RAO.[64–67] Fluticasone is considered the most potent and longest-acting inhaled glucocorticoid available.[3] Fluticasone doses of 1980 μg (9 actuations) every 12 hours have been recommended.[26] At the currently recommended dose (500 μg every 12 hours), inhaled beclomethasone suppresses adrenocortical function in horses, but use is not associated with clinically apparent adverse effects.[65] Most clinicians recommend administering a bronchodilator before the glucocorticoid formulation in the initial treatment of RAO or IAD.[26] Once the disease is in remission, chronic therapy can be continued with glucocorticoids in conjunction with environmental management.

4.4.3 Chloride Channel Blockers

Cromolyn sodium and *nedocromil sodium* are chloride channel blockers that modulate mast cell mediator release and eosinophil recruitment. Cromolyn was previously available as an equine formulation for nebulization and was efficacious as prophylactic therapy in horses with RAO.[68–71] Cromolyn and nedocromil have strong human safety profiles and similar efficacy.[72] Currently, they are available in MDIs. In human beings the clinical response to either drug is less predictable than the response to corticosteroids.[73] Because cromolyn and nedocromil must be administered prophylactically, they have limited use for treating chronic RAO and IAD.

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5 CHAPTER 5 APPLIED NUTRITION

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Basic knowledge of the proper method for feeding horses is important to veterinarians so that they may assist their clients in detemining feed choices that are wholesome, economical, and tailored to the workload or intended use of the horses. In addition, many veterinarians operate a boarding or breeding facility and need to provide balanced rations to the horses in their care. To help the client best, the veterinarians should know the number, type, and use of the horses. Client preference regarding feeds is also important because the choice may be determined or governed at least partially by the amount of staff available and the ability of the staff to comply with detailed instructions.

The basic considerations for the amount and type of feed are determined by the age, size, and use or workload of the horses.[1,2] Broodmares have needs different from growing horses, which are different from horses in heavy work. The nutritional management of orphaned foals and sick neonates or adult horses with serious infectious diseases or neoplasia requires special dietary consideration.

5.1 Feeds

5.1.1 FORAGES

The most common forms of forage fed to horses are pasture and hay. Silage, haylage, and "green chop" also have been used to a much lesser extent. The choice of forage type depends on several factors: land availability, fertility of the soil, storage capability, and climate. Regardless of the form, the quality of the forage fed is dictated by the species, soil fertility, temperature, degree of rainfall, method of harvesting, and most important, the stage of maturity at harvest. The best-quality forages are cut before seed heads (boot stage in grasses) and blooms (bud stage for legumes) appear. This results in a higher proportion of leaves, a decrease in the poorly digestible cell wall content, and consequently a more nutritious product. Hay cubes are fed less often but can be useful when limited storage space is available.

5.1.1.1 Grasses

The grass species generally are divided into two groups: the cool-season and the warm-season grasses. The cool-season grasses include Kentucky bluegrass, smooth bromegrass, orchard grass, tall fescue, timothy, and oats. These grasses are grown predominantly in the northern half of the United States. These species are of greatest value in the spring and fall, providing a forage stand that is lush and high in nutritive value and palatability.

5.1.1.1.1 Kentucky Bluegrass

Long favored by horsepersons everywhere as the ideal pasture grass, bluegrass is highly palatable and winter hardy and is a ready volunteer, producing high yields in the cooler months. Bluegrass seems to handle close grazing well; however, it does not do well in the summer months or under heavy traffic. Kentucky bluegrass is used exclusively in pastures.

5.1.1.1.2 Smooth Bromegrass

Like bluegrass, bromegrass forms a dense sod and is winter hardy. Unlike bluegrass, bromegrass is heat and drought tolerant. Its good palatability, even when mature, makes bromegrass a good choice for pasture or hay when seeded with alfalfa. Bromegrass does not tolerate overgrazing or frequent harvesting and is slow to regrow.

5.1.1.1.3 Orchard Grass

Another common grass species in the temperate zones, orchard grass produces early growth; is more heat, 235
shade, and drought tolerant than most grasses; and recovers rapidly after harvest or grazing with good- 236
quality growth. Its early maturity makes orchard grass compatible in seedings with alfalfa. Orchard grass is less winter hardy than other species.

5.1.1.1.4 Tall Fescue

Of the grasses, tall fescue is the most traffic and drought tolerant. Fescue adapts to a wide range of soil and climate conditions and is persistent. Palatability is generally not high but improves in the spring and following the first frost. The biggest drawbacks to using tall fescue in pastures are the high concentration of alkaloids and the more recent problems associated with endophyte infestations or fescue toxicosis that have serious consequences in broodmare bands.[3] The problem often affects broodmares in late pregnancy and young growing horses. The endophytic fungus *Acremonium coenophialum* affects growing pasture and sometimes hay made from these pastures. (For additional information, see Chapter 20.)

5.1.1.1.5 Timothy

The most well known of the grasses and the most popular of the grass hays, timothy is winter hardy, easy to establish, and widely adapted. Timothy is generally a clean, dust-free hay and is consumed readily by horses. Timothy is not a good pasture grass because of its low tolerance for heavy grazing or cutting and the production of an open, clumpy sod. Like most grasses, timothy is not as heat or drought tolerant as legumes.

5.1.1.1.6 Oat Hay

A popular forage in the western United States and Canada, oat hay is best if harvested when the grain is at the late milk or dough stage. When cut at the proper time, the protein content can be as high as 13%. A valid concern is the potential for nitrate poisoning if oat hay is harvested at a young stage, and testing for nitrate concentration is recommended if oat hay is going to be used as the predominant forage source. Concentrations of nitrates between 0.4% and 0.6% are considered safe for horses.[1] For further discussion, see Chapter 20.

The warm-season grasses Bermuda grass, Bahia grass, and pangola grass do well in sandy soils, are more drought tolerant, but are generally lower in digestibility than the cool-season grasses and often produce lower yields. These forages are found primarily in the southern coastal and southeastern regions of the United States. Most are used as pasture grasses. The exception is Bermuda grass, which also can serve as an easily harvested, high-yielding species for hay production.

5.1.1.2

Legumes

Alfalfa and clover are the predominant legume species. In some areas of the country, stands of bird's-foot trefoil and lespedeza (also called *Sericea*) still are grown. The deep-rooted characteristic of legumes is what contributes to their drought tolerance. Their high protein and calcium content is useful in improving the nutritional value of grass pastures. A disadvantage of legumes is their intolerance to heavy traffic, overgrazing, and poor drainage.

5.1.1.2.1

Alfalfa

Alfalfa has become the predominant legume grown in the United States. Alfalfa is leafy and fine stemmed, produces high yields, is resistant to many pests and diseases, and is high in digestibility and palatability. Despite these advantages, many wives' tales and myths exist that make its use controversial. No data support the long-held contention that the protein content makes it too “rich” for horses and, likewise, that it causes kidney damage. The high protein and mineral content of alfalfa stimulates an increase in water intake and a concomitant increase in urine volume and nitrogen and calcium excretion. Alfalfa also has been called a “hot” feed, but research has shown that no difference exists in sweat production or body temperature in horses fed alfalfa or timothy hay.[1]

The major concerns with alfalfa are the wide calcium-to-phosphorus ratios that can occur, the potential for overfeeding, and the problems with blister beetle infestation. Most alfalfa hays have calcium-to-phosphorus ratios between 3:1 and 5:1, but ratios as high as 15:1 have been observed.[1] Mature horses can tolerate ratios of the total diet as high as 6:1, but some mature horses have developed a propensity for dirt-eating or rock-chewing suggestive of pica at calcium-to-phosphorus ratios of 3:1. Ratios higher than 3:1 are not advised for young, growing horses.[2] When the use of alfalfa results in undesirable ratios, adding phosphorus to the diet or reducing the amount of alfalfa by replacing it with a grass hay should narrow the ratio.

The digestible energy content of alfalfa is generally higher than other forages, making it much easier to overfeed. Fourteen pounds of alfalfa hay is equal in energy content to 18 lb of timothy hay. Horses fed high-quality alfalfa (>55% total digestible nutrients) at 2% of their body weight can become fat quickly, consuming in some cases 50% more calories than required for maintenance. One can feed less alfalfa and still meet the needs of the animal. Supplying some grass hay should help to appease boredom if additional forage seems indicated.

Cantharidin toxicosis from the ingestion of blister beetles was first reported in horses in 1978.[4] (See Chapter 20 for a thorough discussion of cantharidin poisoning.)

5.1.1.2.2

Red and White Clover

Red clover still is preferred by some horsepersons but has declined in popularity. When harvested properly, red clover can be as high in nutritive value as alfalfa. Because cutting clover at an earlier stage of maturity reduces yield, most is cut well beyond the bloom stage. This results in a coarse, thick-stemmed forage that becomes moldy, dark, and dusty when baled.

Red clover also can be infected by *Rhizoctonia leguminicola,* a fungus that produces an alkaloid called slaframine, resulting in the condition known as “slobbers.” Excessive salivation is the most consistent 236

clinical sign, though abortion in a mare also has been reported.[5] Once the affected forage is removed, salivation ceases. White and ladino clovers, known for their high proportion of leaves, are used primarily as pasture forages. Neither clover is particularly winter hardy or shade tolerant.

5.1.1.2.3 Bird's-Foot Trefoil

Bird's-foot trefoil still is grown in parts of the temperate zone but has lost favor with many producers because it is difficult to establish, is weak-stemmed, and is slow to recover from grazing. The forage was named for the crow's foot–like seed pod that develops in the fall. Like the other legumes, bird's-foot trefoil can be high in feeding value when harvested properly.

5.1.1.2.4 Alsike and Sweet Clover

Alsike and sweet clover are not commonly fed to horses and for good reason. Mild to severe photosensitization reactions and hepatitis have been reported in horses consuming Alsike clover.[6] The stemmy consistency of sweet clover makes it difficult to cure for hay, increasing its susceptibility to mold. Common molds such as *Penicillium* species can convert coumarin, a nontoxic substance in sweet clover, to dicumarol. Dicumarol interferes with the activation of vitamin K, making it a potent anticoagulant. One should avoid using these two forages if at all possible.

5.1.2 PASTURE

When well cared for, pasture is the most inexpensive and nutritious form of forage. A combination of grasses and legumes typically are used, taking advantage of the drought-resistant, high-calcium content of the legumes and the traffic resistance and early growth characteristics of grasses. The perfect or ideal pasture is obtained by cultivating those species that have adapted to that area and are compatible with the soil and drainage conditions. The best sources for this information are extension agencies or agronomy departments at universities.

Well-managed pastures can provide ample forage stocked at a rate of one horse per acre. Where space is limited or management is difficult, nearly 8 to 10 acres per horse may be needed. To maintain good, safe pastures, agronomists suggest (1) routine soil testing; (2) clipping to reduce weeds and stimulate new growth; (3) removal of toxic weeds; (4) rotation, if possible; (5) maintaining optimal stocking rates; (6) the proper mixture of grass and legume species; and (7) the spreading or removal of manure to prevent burning and increase the usable acreage.

5.1.3 HAY

Any hay can be the best hay for horses as long as it is cut at the proper stage of maturity. As forage matures, its nutrient content, especially the protein content and protein digestibility, decreases. The more youthful the plant, the higher the leaf content and the higher the nutritive value, for 80% of the nutrients are found in the leaves. Quality hay is free of mold, dust, and weeds; has few if any seed heads; is soft to the touch; and has a pleasant aroma. Probably the best determinant of quality is intake; horses devour high-quality hays with relish.

Hay can be chopped, pelleted, cubed, or fed long stem from the square or round bale. Alfalfa cubes are usually of good quality because of the amount of leaf that is needed for cubing. Waste and storage space are less with cubes. One disadvantage is the boredom that may occur because it takes the horse less time to eat the cubes than the equivalent weight in long-stem hay. Choking is a frequently cited concern but would seem to be reserved for

the greedy eater. Before purchasing cubes, one should ask for the name and address of the manufacturer and should inspect a sample for the presence of foreign materials such as twine, newspaper, or insects. Alfalfa pellets are also available but have their greatest use in mashes. The pellets tend to be more dusty and crumble more easily, increasing wastage. Because of their small size, horses consume pellets much more quickly, which may result in an increase in delinquent activities such as wood chewing, cribbing, or consumption of bedding to appease the desire to chew if alfalfa pellets are the sole source of forage. Chopped hay has been shown to decrease the rate of consumption when mixed with grain[1] and has been used successfully in group-feeding situations to prevent overeating.

5.1.4 GRASS CLIPPINGS

One can feed fresh lawn or pasture trimmings to horses, with several provisos. Anyone who has prepared compost is familiar with the heat that is produced in the center of the pile as a result of the favorable microbial environment; that is, dark, moist, high in carbohydrates, and ultimately anaerobic.

Pockets of mold can develop within the mass that may have unfavorable consequences for the horse. One can prevent mold growth by spreading out what the horse will consume in 30 to 60 minutes and discarding any remaining. Moreover, grass from well-tended yards may contain high concentrations of nitrates from frequent fertilizer applications and should be fed judiciously.

5.1.5 GRAINS

Grains provide a more concentrated source of energy. Compared with some forages, such as grass hays, grains can provide 50% to 100% more digestible energy per pound and in a smaller volume. The feed grains vary in size, weight per unit volume, and nutrient content. For this reason, one should feed grains by weight and not volume (e.g., coffee can or scoop). Processes such as rolling, crimping, cracking, steam flaking, and popping can
improve digestibility, particularly for foals and horses that have difficulty chewing or have poor teeth. One 237
usually can obtain the nutrient value of grains or grain mixtures from the feed tag or feed tables or by requesting 238
a nutrient analysis from the manufacturer or feed dealer. If a guaranteed analysis is not available, the veterinarian should encourage the client to have the feed analyzed.

5.1.5.1 Oats

Oats have long been the favorite and preferred grain of horsepersons. Oats contain approximately 10% fiber, the highest of the feed grains. Oats are therefore bulkier than the other grains, helping to reduce the risk of overconsumption. Protein content varies from as low as 8% for "light" oats to as high as 12% for the heavy Swedish oats. When one purchases oats, they should be large, plump, and clean. The heavier the oat weight per bushel, the higher the energy content. Top-quality oats today can weigh as much as 42 lb per bushel compared with the standard bushel weight of 32 lb. "Clipped" oats have had the ends of the hulls removed. Crimping or rolling is recommended only for foals and horses that cannot chew their food well.

The claim that oats make a horse high or excitable ("feeling their oats") is old and is unfounded. No substances in oats are known to produce this effect other than extra calories making the horse feel more energetic.

5.1.5.2

Corn

The use of corn is increasing as the cost of oats becomes more prohibitive. Corn is energy dense, containing approximately 1.5 Mcal/lb. The reputation of corn as a hot feed is unsubstantiated, other than through wives' tales, producing no more heat from digestion than any other grain and far less than hay. Corn is the heaviest grain per unit volume and therefore has the highest risk of being overfed if not managed properly. Corn is low in protein (8% to 9%) and low in fiber (2% to 3%) compared with the other feed grains but is the only grain used commonly in horse feeds that contains vitamin A. Corn also is graded by weight, the top grade weighing a minimum of 56 lb per bushel. Corn most often is fed whole or cracked, but steam-rolled, steam-flaked, and steam-popped corns are gaining in popularity for use in textured ("sweet") feeds. Ground corn is not recommended because it is dusty. One also may feed corn from the cob, but one should watch horses carefully because cobs have become lodged in the mouths and throats of horses.

Of the grains, corn seems to have a greater potential for supporting mold growth. More recent concerns have involved outbreaks of aflatoxicosis and leukoencephalomalacia in horses caused by toxins produced by *Aspergillus flavus* and *Fusarium moniliforme,* respectively.[7] In response, feed companies voluntarily began screening corn shipments for the presence of aflatoxins, reducing the threat of commercial feed contamination. To stop the use of corn in feeds based on these isolated incidents is not reasonable. As with all grains, one should never use feed with an off odor or that appears moldy.

5.1.5.3

Barley

Barley is used more on the West Coast and in the far north. This grain is an excellent choice for horses. The protein and digestible energy content of barley falls between corn and oats. Because of the small size and tenacious seed coat, barley should be crimped or rolled to improve digestibility.

5.1.5.4

Sorghum or Milo

This southern-grown feed grain is not used widely as an energy source for horses. Like corn, sorghum is high in energy and low in fiber, and therefore the same cautions regarding overfeeding apply. Sorghum also should be rolled, crimped, or steam flaked for improved digestibility.

5.1.5.5

Rye

Rye has been fed to horses but is not used commonly today. Rye is similar in nutrient content to barley. The low palatability, concern of ergot poisoning, and the bounty of other more suitable grains have reduced its use in feeds.

5.1.5.6

Wheat

Wheat is occasionally available for use as feed, though it is expensive. Wheat is similar in nutrient content to barley. Because of its small size, processing by steam crimping or rolling is highly recommended. Wheat has a reputation of forming doughy masses in the stomach and is generally limited to one third of the grain mix.

5.1.6 PROTEIN SUPPLEMENTS

The level of protein required in the diet of horses is influenced by physiologic status (e.g., growth, maintenance, and gestation), protein source, and feed intake. The plant protein sources are used most often in horse feeds and include soybean, cottonseed, linseed, peanut, and sunflower meals. Soybean meal is by far the leading protein supplement, contains 44% protein, and is the highest in lysine (2.8% to 3.0%), an essential amino acid. Soybeans must be toasted to inactivate trypsin inhibitors but not overheated so as to reduce amino acid availability. Interestingly, soybean meal has been found to contain goitrogens, anticoagulant factors, and phytoestrogens. These do not appear to result in problems in horses.

Other seed meals are used to a much lesser degree and tend to be regional. Cottonseed and peanut meal is used mostly in the South. Though cottonseed can be a good protein source when supplemented with lysine,[8] the 238
presence of gossypol, an antioxidant and cardiotoxin,[9] reduces its acceptability by horsepersons. Peanut meal is 239
also limited in lysine, somewhat expensive, and risky to feed because it can be a source of aflatoxins.[10] Sunflower meal is low in lysine but higher in methionine.

Linseed meal is known more commonly for its ability to impart a sheen to the hair coat than as a protein supplement. Immature seed can contain the cyanogenic glycoside linamarin and its enzyme linase, both of which are destroyed by heat-processing.[11] Linseed meal is also low in lysine. Low production and expense have reduced its use in animal feeds.

Urea also has been used as a nonprotein nitrogen source for adult horses but has no advantage over more common sources.[12,13] Horses are not as efficient in using urea as are cattle and are much more tolerant to excessive levels. Urea toxicity is less likely in horses because urea is absorbed by the gastrointestinal tract and excreted in the urine before reaching the hindgut, where the microbial population hydrolyzes urea to ammonia and carbon dioxide via urease. Urea toxicosis has been produced in ponies experimentally by oral dosing of 5 g urea per kilogram of body mass.[14] The clinical signs of urea toxicity are confined to the central nervous system, with muscle tremor, incoordination, and weakness. Death is the result of ammonia intoxication.

5.1.7 BY-PRODUCT FEEDS

A variety of feedstuffs are "waste" products of the feed and food industry. Some can add extra fiber, and others serve as energy sources. These by-products include cottonseed hulls, peanut hulls, soybean hulls, wheat bran, wheat middlings, dried brewer's and distiller's grains, citrus pulp, and dried whey, to name but a few. Other by-products may be available by region. One by-product that is becoming increasingly popular is beet pulp. A by-product of the sugar industry, beet pulp is added to textured feeds as a fiber source. The primary markets for these feeds have been owners of racing horses and horses with hay allergies or respiratory diseases, most notably heaves. Beet pulp is high in digestible energy and provides some dietary calcium. Though feeds containing beet pulp are bulky, horses do develop vices and bad habits from grazing boredom if some long-stem hay is also not made available.

5.1.8 VITAMIN AND MINERAL SUPPLEMENTS

The National Research Council (NRC) recommendations for vitamins in horses are restricted to vitamins A, D, and E; thiamine; and riboflavin. Table 5-1 lists the sources commonly used to supplement vitamins in most feeds. As ingredients, vitamins vary in price, regional availability, solubility, potency, and stability.

For minerals the sulfate forms of supplements are generally higher in availability than the carbonate or oxide form. Newest additions to the trace mineral supplements are the chelated minerals. These are trace elements bound to amino acids, peptides, or polysaccharides, with the goal of decreasing the opportunity for mineral antagonisms and increasing absorption. Whether they are in fact superior to inorganic forms in availability or efficacy remains to be determined in the horse. Chelated minerals are more expensive than the inorganic forms but may prove advantageous in improving the palatability of feeds containing high concentrations of minerals. Table 5-2 provides a list of mineral sources.

TABLE 5-1 Commonly Used Vitamin Sources in Horse Feeds

VITAMIN	SOURCE
Biotin	*d*-Biotin
Choline	Choline chloride
Cyanocobalamin (B_{12})	Cyanocobalamin
Folacin (folic acid)	Folacin
Niacin	Nicotinic acid
	Nicotinamide
Pantothenic acid	Calcium pantothenate
Pyridoxine (B_6)	Pyridoxine hydrochloride
Riboflavin (B_2)	Riboflavin
Thiamine (B_1)	Thiamine hydrochloride
	Thiamine mononitrate
Vitamin A	Vitamin A acetate
	Vitamin A palmitate
	Vitamin A propionate
	β-Carotene (precursor)
Vitamin C	Ascorbic acid
Vitamin D	Activated animal sterol (D_3)
	Cholecalciferol
	Ergocalciferol (D_2)
Vitamin E	α-Tocopherol acetate
	α-Tocopheryl acetate
Vitamin K	Menadione sodium bisulfite

5.2 Nutrition of the Mature Horse

One can meet the nutrient requirements of the mature horse under most circumstances by feeding a good-quality hay, water, and a salt block supplemented with trace minerals. The rule of thumb of providing 2% of body weight per day in dry feed is still a good rule to follow. Contrary to the practices of most owners, grain is needed only when hay alone cannot meet energy needs. Supplementation with grain may be necessary when the exercise plane increases or forage quality decreases. Overnutrition from feeding excess grain and empirical supplementation of vitamins and minerals are of more concern today than are dietary deficiencies. Table 5-3 summarizes the nutrient requirements of the mature horse.

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TABLE 5-2 Commonly Used Mineral Sources in Horse Feeds

MINERAL	SOURCE	MINERAL CONTENT
Calcium, phosphorus	Limestone	38% Ca
	Dicalcium phosphate (dynafos)	20% Ca, 18% P
	Mono-and dicalcium phosphate (biofos)	18% Ca, 21% P
	Tricalcium phosphate	38% Ca, 18% P
	Monosodium phosphate (monofos)	22% P, 17% Na
	Sodium tripolyphosphate	25% P, 31% Na
Magnesium	Magnesium sulfate	20% Mg
	Magnesium oxide	28% Mg
Potassium	Potassium chloride	52% K
	Potassium sulfate	45% K
Sodium	Sodium chloride	39% Na, 61% Cl
Copper	Copper sulfate	25% Cu
	Copper oxide	80% Cu
	Copper carbonate	57% Cu
	Copper proteinate	10%-13% Cu
Manganese	Manganese sulfate	23% Mn
	Manganous oxide	77% Mn
	Manganous sulfate	32% Mn
	Manganese proteinate	15% Mn
Cobalt	Cobalt carbonate	49% Co
	Cobalt sulfate	38% Co
	Cobalt proteinate	9%-11% Co
Iron	Ferric carbonate	48% Fe
	Ferric chloride	21% Fe
	Ferric ammonium citrate	17% Fe
	Ferrous fumarate	33% Fe
	Ferric oxide	70% Fe
	Ferrous sulfate	20%-30% Fe
	Iron dextran	2%-5% Fe

Zinc	Zinc sulfate	22%-36% Zn
	Zinc oxide	80% Zn
	Zinc proteinate	15%-22% Zn
	Zinc methionine	Not available
Iodine	Ethylenediaminedi-hydroiodide	77% I
	Potassium iodide	76% I
	Calcium iodide	65% I
Selenium	Sodium selenite	45% Se

5.2.1 ENERGY

The energy needs of the mature horse can vary. The digestible energy (DE) requirement for field maintenance allows for what is necessary to maintain body mass (BM) and daily, nonworking activity (searching for food, socialization). For horses under 600 kg, one can calculate the DE from the following equation[15]:

$$0.021(BM_{kg}) + 0.975 = \text{Mcal DE per day}$$

For horses greater than 600 kg, one can use the following equation:

$$0.0383(BM_{kg}) - 0.000015(BM_{kg})^2 + 1.82 = \text{Mcal DE per day}$$

The latter equation allows for the decrease in voluntary activity observed in the larger breeds.[2] Both of these estimates apply to horses maintained out of doors in pastures, paddocks, or dry lots. For stall-bound horses, the amount of energy expended for activity and thermoregulation has been reduced. The resting energy expenditure of horses in stalls calculated from the following equation is probably closer to the energy needs of most pleasure and halter horses.[15]

$$0.021(BM_{kg}) + 0.975 = \text{Mcal DE per day}$$

For owners periodically to obtain the body weight and to assess the condition of their horses to prevent undesirable weight gain is important. Cloth weight tapes provide a quick and reasonably accurate estimate. One also can calculate the body weight using the equations based on chest girth[16] (Box 5-1). For accuracy, one should take this measurement at the base of the withers and pull the tape snugly without embedding the tape in the skin. One also should evaluate body condition scores. Table 5-4 provides descriptions of body conditions rated on a scale of 1 to 9.[17] Horses, regardless of age, are kept best at a score of 4 to 5, so that one can barely see but easily feel the ribs.

The requirements for work expenditure have been calculated using the weight of the horse, rider, and tack and the workload.[18,19] For practical purposes, increases over maintenance of 25% for pleasure and equitation; 50% for roping, cutting, and jumping; and 100% for distance training and polo should be a reasonable guide. One should base adjustments in feed intake on body condition.

5.2.2 PROTEIN

The protein requirement of the mature horse is roughly 1.3 g of crude protein per kilogram of body mass or 40 g/Mcal of DE.[2] However, because protein is not used as an important fuel source in horses, a level of 12% of dry matter is adequate for most horses. Quality of protein is less important for mature horses than for the growing horse but should not be overlooked. Despite a widespread belief, excess protein does not cause kidney damage in healthy horses. Feeding more protein than is necessary is expensive for the owner and only harmful to the pocketbook.

5.2.3 MINERALS

For the mature horse the major mineral needs are met with the consumption of good-quality feed to meet energy and protein needs. Trace mineral salt blocks are recommended strongly to provide the sodium, zinc, and iodine
not found in most feeds. In areas where selenium is deficient, a salt block with added selenium is required. 240
Unlike salt used for human consumption, most white salt blocks do not contain iodine and should be replaced 241
with trace mineral blocks containing iodine. The label found on the block or brick provides the mineral content.

TABLE 5-3 Daily Nutrient Requirements of the Mature Horse*

NUTRIENT	STALL REST	MAINTENANCE	PROVIDED BY 13 lbALFALFA† 2 ozTM SALT‡	PROVIDED BY 16 lb GRASS† 1 lb PVM§
Digestible energy, Mcal	11	15	14	14
Crude protein, g	416	596	944	654
Calcium, g	18	18	65	30
Phosphorus, g	13	13	15	24
Potassium, g	23	23	145	121
Sodium, g	6	6	28	13
Magnesium, g	7	7	15	18
Copper, mg	60	60	65	126
Iron, mg	300	300	1870	1254
Manganese, mg	300	300	400	380
Zinc, mg	300	300	347	340
Cobalt, mg	1.5	1.5	5	3
Iodine, mg	1.5	1.5	2	2
Selenium, mg	1.5	1.5	7	2
Vitamin A, IU	20,500	20,500	130,000	137,070
Vitamin D, IU	1770	1700	10,620	12,706
Vitamin E, IU	295	295	60	210
Vitamin K, IU	NA	NA	NA	NA
Thiamine, mg	18	18	22	17
Riboflavin, mg	12	12	74	67
Niacin, mg	NA	NA	224	209
Folic acid, mg	NA	NA	22	15
Pyridoxine, mg	NA	NA	24	4
Pantothenic acid, mg	NA	NA	155	59
Biotin, mg	NA	NA	2	1
Cyanocobalamin, μg	NA	NA	NA	25

* Calculated for a 450-kg horse.

† Sun-cured in midbloom.

‡ Trace mineral (TM) salt containing 120 ppm selenium.

§ Average analysis for protein-vitamin mineral (PVM) pellet such as MannaPro Spur, Tizwhiz 30 Plus, Wayne Propel, Buckeye Gro'N Win.

5.2.4 VITAMINS

A horse consuming good-quality hay or pasture receives all the necessary vitamins directly from the feed, body stores (fat-soluble vitamins), or by microbial synthesis in the hindgut. Naturally occurring B vitamin deficiencies have been reported only in cases in which dietary or chemical antagonists were present.[20,21] When one feeds poor-quality hay, the addition of a commercial feed to supplement energy intake provides the needed vitamins.

5.2.5 GENERAL GUIDELINES

Providing 1.5% to 2.0% of the body weight in feed per day meets the needs of most horses. For grass hays, a
1000-lb horse kept on dry lot or minimal pasture needs approximately 18 lb/day. If one feeds alfalfa hay, only 14 241
lb provides the same amount of energy for field maintenance because of the higher energy content. If one feeds 242
grain, the ratio by weight of hay to grain should not exceed 50:50. Plenty of clean, fresh, cool water should be available at all times. Water intake appears to be related directly to dry matter intake.[22] Most horses consume a minimum of 1 L/Mcal of DE or 2 to 3 L/kg dry feed.

5.2.5.1 BOX 5-1 EQUATIONS FOR ALCULATING BODY WEIGHT FROM CHEST GIRTH (CG) MEASUREMENTS*

Chest girth >66 inches:	
Males	0.14475 (CG_{in}) = Body weight, lb
Females	0.14341 (CG_{in}) = Body weight, lb
Chest girth <66 inches (including foals):	
Colts	0.1387 (CG_{in}) + 0.400 = Body weight, lb
Fillies	0.1382 (CG_{in}) ++ 0.344 = Body weight, lb

Modified from Willoughby DP: *Growth and nutrition in the horse,* Cranbury, NJ, 1975, AS Barnes.

* Use the adult formulae for mature ponies.

TABLE 5-4 Description of Body Condition Scores

SCORE	DESCRIPTION
1 Poor	Animal extremely emaciated; spinous processes, ribs, tailhead, tuber coxae, and tuber ishcii project prominently; bone structure of withers, shoulders, and neck easily noticeable; no fatty tissues can be felt.
2 Very thin	Animal emaciated; slight fat covers over base of spinous processes; transverse processes of lumbar vertebrae feel rounded; spinous processes, ribs, tailhead, tuber coxae, and tuber ischii prominent; withers, shoulders, and neck structures faintly discernible.
3 Thin	Fat buildup about halfway on spinous processes; transverse processes cannot be felt; slight fat covers over ribs; spinous processes and ribs easily discernible; tailhead prominent, but individual vertebrae cannot be identified visually; tubera coxae appear rounded but easily discernible; tuber ischii not distinguishable; withers, shoulders, and neck accentuated.
4 Moderately thin	Slight ridge along back; faint outline of ribs discernible; tailhead prominence depends on conformation, and fat can be felt around it; tuber coxae not discernible; withers, shoulders, and neck not obviously thin.
5 Moderate	Back is flat (no crease or ridge); ribs not visually distinguishable but easily felt; fat around tailhead beginning to feel spongy; withers appear rounded over spinous processes; shoulders and neck blend smoothly into body.
6 Moderately fleshy	May have slight crease down back; fat over ribs is spongy; fat around tailhead feels soft; fat beginning to be deposited along the sides of the withers, behind the shoulder, and along the side of the neck.
7 Fleshy	May have crease down back; individual ribs can be felt, but filling between ribs with fat is noticeable; fat around tailhead is soft; fat deposited along withers, behind shoulders, and along the neck.
8 Fat	Crease down back; difficult to feel ribs; fat around tailhead very soft; area along withers filled with fat; area behind shoulder filled with fat; noticeable thickening of neck; fat deposited along inner thighs.
9 Extremely fat	Obvious crease down back; patchy fat appearing over ribs; bulging fat around tailhead, along withers, behind shoulders, and along neck; fat along inner thighs may rub together; flank filled with fat.

Modified from Nutritional Research Council: *Nutrient requirements of horses,* Washington, DC, 1989, National Academy Press.

5.3 Nutrition of the Stallion and Broodmare

5.3.1 STALLION

Nutritional management of the stallion differs little from that described for the mature horse. During breeding season, an increase in energy intake generally is needed to compensate for the increase in activity and stress. Adjustments in feed intake should be gradual. One should increase feed to provide enough energy to maintain the stallion at the desired weight and condition. The horse may need an average increase of 25% more than

maintenance. The usual energy requirement for stallions in the nonbreeding season is about 15 kcal/lb body weight and about 19 kcal/lb body weight during the breeding season.

Obesity is not an uncommon problem in stallions, particularly in the off season. Obesity occurs when the quantity of feed is not reduced when activity level decreases, resulting in overfeeding. The consensus of stallion managers is that stallions kept in good body condition through dietary control, exercise, or both are more sound, more responsive, and fertile and remain healthier than stallions that are permitted to become obese. To help maintain optimal body weight during the breeding season, one should turn out stallions or exercise them in hand for a few hours each day.

Contrary to popular belief, no evidence indicates that extra minerals or vitamins are required for breeding stallions other than those for maintenance. Stallions with access to well-managed pasture or that are receiving good-quality hay and trace mineral salt derive little benefit from additional supplementation. The testicular degeneration reported in rats fed vitamin E–deficient diets has not been observed in horses, and no evidence indicates that vitamin E, A, or C supplementation improves libido or prevents sterility in the stallion.[23,24] However, no evidence indicates that supplementation of these vitamins is harmful.

5.3.2 OPEN MARE

One should feed mares with a condition score of 5 or 6 according to the guidelines for the mature horse. One should place mares that have retired recently from athletic competition or may be in poor condition in a weight- 242
gaining plane before breeding to increase the likelihood of conception and improve reproductive efficiency. 243
Recent research indicated that "flushing," or increasing the energy intake approximately 10% to 15% more than maintenance, increased the chance of conception and reduced the number of covers for mares with a body condition of 4 or lower.[17] No advantage accrues to overfeeding the fat mare. Obesity has been cited as a contributing cause to angular limb deformities in foals and reduced conception rates.[25,26] As in stallions, no evidence indicates that supplementation of other nutrients beyond requirements enhances reproductive performance.

5.3.3 PREGNANT MARE

One should feed the nonlactating mare in early and midgestation to meet maintenance requirements (Table 5-5). The early growth of the fetus is minimal and does little to increase nutrient demand. Often mares are "fed for two" early in pregnancy, which can result in higher feed costs and, more commonly, undesirable weight gain. Recent research suggests that weight gain occurs during midgestation for use as a source of energy later in gestation,[26,27] but no advantage seems to accrue from gains greater than 12% to 15%. One should include additional calories and other nutrients to support lactation and athletic activity in the dietary plan.

The greatest increase in fetal growth and nutrient demand occurs during the last 3 to 4 months of gestation[28–30] (Table 5-6), necessitating increases in most nutrients to support growth and development. Supplying the extra nutrients is confounded, however, by the reduction in digestive capacity as a result of the increasing size of the fetus. The solution is to increase the nutrient concentration and, in some cases, the quality of the feed.

The increases in energy intake for the mare in the last trimester were based on the energy content of equine fetal growth in the last 3 months of gestation. Estimates of the DE requirements for broodmares are 1.11, 1.13, and 1.2 times the maintenance requirement for the ninth, tenth, and eleventh month, respectively,[2] but may be higher for mares in poor condition.[31] For the 1100-lb mare at field maintenance, this increase would equal an additional

1.64, 2.10, and 3.28 Mcal/day during months 9, 10, and 11, respectively. One can meet these increases easily by an additional 11 lb of fresh pasture, 2 to 3 lb of hay per day, or 2 lb of grain, demonstrating that large quantities of feed are not required. In cases in which grass hay is the primary forage, a proportional increase in concentrate to 20% to 30% and a decrease in roughage to 70% to 80% usually provides the energy needed. One should monitor mares as previously described to prevent undesired increases in condition score. Decreases in energy intake to encourage weight loss (>10%) in obese mares is not recommended because of the potential risk of hyperlipemia.[32]

TABLE 5-5 Nutrient Requirements of the Gestating and Lactating Mare[*]

NUTRIENT	EARLY GESTATION	LAST TRIMESTER	LACTATION
Digestible energy, Mcal	15–19	17–22	26–32
Crude protein, g	600–760	750–1000	1300–1700
Calcium, g	18–24	36–45	50–65
Phosphorus, g	13–18	27–35	32–45
Potassium, g	22–30	30–40	40–55
Magnesium, g	7–9	10–11	10–12
Sodium, g	6–15	7–16	8–18
Copper, mg	100–150	250–300	250–300
Iron, mg	400–500	400–500	400–500
Manganese, mg	400–500	600–700	600–700
Zinc, mg	400–500	700–800	700–800
Cobalt, mg	1–2	1–2	1–2
Iodine, mg	1–2	2–3	2–3
Selenium, mg	1–2	2–3	2–3
Thiamine, mg	27–30	30–35	35–40
Riboflavin, mg	18–20	20–22	22–24
Pyridoxine, mg[†]	—	—	—
Niacin, mg[†]	—	—	—
Pantothenic acid, mg[†]	—	—	—
Folic acid, mg[†]	—	—	—
Biotin, mg[†]	—	—	—
Cyanocobalamin, μg[†]	—	—	—

* Mares weighing 450 to 600 kg. Energy need may vary according to age, desired body condition, and milk production.

† No requirement established.

A 10% increase in protein intake has been recommended for protein accretion of the fetus.[2] In nearly all cases, the additional feed provided to meet energy needs supplies the extra protein. The effects of higher intakes of protein on fetal health have not been evaluated, but the fact that many mares fed alfalfa consume nearly twice their requirement for protein and produce healthy, vigorous foals suggests that there should be little concern.

Recent studies of fetal growth, body composition, and postnatal development have provided some estimates of mineral needs during the last 3 to 4 months of pregnancy.[33] Data suggest that to provide for fetal mineral deposition, the calcium and phosphorus intake must increase by nearly 80% more than maintenance; magnesium and potassium must increase by 25%.[2] Because most maintenance diets provide nearly twice the NRC recommendation for calcium, phosphorus, and magnesium and nearly 6 times that for potassium, supplementation rarely is required. However, mares fed diets of grass hay and unfortified grain are likely to be 243
deficient in calcium and phosphorus. One should compare current intake with the recommended levels in Table 244
5-5 to determine how much extra calcium or phosphorus is needed.

TABLE 5-6 Length and Weight of Equine Fetuses by Gestational Age[*]

AGE (DAYS)	CROWN-RUMP LENGTH (cm)[*]	MASS (WEIGHT)
60	6	17 g
90	16	160 g
120	25	700 g (1.5 lb)
150	35	1.6 kg (3.5 lb)
180	48	4.0 kg (9.0 lb)
210	60	10 kg (22 lb)
240	75	17 kg (37 lb)
270	85	20 kg (44 lb)
300	95	29 kg (64 lb)
330	100	42 kg (92 lb)
Average birth weight		42–55 kg (92–120 lb)

Modified from Bergin WC, Gier HT, Frey RA et al: Developmental horizons and measurements useful for age determination of equine embryos and fetuses. Proceedings of the thirteenth annual meeting of the American Association of Equine Practitioners, New Orleans, 1967, pp 179–196; and Platt H: Growth of the equine foetus, *Equine Vet J* 16:247–252, 1984.

* Straight line from tip of forehead to base of tail.

Researchers have had a great deal of interest in work concerning trace mineral supplementation of the late-gestation broodmare and its role in the developmental orthopedic diseases (DODs) of foals.[34,35] Though additional studies are needed, recent research has demonstrated that supplementation of copper to mares during the last 90 days of gestation and to their foals reduced the frequency of cartilage abnormalities in the foals compared with mares and foals consuming NRC-recommended concentrations of copper.[35] Another important note is that copper was not the only nutrient increased in this study. Manganese, zinc, and selenium concentrations also were increased between diets to prevent antagonisms, but nutrient ratios extrapolated from

NRC recommendations were maintained. Therefore adding copper or any mineral alone without evaluating the other minerals is not likely to be of benefit and is not recommended. Selenium and iodine are also of importance to the broodmare, but one should exercise caution to avoid oversupplementation, particularly with iodine. Foals from mares fed from 35 to 48 mg iodine per day were born with enlarged thyroid glands, characteristic of iodine toxicity.[36,37] The author currently recommends the levels of trace minerals listed in Table 5-5 for broodmare diets on farms where nutrition is believed to be a contributing factor to the occurrence of the DODs. In most situations, the recommendations given for early gestation may be sufficient.

Of the vitamins, A, E, and folate are of greatest concern. Studies have demonstrated that serum concentrations of these vitamins are higher in pastured horses and decrease when diets of hay and grain are fed,[38,39] suggesting the potential for deficiencies. However, the decreases in serum concentrations were not associated with clinical evidence of vitamin deficiency, possibly because of the mobilization of body stores. One should evaluate the total dietary vitamin intake before recommending supplementation, and one should follow the NRC recommendations.[2] β-Carotene (1 mg = 400 IU vitamin A) has been recommended as the vitamin A supplement of choice because of its lower potential for toxicity compared with vitamin A. The authors also suggest that folate supplementation should not exceed 100 mg/day.[40]

5.3.4 LACTATING MARE

Energy requirements for the lactating mare depend largely on the volume of milk she is capable of producing. Production also is influenced by the number of previous lactations, the stage of lactation, genetic potential, nutrient supply (most notably energy and water), and foal intake.

During the first 3 months of lactation, one can expect most mares to produce between 3.0% and 3.5% of body weight in fluid milk each day, with pony mares producing slightly more at 4%.[41–43] Field experience suggests that maiden mares tend to have lower milk yields than multiparous mares. One can expect peak lactation to occur between 6 and 10 weeks post partum[41,44–46] but the peak of maximum production may vary and has occurred as early as 30 days.[41] As lactation continues, milk yields decline to approximately 2% of body weight.

Nutrient composition also changes during lactation (Table 5-7). Mare's milk becomes more nutrient dilute as lactation progresses. Protein, fat, vitamin, and mineral content decreases and lactose increases.[41,42,47–50] The increase in lactose content is not high enough to offset the decreases in protein and energy-dense fat, resulting in a lower calorie content.

Recent analyses of mare's milk have further characterized its unique protein distribution and amino acid profile.[51,52] Milk varies between species in its proportion of whey to casein.[53] The ratio in mare's milk is roughly 50:50 during lactation, though it is higher in whey content (85:15) during the early hours post partum.[51] This ratio is in contrast to bovine milk protein (whey- to-casein ratio of 16:82) which is primarily casein protein.[53,54] Amino acid patterns are also different among species.[52] Table 5-8 summarizes the amino acid patterns recently reported for milk from the cow, goat, sow, and mare. Unlike cow's, goat's, and sow's milk, mare's milk protein is nearly 2 times higher in arginine, a finding suggested by a study that examined growth and plasma amino acid 244
concentrations in foals receiving mare's milk or a defined formula containing bovine protein.[55] The true 245
importance of this high level of arginine remains to be determined. If the arginine is needed for optimal growth, the low level of arginine in bovine protein and consequently in most commercial milk replacers may help to

explain the less-than-optimal performance often observed in orphan foals compared with their nursing counterparts.[56,57]

TABLE 5-7 Nutritional Profile of Mare's Milk (per Liter)*

NUTRIENT	WEEKS 1–4	WEEKS 5–8	WEEKS 9–12
Gross energy, kcal	570	515	470
Protein, g	28.5	22	20.2
Fat, g	18.6	15.4	12.5
Lactose, g	65	67	66
Ash, g	5.3	4.8	3.0
Calcium, g	1.2	0.98	0.77
Phosphorus, g	0.6	0.5	0.4
Potassium, g	0.7	0.5	0.4
Sodium, g	0.2	0.2	0.15
Magnesium, g	0.09	0.07	0.05
Copper, mg	0.6	0.3	0.26
Iron, mg	0.9	0.7	0.5
Manganese, mg	1.1	1.2	1.1
Zinc, mg	2.6	2.5	2.2
Cobalt, mg	0.04	0.05	0.02
Iodine, mg	NA	NA	NA

Selenium, mg	0.008	0.005	NA
Vitamin A, IU[†]	40		
Vitamin D, IU[†]	<80		
Vitamin E, mg[†]	0.48		
Thiamine, mg[†]	0.32		
Riboflavin, mg[†]	0.61		
Pyridoxine, mg[†]	0.41		
Niacin, mg[†]	0.93		
Pantothenate, mg[†]	8.9		
Folic acid, μg[†]	82		
Biotin, μg[†]	8.8		
Choline, mg[†]	73		
Vitamin B_{12}, μg[†]	3.1		
Density, g/ml	1.03		

* Data from references 41, 42, and 46 to 48.

† Data from Ross Products Division, Abbott Laboratories, Columbus, Ohio.

The nutrients in greatest demand are water and energy, followed by protein, calcium, and phosphorus. Water is the major constituent of mare's milk, being roughly 90%. A 500-kg mare producing 15 kg milk daily would have to increase her water intake nearly twofold to replenish this loss. Additional calories also are needed. Early in lactation, mare's milk contains approximately 560 kcal/kg fluid milk.[41,42,47] Assuming that mares convert 60% of feed DE into milk gross energy, the mare must consume an extra 792 kcal of DE for every kilogram of milk produced, or an extra 11.9 Mcal in the case of the 500-kg mare, an increase of 72% more than maintenance. Protein needs have been estimated to more than double from those of maintenance from 1.3 to 2.8 g of crude protein per kilogram of body mass.[2]

TABLE 5-8 Reported Amino Acid Composition of Selected Mammalian Milks*

AMINO ACID	HORSE	COW	GOAT	PIG
Alanine	37 ± 2	32 ± 1	34 ± 5	36 ± 2
Arginine	60 ± 2	34 ± 1	29 ± 1	44 ± 1
Aspartic acid	95 ± 5	70 ± 5	75 ± 1	78 ± 5
Cystine	11 ± 2	9 ± 1	9 ± 1	16 ± 1
Glutamic acid	217 ± 8	208 ± 2	209 ± 15	208 ± 5
Glycine	16 ± 1	18 ± 1	18 ± 2	32 ± 1
Histidine	22 ± 2	24 ± 1	26 ± 1	24 ± 1
Isoleucine	39 ± 1	47 ± 1	48 ± 1	40 ± 2
Leucine	93 ± 3	99 ± 1	96 ± 3	89 ± 4
Lysine	73 ± 5	86 ± 2	80 ± 10	79 ± 3
Methionine	22 ± 1	26 ± 1	25 ± 2	22 ± 1
Phenylalanine	43 ± 2	50 ± 1	47 ± 1	43 ± 3
Proline	91 ± 8	100 ± 4	106 ± 8	117 ± 3
Serine	52 ± 8	56 ± 1	49 ± 5	51 ± 3
Threonine	39 ± 2	42 ± 1	49 ± 1	37 ± 1
Tyrosine	45 ± 4	47 ± 1	38 ± 1	39 ± 1
Valine	47 ± 2	52 ± 2	61 ± 1	46 ± 1

Modified from Davis TA, Nguyen HV, Garcia-Bravo R et al: Amino acid composition of human milk is not unique, *J Nutr* 124:1126–1132, 1994.

* Milligrams per gram of total amino acids.

Calcium and phosphorus losses during lactation can be significant, making supplementation of these minerals essential. Mare's milk in early lactation contains 120 mg calcium and 60 mg phosphorus per kilogram of fluid milk, decreasing to 80 mg and 40 mg, respectively, in later weeks. Because only 40% to 50% of the calcium and phosphorus is available from dietary sources, supplementation of 240 mg calcium and 150 mg phosphorus per kilogram of milk seems likely. To date, no comprehensive studies have been done on the effects of dietary calcium and phosphorus intake on milk mineral content or composition.

The trace mineral and vitamin content of mare's milk is available from a limited number of studies.[48–50,58] Because no overt signs of vitamin deficiencies have been observed in nursing foals, sufficient quantities are assumed to be available. All of the trace minerals are low in milk (see Table 5-7). Increased efficiency of absorption and reliance on prenatal reserves are likely important in preventing the development of deficiencies until solid food intake begins.[48]

The point at which mare's milk and hepatic stores can no longer provide for the needs of the foal is not known. 245
Growth rate of the foal and the pre- and postpartum nutrition of the mare are two likely determining factors. The 246

fact that selenium-responsive nutritional muscular dystrophy most often occurs in nursing foals[59] and that more recent evidence demonstrates a reduction in cartilage defects following trace element supplementation at 3 months of age[35] suggests that supplementation may be needed earlier for some minerals.

Generally, body condition score is the best gauge for determining the energy needs of the lactating mare. The habit of "feeding the stall" rather than the horse often results in underfeeding the good producers and overfeeding the poor producers. Mares that lose weight during lactation should be provided with more energy, a higher quality of feed, or both, if clinical examination reveals no underlying cause. Mares that continue to gain weight during lactation are storing the energy as fat rather than channeling it toward milk production.

5.4 Nutrition of the Growing Horse

5.4.1 FOALS

The precocious nature of foals requires that they receive nutrients within hours of birth to survive. Body composition studies have shown that foals have little in energy reserves compared with the adult horse.[29,33] A readily available food source therefore is needed to fuel organ systems and red blood cells and to support thermoregulation and growth. These energy needs are met by frequent nursing,[60] an inherent ability for a high rate of water turnover,[42] and physiologic lengthening of the small intestine to increase available surface area for greater absorption.[61] Nutrition becomes of greater importance for the foals born of mares that are malnourished or that may have chronic placental insufficiency or uterine sepsis, all of which may result in small foals with low nutrient reserves.[62] Because of the increase in nutrient demand following injury or disease, supplying ample, balanced, and complete nutrition is critical for health, development, growth, and recovery.

Data collected from several studies have provided reasonable estimates for evaluating growth and growth rates of foals.[63–67] The greatest increase is in body weight. At birth, foals are approximately 11% of their adult body weight; normal birth weights for most light breeds are between 90 and 110 lb. The period of most rapid growth is the first 30 days when birth weight generally doubles. Average daily gain for the first week is 1.6 kg/day, which decreases steadily with increasing age, to 1.2 kg/day by the fourth week. Tables 5-9 and 5-10 present the reported body weights, rates of weight gain, and relationship to adult weight for growing foals.

Foals are roughly 60% of their adult height at birth.[1] Accurate measurement of wither heights at birth is complicated by the tendon laxity frequently encountered in neonates, but most foals are between 38 and 42 inches high. As with weight, growth rate in height is greatest the first week and then steadily declines. Tables 5-9 and 5-10 show wither heights, rates of height gain, and comparisons with adult measurements.

TABLE 5-9 Reported Growth Rates for Healthy Foals

AGE	WEIGHT (kg/DAY)	HEIGHT (cm/DAY)
First week	1.6	0.4
Second week	1.5	0.35
Birth to 4 weeks	1.4	0.32–0.33
4–8 weeks	1.3	0.25–0.26
8–12 weeks	1.1	0.20–0.25

Data from references 63 to 65 and D.A. Knight, Columbus, Ohio.

Data collected from lactation and growth studies have provided some estimate of the energy, protein, calcium, and phosphorus needed to support early growth in healthy foals. Nursing foals consume approximately 150 kcal gross energy per kilogram of body mass during the first week.[42,43,46] This consumption equals 13 to 14 qt of mare's milk per day for light horse foals or 15 to 17 qt/day for large Warmblood or Draft foals. Similarly, protein needs are high: 6 to 7 g/kg body mass. As the foal ages, the growth rate slows and the energy and protein needed as a function of body weight decreases accordingly (Table 5-11). However, because the larger foal has more total mass to support, the volume of milk needed to meet the requirements increases.

TABLE 5-10 Estimated Body Weight and Wither Height Ranges for Growing Horses of Light Breeds

AGE	MASS (kg)	PERCENT OF MATURE MASS*	HEIGHT (cm)	PERCENT OF MATURE HEIGHT*
Birth	50 ± 9	10	96 ± 5	60
14 days	73 ± 14	14	104 ± 5	65
1 month	91 ± 14	18	109 ± 5	68
2 months	136 ± 14	27	114 ± 5	71
3 months	163 ± 14	33	119 ± 5	75
4 months	191 ± 14	38	123 ± 5	76
5 months	214 ± 14	43	127 ± 5	79
6 months	240 ± 14	48	129 ± 5	81
9 months	295 ± 23	59	142 ± 5	89
12 months	340 ± 23	68	147 ± 5	92
18 months	409 ± 23	82	152 ± 5	95

Data from references 16, 56 to 60, and 63 to 67; H.F. Hintz, Ithaca, New York; and D.A. Knight, Columbus, Ohio.

* Age at maturity is 5 years (60 months).

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TABLE 5-11 Estimated Calories and Volume of Mare's Milk Needed to 2 Months of Age by a 55-kg Foal at Birth

		VOLUME		
AGE	**KILOCALORIES**	**QUARTS**	**ml/hr**	**kcal/kg**
Birth to 7 days	7500	14	550	145–150
8–14 days	9800	18	700	135–145
15–28 days	11,000	20	800	100–135
1–2 months	12,500	23	900	80–100

The calcium and phosphorus concentration of mare's milk appears to be adequate in meeting the early needs of the growing foal. Because foals gradually begin to consume hay and grain during the time they are nursing, at what point these mineral needs are no longer met by milk alone is unknown. Apparently, trace element and fat-soluble vitamin stores are supplied for the short term by prenatal stores.[48–50] Trace element insufficiencies have been suggested to be responsible for some of the DODs present in growing foals and weanlings,[35,68] requiring supplementation of these nutrients before weaning.[35]

From birth the equine digestive tract undergoes anatomic and physiologic adaptions that favor the use of carbohydrates. Lactase, maltase, and sucrase activities have been measured in the small intestine of fetuses and foals.[69] Fetal disaccharidase activity is low, but lactase begins to rise within a few hours of birth. Lactase continues to be the predominant disaccharidase in the equine small intestine until 3 to 4 months of age, when the activity of maltase equals that of lactase. Maltase activity continues to rise, becoming the primary disaccharidase of the mature small intestine. As mentioned previously, the growth of various portions of the gastrointestinal tract seems to parallel these changes in enzyme activity.[61] Significant increases in the length and diameter of the small intestine occur within the first month, increasing the available villous surface area and making it possible for the foal to process increasing volumes of milk to meet its needs. During the next 5 months, growth of the small intestine continues but the greatest increases occur in the lengths of the cecum and large colon at a time when foals traditionally begin mimicking adult grazing behavior and increasing their intake of hay and grain.[70] From 6 months on, most of the growth occurs in the cecum and large intestine, allowing for the increased consumption and use of fibrous feeds.

The actual nutrient requirements of nursing foals have not been determined. From studies of foals 4 months and older, nutrient requirements have been estimated. Table 5-12 show the nutrient recommendations for foals from 4 to 6 months of age.

5.4.2 FEEDING OF ORPHAN FOALS

One can graft orphan foals onto a nurse mare or raise them by hand. One makes the best decision by evaluating several factors with the personnel who will share in the responsibility of caring for the foal.

The nurse mare has the advantage of being the most natural substitute. Nurse mares usually are purebred Draft or Draft crosses, are mild in temperament, and provide the foal with an appropriate role model. Average cost at the time of this writing was between $10 and $15 per day in the United States. In the author's experience, nurse mares are best recommended for farms where labor or technical expertise is limited and turnout is a major portion of the management. However, nurse mares have some disadvantages. Nurse mares are not readily available in many parts of the country. Not all mares are immediately compatible with the orphan, especially if the mare has had sufficient time to bond to her own foal. In some cases, several mares may be needed before a successful graft takes place. The transient nature of nurse mares increases their exposure to infectious disease and the possibility of disease transmission to the privately owned broodstock. Moreover, most leasing agreements require that the nurse mare be returned in foal, which may be difficult for farms without access to a stallion.

If one selects a nurse mare, one should ask for her foaling date and whether her foal is still at her side. A mare 4 to 6 weeks into lactation may not have the quality of milk to sustain a neonate and will have bonded to her own foal, making grafting difficult. For nurse mares to arrive at farms nearly devoid of milk because of stress or lack of mammary stimulation also is not uncommon if her foal died or was removed prematurely. Reinduction of lactation is difficult and not always successful.

Rearing by hand is accomplished by teaching the foal to drink milk substitute from a bottle or, preferably, a bucket. Teaching a foal to drink from a bucket is preferable to bottle feeding because it is less labor intensive for the owner and allows the foal to drink milk on demand. Feeding from a bucket also may help to prevent some of the behavioral problems frequently encountered with foals that have bonded to human beings. Although some of these behaviors are entertaining when the foal is small, a growing foal can cause serious injury to handlers if not reprimanded early. Foals older than 7 to 10 days of age are more difficult to train to feed from a bucket, possibly because the nursing reflex has been well established. Consideration of a nurse mare for these foals may be best.

Several milk substitutes for foals are available (Table 5-13). Selection should be based on quality, performance, acceptability, and nutritional profile. Cow's milk, goat's milk, and calf, lamb, and kid milk replacers also have 247
been used. These substitutes are different from mare's milk in several respects and are not always nutritionally 248
complete or balanced for foals (see previous discussion on feeding the lactating mare). Though goat's milk has been advocated by subjective appraisal as a suitable replacer for foals, poor weight gains and metabolic acidosis recently were reported in neonatal foals fed goat's milk to provide 135 kcal/kg/day.[71] Products containing maltodextrins, corn syrups, oligosaccharides, and glucose polymers are not recommended for foals less than 3 weeks of age because of the low level of maltase activity present in the small intestine of the foal.[69] Carbohydrates that are not digested are fermented by the microbial population of the gut and may result in excessive gas production or an osmotic diarrhea.

TABLE 5-12 Nutrient Recommendations for the Foal, Weanling, and Yearling*

NUTRIENT	FOAL†	WEANLING	YEARLING
Feed intake,% body weight	2.5–3.0	2.2–2.5	2.0–2.2
Digestible energy, Mcal	13–15	15–18	18–20
Crude protein, g	650–800	750–850	850–1000
Calcium, g	35–45	40–50	40–50
Phosphorus, g	25–35	30–40	30–40
Potassium, g	10–15	15–20	20–25
Magnesium, g	4–5	5–6	6–7
Sodium, g	4–6	6–8	8–9
Copper, mg	100–150	150–180	175–200
Iron, mg	300–350	350–450	350–450
Manganese, mg	250–300	300–350	350–400
Zinc, mg	350–450	400–475	475–500
Cobalt, mg	0.5–1.0	0.5–1.0	0.5–1.0
Iodine, mg	0.5–1.0	0.5–1.0	1.0–2.0
Selenium, mg	1.0–1.5	1.0–2.0	1.0–2.0
Vitamin A, IU	15,000–20,000	20,000–25,000	25,000–30,000
Vitamin D‡	—	—	—
Vitamin E, mg	300–400	500–650	700–800
Vitamin K‡	—	—	—
Thiamine, mg	10–15	15–20	20–25
Riboflavin, mg	7–10	12–15	15–18
Pyridoxine, mg‡	—	—	—
Niacin, mg‡	—	—	—
Pantothenicacid, mg‡	—	—	—
Folic acid, mg‡	—	—	—
Biotin, μg‡	—	—	—
Cyanocobalamin, μg‡	—	—	—

* Horses with a mature mass of 400 to 500 kg.

† Foals from 4 to 6 months of age.

‡ No requirement has been established.

Bucket training begins by isolating the foal in a deeply bedded stall for approximately 2 to 4 hours after its last meal. This acts to stimulate appetite, making the foal more willing to accept the new diet. If the foal is dehydrated, one should offer an appropriate electrolyte solution first. One should prepare the milk substitute according to package directions. A wire kitchen whisk is recommended to aid in blending and speeding the mixing process. As with any change in diet, one should offer the foal roughly half of the recommended volume for the first day or 2 rather than alter the concentration. A standard 2-gal plastic or rubber bucket is ample for most foals. For the first attempt, a full bucket is recommended to make the milk more accessible to the foal. One should encourage the foal to nurse from a finger or nipple and gently guide the foal to the bucket. Tipping the bucket slightly makes it easier for the outstretched head of the foal to contact the milk surface. Several attempts separated by rest periods usually are required before the foal nurses from the bucket on its own. Most foals learn to drink within 12 hours, but some take as long as 24 hours. Once the foal has consumed milk on its own, one should hang the bucket so that the rim is slightly higher than the point of the shoulder and assist the foal when needed. If the foal fails to nurse within 4 hours, tube feeding may be necessary because the energy reserves are minimal.

Foals generally consume 20% to 30% of their body weight in milk each day or approximately 120 to 135 ml/lb 248
of body weight for formulae with a caloric density of 500 to 650 kcal/L. As a general rule, most foals of light- 249
horse breeding need 4 or 5 gal of milk substitute each day; draft foals require 6 to 8 gals. Many foals can be fed 2 to 4 times a day. For foals requiring more observation, one may initiate a more rigorous schedule of 6 to 8 times a day with smaller volumes, with the volume increased and number of feedings decreased as the condition of the foal improves. One should mix milk replacer only as needed and not store it. One should discard milk that has not been consumed after 12 hours. One should keep all feeding equipment scrupulously clean to avoid bacterial growth and contamination.

TABLE 5-13 Comparison of Milk Substitutes and Formulae (per Liter)[*]

	MARE	GOAT	NUTRIFOAL[†]	FOAL-LAC[‡]	KID REPLACER[§]
NUTRIENT					
Energy (kcal)	550	690	700	650	600
Proteins, g	25	33	45	36	38
Carbohydrates, g	62	47	60	93	49
Fats, g	21	40	31	27	29
Calcium, g	1.4	1.4	2.0	1.7	0.9
Phosphorus, g	0.9	0.9	1.4	1.4	1.2
Ca:P ratio	1.5:1	1.5:1	1.4:1	1.2:1	0.75:1
Iron, mg	0.8	0.5	19	17	16
Copper, mg	0.5	0.2	7.0	5.0	2.0
Manganese, mg	1.3	0.1	10	13	5.0
Zinc, mg	3.0	4.0	18	15	12
Selenium, mg	0.007	0.01	0.05	Not known	Not known
Total solids,%	11	13	14	17	14
Osmolality (mOsm/kg water)	350	280	300	Not known	Not known
Carbohydrate source	Lactose	Lactose	Lactose	Lactose, Maltodextrins, corn syrup	Lactose
Casein-to-whey ratio	50:50	86:14	50:50	Not known	Not known
CALORIES FROM					
Proteins (%)	20	18	26	19	25
Carbohydrates (%)	47	28	31	49	32
Fats (%)	33	53	43	32	43
NPCal/N ratio	105:1	107:1	75:1	107:1	75:1
Cal/N ratio	132:1	131:1	100:1	132:1	100:1

* *NPCa1/N ratio,* Nonprotein calories-to-nitrogen ratio; *Cal/N ratio,* calories-to-nitrogen ratio.

† KenVet, Ashland, Ohio.

‡ Pet-Ag, Inc, Elgin, Ill.

§ As analyzed, Land O'Lakes, Inc., Fort Dodge, Iowa.

One should introduce foals to solid feeds as soon as possible. Once the foal has mastered drinking from a bucket, one should place several handfuls of high-quality hay and 0.5 lb of milk-based pellets nearby. Few foals show any interest in these feeds, but their inherent curiosity allows them to become familiar with the aroma and texture. One should increase the quantity of milk-based pellets as indicated by appetite, up to a maximum of 2 lb/day. On reaching this goal, one can add a balanced, high-quality 16% to 18% foal feed. By 2 months of age, one should discontinue liquid milk feeding; most foals are eating 2 to 2½ lb of concentrate and 2 to 3 lb of hay. By 4 to 5 months, milk-based pellets are no longer needed and can be replaced with the foal feed of choice. By 6 months, foals can consume 6 to 8 lb of foal feed and 4 to 5 lb of hay. One should feed foals to the condition in which ribs are palpable but not visible. Owners frequently confuse the presence of a potbelly with obesity. Potbellies in young horses can result from intestinal parasite burden, poor-quality feed, underfeeding, overconsumption of hay, or lack of exercise.

Ample exercise is necessary to encourage growth and proper musculoskeletal development and to ease boredom. Behaviors such as suckling the umbilical stump, prepuce, penis, stifle, or ears of other foals or animals are not uncommon and generally cease with time. Placing orphans with other weaned foals of similar age or with a placid, tolerant gelding or mare provides companionship and tutoring in equine social skills.

5.4.3 WEANLINGS

For a young horse to grow and develop properly, the diet must be in sufficient quantity and contain the appropriate balance of nutrients (see Table 5-12). Weanlings generally consume between 2.0% and 2.5% of their 249
body weight in feed per day, depending on the quality and quantity of feed available and the desired growth rate. 250
To meet their energy needs, younger weanlings should receive diets consisting of 30% to 40% grain and 60% to 70% hay. As weanlings increase in body size and digestive capacity, the proportion of grain to hay should shift in favor of more hay if the quality of the hay is high. By 12 months of age, a diet of 50% hay and 50% grain by weight is usually adequate. With the exception of exceptionally large, active individuals and the feeding of poor-quality hay, grain feeding in excess of 8 to 10 lb/day is not necessary or recommended.

Growth rates of weanlings from 6 to 12 months of age continue to decline. Weight gain decreases from 0.8 kg/day during the sixth month to 0.55 kg/day during the twelfth month. Cumulative gain in wither height drops to 0.07 cm/day. The amount of digestible energy per kilogram of body mass needed to support growth decreases to 60 to 65 kcal.[2]

Daily exercise is important for skeletal growth, development, and appetite stimulation. Weanlings that are feed as a group should have ample feed space provided to prevent dominance by any one youngster. One can mix chopped hay with the grain to slow the feeding rate and increase the bulk, reducing the chance of grain engorgement and the risk of gastric dilation and rupture.[2,72] Individual feeding provides a greater opportunity for control of nutrient intake and supplementation when needed but is more labor intensive.

Overfeeding is a common problem in the management of growing horses. The reasons that young horses are overfed are, presumably, to enhance growth rate and, in the case of show horses, to give them a smoother appearance. Several studies comparing the growth of weanlings fed energy and protein levels as high as 150% to 200% greater than the 1979 NRC recommendations have been conducted,[73–76] but differences in design make them difficult to compare. The most consistent effect of overfeeding is an increase in average daily weight of 12% to 36%. Average daily gains in wither height have been reported not to differ[73,74] or to increase by 7% to 12%.[75,76] In summary, no advantage accrues from feeding a weanling in excess of its needs.

The DODs physitis, osteochondrosis, and contracted tendons frequently occur and are diagnosed just before or after weaning,[77–79] though the initiation of the defect probably occurs much earlier.[35] Diet analysis and correction of any imbalances or inadequacies is recommended as a conservative treatment to prevent further lesion development.[79]

5.4.4 YEARLINGS

Diets for yearlings should contain the same balance and fortification established in the weanling program (see Table 5-12). Growth rate continues to slow; weight gain has decreased to 0.5 kg/day, wither height to 0.04 cm/day. Yearlings have attained nearly 90% of their adult height by 12 months and 95% by 18 months. Overfeeding in preparation for sale or show may aggravate existing disease or result in conditions that may not be manifested in the short term[76] and therefore is not recommended. The presence of physitis, osteochondrosis, or cervical vertebral stenotic myelopathy is not uncommon in this age group.[78,80,81] With the exception of physitis, changes in the diet at this stage are not likely to improve clinical outcome but may arrest further lesion development and are recommended.[35]

5.5 Problems Related to Nutrition

5.5.1 OBESITY

Obesity in horses is a condition that currently is not recognized as a medical problem, is rarely treated, but is likely to have as major an effect on the health and longevity of horses as it does in other species.[82–84] To expect that excess condition in horses is just as likely to aggravate bone and joint injuries, reduce exercise tolerance, decrease heat tolerance, increase susceptibility to disease, and adversely affect reproductive performance would seem logical.

When is a horse considered to be obese? A person is considered to be obese whose body weight is 20% greater than the standard for height and frame; at 10% the person is simply overweight. No criteria for obesity have been established for horses as have been for dogs and cats.[84] Body condition scores of 6 to 9 describe horses with increasing degrees of fat deposition. In one study, an increase in body mass of 30 kg in a group of mares was associated with an elevation in condition score from moderately thin to fleshy.[17] The increase was only 6% of body weight, indicating that different criteria may be needed for the horse. One should use subjective appraisal by body condition scores and breed standards to provide a reasonable guide until more data are available.

The primary cause of obesity in horses is overfeeding or supplying calories in excess of use. Ponies, pleasure horses, and broodstock seem to be affected most often. Horses on lush pasture and with little exercise (i.e., broodmares) can become heavy; this situation is understandably difficult to control. Endocrine (i.e., hypothyroidism), metabolic, and genetic factors also may be involved.

With the exception of halter horses or market yearlings, feeding horses to an overweight or obese state is not intended. The problem and its treatment are a matter of dietary management rather than the dietary constituents per se. Some factors involved in obesity may be the following:

1. *Lack of knowledge concerning energy content of feeds and feed-weight-per-unit volume*. Few if any owners know the energy content of feedstuffs. Most can say that corn is higher in energy than oats but 250 251

cannot give the actual difference. This lack of knowledge is underscored further by the habit of feeding grain and hay by the convenient unit rather than weight: the scoop, coffee can, quart, and cup for grain and flake, section, and bat for hay, regardless of quality or type.

2. *Overestimation of maintenance needs*. Few owners know or have ever measured the weight of their horses. If the horse is one of the light breeds, the owner assumes it weighs 1000 lb and feeds it according to the recommendations for the 1000-lb reference horse at maintenance. As discussed previously, probably two levels of maintenance exist for most horses. The one referred to most often in feeding directions and textbooks has been discussed in the context of this chapter as field maintenance. For horses that spend a good deal of the time in stalls, feeding at this level may exceed their daily energy expenditure by as much as 30%.

3. *Inability to assess body condition*. Owners rarely admit that their horses are carrying more weight than is ideal and most are probably not sure how to know. Little emphasis is placed on the value or the "how to" of evaluating body condition in horses. For example, "hay bellies," more the result of poor-quality hay and little exercise, often are assumed to be a sign of obesity in horses, particularly young horses.

4. *Overestimation of exercise intensity*. An hour of leisurely trail riding at a walk and slow trot burns about 1 Mcal, resulting in minimal energy expenditure. Many owners feel this constitutes work and increase caloric intake. The presence of sweat often is viewed as evidence that the horse has worked hard, and owners may give extra feed to make up for this perceived increase in energy expenditure.

5. *Animal-human bond*. Like dogs and cats,[84] horses frequently are rewarded for compliance or their friendship by receiving feed: carrots, apples, horse treats, handfuls of grain, extra flakes of hay. Feeding establishes a direct relationship between owner and horse. The horse soon associates the presence of the owner with feed; the owner views the interest as affection and the relationship continues.

If one can find no medical cause for excessive weight gain, one should discuss treatment by dietary means with the owner. One should obtain the current weight and condition score of the horse. To encourage weight loss requires a reduction in calories below expenditure. A method the author has used with success begins with the formulation of a diet that provides 70% of the maintenance energy requirements of the horse *at its ideal weight*. Grass hay is suggested as the diet base because it is lower in digestible energy per kilogram than legume hay and also weighs approximately half as much per unit of volume as alfalfa hay, so one can feed more sections or flakes. This helps to reduce the concern of owners that they are starving their horses. One can supply vitamins and minerals by a supplement and trace mineral salt.

Exercise is an integral component of a weight loss program through stimulating the loss of fat rather than lean body mass, which may not be possible if the horse is lame or has respiratory compromise. Any increase in exercise, regardless of intensity, should be done slowly and monitored frequently.

Giving the owner some idea of how long the horse will take to reach its goal weight also is helpful. In most cases, the horse may take 3 to 5 months to reach goal weight. By the veterinarian's giving the owner an honest appraisal, the owner will know what to expect and will be more willing to comply. One should schedule periodic evaluations to monitor the progress of the horse. Once the horse has reached its goal weight, one can adjust the caloric intake for body maintenance and planned activity level.

5.5.2 DIARRHEA

This discussion is limited to common nutritional diarrheas in the horse. These diarrheas are usually more of a nuisance and unaesthetic than of clinical concern. No studies of their pathophysiology appear in the literature. Most likely, the diarrheas of dietary origin are osmotic and appear to result from an increase in carbohydrate fermentation and change in the microbial population. Osmotic diarrhea usually occurs when an excess of nonabsorbable, water-soluble, low-molecular-weight solutes is present in the bowel, drawing water into the gut. This type of diarrhea is usually self-limiting if the horse is given time to adapt to the change.

Sudden and large increases in grain intake can result in diarrhea in some horses. An increase in rate of passage presents a large quantity of partially digested and undigested carbohydrates to the colon, where the carbohydrates undergo rapid fermentation by the hindgut microflora. The rise in lactate and volatile fatty acid concentrations results in an increase in osmolality of the colonic fluid, causing an influx of water into the colon. If lactate production exceeds the buffering capacity of the colon, mucosal damage may occur, increasing toxin absorption. [85]

Fresh pasture is high in water and soluble carbohydrates. The loose, watery feces seen after horses are turned out to pasture likely are caused by a combination of the fermentation of the available carbohydrates, a more rapid rate of passage reducing water absorption, the high water content of the ingesta overwhelming absorptive capability, and the lack of long fiber to produce formed feces. 251

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The soft feces that develop when some horses are fed alfalfa hay happens most often when an abrupt change has occurred from a grass hay to alfalfa hay or when the quality of the alfalfa hay has improved greatly. In this case the diarrhea probably is caused by the increased mineral content of alfalfa causing an osmotic diarrhea.[22] Fiber content also has decreased, reducing the dry matter content of the feces.

Show horses and racehorses frequently develop diarrhea during shipping or before performances. Although possibly caused by changes in feed and water, this diarrhea more likely is caused by stress, resulting in excessive stimulation of the parasympathetic nervous system, increasing gut motility and mucous secretion. However, the most common stress-induced diarrhea in horses is associated with *Salmonella* species (see Chapter 2).

5.5.3 COLIC

As with obesity, the nutritional causes and nutritional methods of prevention of colic are limited. In cases in which poor-quality or tainted feed is fed accidentally, colic may result, and one should caution the owner more about dietary management than about the diet itself. The horse evolved as a continuous grazer, consuming small meals frequently.[70] Most horses today are raised in confinement with little or no access to pasture and are fed high-concentrate meals according to schedules devised by the owner.[70,85] All too often not enough hay is given at stables, and water is not always clean and readily available. Without hay to keep their grazing instinct satisfied, horses may begin to eat wood or bedding or develop other vices such as weaving or cribbing to assuage boredom.[70,86] Only recently has evidence been provided to support the contention that current feeding practices alter physiologic responses and contribute to the occurrence of digestive upsets in the horse.[85]

Several ways exist to reduce the risk of some colics by dietary means. A discussion of the current practices with the owner or manager will help to identify those of most importance and narrow the focus.

1. Provide sufficient quantities of free-choice, good-quality, long-stem hay. Horses fed hay ad lib spend more time eating than horses fed pelleted feeds or all-grain diets.[86–88]
2. Increase the frequency of feeding and reduce the meal size.[85]
3. Avoid abrupt changes in hay or grain type or quantity.[1,85]
4. Avoid abrupt changes in times of feeding or order of feeding.[85]
5. Reduce the grain meal by half when the feeding schedule has been interrupted.
6. To slow the feeding rate, offer hay first to reduce the initial hunger urge.[1]
7. Provide plenty of clean, fresh, cool water.

5.5.4 DEVELOPMENTAL ORTHOPEDIC DISEASES

The DODs are conditions of the young, growing horse that occur with a disturbance in the conversion of cartilage to weight-bearing bone. Included in this group are osteochondrosis, osteochondritis dissecans (OCD) of cartilaginous origin, acquired angular limb deformities, physitis, subchondral cystic lesions, some cervical vertebral stenotic myelopathies, flexural deformities, and cuboidal bone malformations.[89]

Age at diagnosis is typically between birth and 2 years.[77,79,81] These diseases appear to occur predominantly in purebred horses, with Thoroughbreds, Quarter Horses, Standardbreds, and Arabians as the breeds most commonly affected.[89] Anecdotal and clinical data tend to support a higher incidence of OCD and cervical vertebral stenotic myelopathy in colts,[77,89] but the gender distribution for the other conditions has not been reported.

The etiologic profile of the DODs is complex. The factors most frequently implicated are (1) genetic predisposition, (2) rapid growth rate, (3) nutrition, (4) biomechanics and conformation, (5) trauma, and (6) metabolic function. Probably no one cause is the culprit, but rather a combination of all or some of these factors.

5.5.4.1 Genetic Predisposition

A growing body of evidence indicates that a heritable factor may be involved in some of these diseases.[90–93] A study of offspring produced by two wobbler stallions out of 12 wobbler mares did not result in neurologic lesions, but a high incidence of contracted tendons (30%), physitis (40%), and OCD (50%) was observed.[90] Radiographic screening of Standardbred trotting and Swedish Warmblood horses identified progeny from particular stallions that had a significantly ($P < 0.001$) higher frequency of OCD in the tibiotarsal joint than offspring of other stallions.[92] These observations have been confirmed by a more recent study in which the incidence of tibiotarsal osteochondrosis was as high as 30% for the progeny of two stallions.[93] The authors of the study point out that neither of these stallions had radiographic evidence of the disease, suggesting that progeny testing may be the best method of identifying genetically unsuitable broodstock.

5.5.4.2 Growth Rate

Energy and protein and the effects of overfeeding these nutrients on accelerating growth rate have long been implicated as the most likely cause of cartilage and bone disease in young horses,[77,94] but only anecdotal data exist.

Increases in energy and protein intake have resulted in greater weight gains compared with controls[75,76] but not compared with gains inferred from available growth data for young horses.[63,65–67,95] Acceleration of 252
vertical growth (wither height) by overfeeding has been less consistent.[73–76] Increased weight gains have been 253
associated with a higher occurrence of conformational defects in yearlings fed ad lib[76] and radiographic evidence suggesting physitis in weanlings[95] but do not appear to be related to contracted tendons[75] or to be necessary for the development of osteochondrosis.[35,91]

5.5.4.3 Nutrition

Several nutrients or dietary factors may be involved in DODs directly or indirectly. The balance and quantity of all dietary elements likely are critical for nutritional intervention.

5.5.4.3.1 Energy and Protein

Endocrine aberrations in response to the overfeeding of energy were implicated in studies in which starch intakes of 4.5 g/kg body mass were shown to result in a transient (60-minute) increase in postprandial circulating levels of triiodothyronine (T_3) and consequent decreases in thyroxine (T_4) concentration compared with starch intakes of 2.5 to 3.5 g/kg body mass.[96,97] Biochemical changes in normal-appearing cartilage from foals with dexamethasone-induced bone lesions[98] were presumed to be characteristic of OCD, and some similarities were found in cartilage from weanlings consuming high-starch diets.[73] Because T_4 is required for cartilage maturation, this led to the hypothesis that lesions of osteochondrosis may result from a high carbohydrate– induced hypothyroid state.[97] The hormones T_3 and T_4 have similar biologic effects.[99] Triiodothyronine has been shown to stimulate chondrocyte maturation and hypertrophy and increase alkaline phosphatase activity in porcine physeal cartilage.[100] Alkaline phosphatase secretion by matrix vesicles in the hypertrophic zone is considered a preparatory step in cartilage mineralization, though its precise role is unclear.[101,102] Therefore increases in T_3 would seem to accelerate cartilage maturation and endochondral ossification rather than retard it. Moreover, the concentrations of circulating T_3 and T_4 and insulin reported for the high-carbohydrate group were well within the normal range for horses between the ages of 4 months and 5 years.[103] One also should note that the weanlings in these studies received their entire caloric intake in two meals. The endocrine response seemingly would be different with the more standard management practice of continuous access to hay or pasture interspersed with two or three grain meals.

High dietary protein content has been linked with high energy in increasing growth rate[77] and has been reported to increase renal calcium excretion, resulting in negative calcium balance.[104] Increases in dietary

protein beyond the requirement in a later study did not increase gains in height or weight, nor were any detrimental effects on calcium absorption observed.[105]

5.5.4.3.2 Calcium and Phosphorus

The importance of calcium and phosphorus in proper bone formation is well known. Low dietary calcium or phosphorus can result in an excess of uncalcified bone and widening of the metaphyseal cartilage.[2] Nutritional secondary hyperparathyroidism, or bighead, and other skeletal abnormalities occur in the presence of low dietary calcium and adequate to high phosphorus.[106,107] Because calcium is absorbed primarily in the small intestine and phosphorus in the small and large intestines,[108] excess dietary phosphorus interferes with calcium by competing for the absorptive site. Physitis, once referred to as rickets, also has been linked to improper calcium and phosphorus balance,[78] though no scientific data prove the relationship.

Conversely, excess dietary calcium and hypercalcitonemia recently have been proposed to cause osteochondrosis in horses[109] based on data from an earlier overnutrition study in Great Dane puppies in which many nutrients (especially vitamin D) were excessive.[110] Studies have demonstrated that young ponies[108] and horses[111] respond to high calcium intake by decreasing intestinal absorption and increasing calcium excretion in the urine and feces and uptake by bone. No deleterious effects attributed to calcium have been noted at 2 times[35,75] to 5 times[112] the requirement when adequate phosphorus (ratio of at least 2:1) was present.

High calcium per se is unlikely to be responsible for DOD, but one should consider its effect on other nutrients. Alfalfa is becoming the predominant forage fed to horses.[1] The calcium content of alfalfa can be between 2 and 10 times higher than that of grass or mixed hay.[2] If alfalfa hay is fed with commercial feeds designed for grass hay, a higher-than-desirable intake of calcium could result[113] and be of concern in the presence of high dietary vitamin D. Excess dietary calcium can interfere with the absorption of zinc[114–116] and manganese,[117] two trace elements that are co-factors in cartilage metabolism.

5.5.4.3.3 Trace Minerals

Low dietary concentrations of copper, manganese, or zinc have been shown to result in cartilage and bone abnormalities in several species,[118–123] including the horse.[35,68,124] The primary roles of these minerals are as constituents of metalloenzymes involved in cartilage synthesis or calcification.[119,121,125]

As early as 1946, copper supplementation was shown to prevent the development of angular limb deformities and articular surface erosions in young show calves.[126] In 1949, articular cartilage defects were observed in normally growing foals fed diets containing 8 ppm copper but not in diets containing 18 ppm copper.[127] The relationship between copper and cartilage abnormalities in foals was supported further by
reports of a copper-responsive osteodysgenesis[128,129] and the presence of severe cartilage ulcerations in 253
hypocupremic foals.[124] Subsequently, copper supplementation to mares in late gestation and to their foals 254
was found significantly to reduce the number and type of cartilage lesions in the foals compared with the number of lesions in foals that along with their dams had been fed diets containing copper concentrations according to the NRC recommendation.[35] Although copper is well known as a component of lysyl oxidase,

the enzyme responsible for the cross-linking of hydroxylysyl residues in collagen synthesis,[130] the mechanism of the apparent effect of copper is not known. Possibly, other biochemical roles for copper in cartilage synthesis and calcification remain to be identified. A 1993 study[68] that should prompt further investigation described biochemical changes in the cartilage of foals fed low-copper diets.

Manganese deficiency causes enlarged joints and perosis in growing chicks.[121,131] The primary cartilage defect in chicks appears to result from a decrease in proteoglycan synthesis caused by a lack of glycosyltransferases, manganese-containing metalloenzymes.[121] Angular limb deformities, congenital fractures, and tendon contractures in foals have been associated with low manganese concentrations in pastures, but the precise biochemical or structural cause was not identified.[132,133]

A decrease in osteoblastic activity with an increase in cartilage matrix was observed in chicks given zinc-deficient diets.[119] Consequently, the activity of alkaline phosphatase, a zinc metalloenzyme, was shown to be reduced in zinc deficiencies.[134] Alkaline phosphatase concentration and activity are high in the resting zone and in matrix vesicles of the zone of hypertrophy.[101,135] Though initially its primary role of alkaline phosphatase was thought to be via the hydrolysis of pyrophosphate, an inhibitor of cartilage calcification,[136] alkaline phosphatase activity now appears to be diverse in cartilage matrix, cleaving a variety of phosphate-containing substances crucial to the initiation of calcification.[101,102]

Most feedstuffs are inherently low in zinc, with an average concentration of 20 to 25 ppm.[2] This concentration is less than the NRC dietary recommendation of 40 ppm. Moreover, the presence of dietary antagonists may further inhibit absorption. Soybean meal frequently is used as the protein source in diets for young horses.[1,2] Bean and seed meals are known to contain high levels of phytate, which has been shown to interfere with zinc availability, particularly in the presence of high calcium concentrations.[114,116] This antagonism could be of concern with forages high in calcium, such as legumes.

5.5.4.4 Biomechanics and Conformation

Excessive compression of growth plates has been shown to result in focal areas of cartilage thickening and, when severe and prolonged, eventual thinning, premature vascular invasion, and closure of the plate.[137,138] When the compression was removed, mineralization and calcification were restored. The primary lesions are believed to have resulted from a cessation of blood flow to the cartilage, reducing nutrient delivery. The focal, cone-shaped thickenings observed in rabbit cartilage undergoing compression have been reported in metaphyseal cartilage of foals and weanlings,[35,139] but their pathologic significance in young horses is not known.

Young horses that are heavily muscled, carrying excess condition, and have some degree of limb malalignment seem to be more prone to develop the firm, metaphyseal enlargement of the distal radius, metacarpus, or metatarsus referred to as physitis.[78,95,140,141] Foals that are toed in with offset knees also seem more vulnerable.[140] Thinning of physeal cartilage with columnar disorganization and the formation of bone bridges has been associated with epiphyseal compression and the formation of exostoses in young horses with clinical evidence of physitis.[141]

5.5.4.5

Trauma

Any damage that compromises vascular penetration and nutrient delivery affects cartilage growth and maturation. Trauma is likely to be responsible for the separation of defective tissue, as in osteochondritis dissecans, but is not believed to be the primary cause.[142] For trauma to account for a majority of the cases of DOD seems unlikely, but one cannot overlook trauma as a cause.

5.5.4.6

Metabolic Function

Cartilage growth and endochondral ossification are orchestrated by the initial or subsequent release of several hormones. Growth hormone, somatomedin C, insulin, T_4, parathyroid hormone, calcitonin, vitamin D, estrogen, and testosterone can stimulate or suppress chondrocyte proliferation, sulfate incorporation, proteoglycan synthesis, or calcification.[135,143] In hypothyroid foals, evidence of faulty endochondral ossification such as osteochondrosis, delayed physeal closure, and ossification of the carpal and tarsal bones has been described.[144,145]

5.6

Nutritional Support of the Sick Horse

Interest in the development of nutritional support protocols for the hospitalized veterinary patient continues to grow. The adverse effects of inadequate nutritional support are well documented and may be a contributing factor to poor response of traditional therapies. Early, aggressive nutritional intervention of critically ill or injured patients helps to prevent unnecessary depletion of nutrient stores, promote repair, reduce the number and severity of secondary complications, improve the response to antibiotic treatment, and increase the chances of a favorable outcome. 254

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5.6.1

METABOLIC CONSEQUENCES OF STARVATION VERSUS DISEASE

Recognizing the differences in the metabolic response of the body to starvation and to disease is critical for understanding the importance of nutritional intervention. In starvation the goal of the body is to economize, to do what is necessary to lengthen life in the face of low or no food supplies. The decrease in circulating insulin following food deprivation results in a reduction of the conversion of T_4 to T_3, lowering the metabolic rate and reducing energy expenditure. Glucagon is released in response to the hypoglycemia, triggering hepatic glycogenolysis to supply the needed glucose. This source is depleted quickly, making amino acids from body protein the predominant sources of glucose in the early phase of starvation. Fats also are mobilized as energy sources, producing fatty acids and glycerol. In the starving horse, most of the fatty acids and glycerol are reesterified to triglycerides and transported out as very-low-density lipoprotein (VLDL).[146] Analysis of the composition of VLDL produced in the fasted state show that it is higher in cholesterol and lower in protein than the VLDL in fed ponies[147] and may explain the hypercholesterolemia observed during fasting. The VLDL is transported to the peripheral tissues where it is cleaved by membrane-bound lipoprotein lipase, providing free fatty acids as energy substrates. Some of the glycerol is used for glucose production, and fatty acids channeled for ketone production can be used by brain and heart as an alternative energy source, sparing glucose and ultimately body protein. When this occurs, stored body fat becomes the main source of fuel, though protein losses still continue at a slower rate. Other effects of starvation include (1) decreased synthesis of albumin, transferrin, and fibrinogen; (2) shrinking of the intestinal villi and decreased digestive enzyme synthesis and

activity; (3) depression of immune response mechanisms, including antibody synthesis, phagocytosis, and neutrophil activation; and (4) muscle atrophy and weakness from continued degradation of muscle protein. Once fat stores have been depleted, protein becomes the primary fuel source. Continued protein degradation results in multiple organ failure and ultimately death.

The response of the body to stress is much different than is its response to starvation. The attempt to conserve is reversed in the presence of trauma and disease. D.P. Cuthbertson[148] has described the response to stress in two phases: the “ebb” phase and the “flow” phase. The ebb phase is characterized as the period immediately following injury when a decrease occurs in peripheral vascular perfusion, cardiac instability, and hypometabolism. Once resuscitation has been successful and perfusion has been restored, the hypermetabolism, amino acid mobilization from skeletal muscle, and significant increases in gluconeogenesis associated with the flow phase of the stress response begin. Several hormones act as key effectors in the flow phase. Catecholamines are released in response to stress, causing an increase in the metabolic rate and the β-adrenergic–stimulated release of glucagon to elevate glucose production. A rise in adrenocorticotropic hormone also occurs, promoting a subsequent increase in glucocorticoid secretion and providing an additional stimulus for glucagon, continuing to drive amino acid mobilization from skeletal muscle and lipolysis. Tumor necrosis factor and interleukin-1, two of several cytokines produced by T cells and macrophages during the acute phase response, initiate the induction of fever by a direct effect on the hypothalamus, thereby increasing energy expenditure approximately 7% for every degree Fahrenheit rise in body temperature. Hyperglycemia frequently is observed in septic patients in the face of normal or increased insulin secretion, possibly because of a combination of cortisol-driven gluconeogenesis and an increased peripheral insulin resistance.[149] Additional demands for glucose and amino acids also are incurred for tissue repair and acute phase protein synthesis. Without protein and energy intake to ameliorate some of the loss, functions such as wound healing and host defense may be sacrificed to support tissues of highest priority for sustaining life.

5.6.2 ENTERAL FEEDING

5.6.2.1 Candidates for Nutritional Support

Not all patients require enteral nutritional support. Most horses that were well nourished before the onset of acute, short-term disease resume eating within a short period (3 to 4 days). However, for the disease process of some horses to worsen is not uncommon, prolonging the inappetence and initiating the catabolic state described previously. The criteria of Butterworth,[150] modified for horses and listed in Boxes 5-2 and 5-3, are suggested to help identify likely candidates for nutritional support.

Horses that have been anorectic for more than 3 days generally are normo- or hypoglycemic, hypertriglyceridemic, hypercholesterolemic, and hyperbilirubinemic (Table 5-14). Though hypertriglyceridemic horses are usually hypophagic, the converse is not always true. The appearance of opaque sera suggests hyperlipemia, a serious condition requiring immediate medical and nutritional intervention. Azotemia may or may not be present but may exacerbate the development of hyperlipemia.[151] Horses that have been ill for some time often have low packed cell volumes but normal mean corpuscular hemoglobin concentrations and mean corpuscular volumes, indicating the anemia of inflammatory disease.[152]

If one has no reason to suspect that the absorptive capacity of the digestive tract has been reduced, one should 255
choose enteral feeding. This route is the most physiologic and the simplest, easiest, safest, and least expensive 256
method of providing nutritional support.

5.6.2.1.1

BOX 5-2 FEATURES OF THE PHYSICAL EXAMINATION SUGGESTING POOR NUTRITIONAL STATUS

1. Cutaneous
   a. Thin, shiny skin
   b. Drying and scaling of skin
   c. Easily pluckable hair
   d. Decubitus ulcers
   e. Follicular hyperkeratosis
   f. Nonhealing surgical wounds
2. Mucous membranes
   a. Pallor or redness of buccal mucosa, gums
   b. Atrophy of lingual papillae
3. Musculoskeletal and neurologic
   a. Joint laxity
   b. Physitis
   c. Growth retardation
   d. Flexural deformities (<1 yr old)
   e. Weakness and atrophy
   f. Ataxia
4. Abdominal and other organs
   a. Hepatomegaly
   b. Ascites
   c. Small bowel distention, gas or fluid
   d. Lymphadenopathy, tumors
5. General appearance
   a. Edema
   b. Obesity

c. Cachectic appearance

Modified from Butterworth CE Jr: Some clinical manifestations of nutritional deficiency in hospitalized patients. In *Proceedings of the second Ross Conference on Medical Research: Nutritional Assessment—Present Status, Future Directions and Prospect,* Columbus, Ohio, 1981, Ross Laboratories.

5.6.2.2

Nutritional Formulae

The first “blenderized” enteral diet for hospitalized horses, pioneered by Naylor, Freeman, and Kronfeld[153] (Table 5-15), provided clinicians with a means by which to meet the energy and protein needs of their equine patients. Unfortunately, this diet and other home brews are not only difficult to procure, mix, deliver, and store but often can be energy-dilute because of the large volume of water needed to deliver them, and they may lack nutritional balance and adequacy. Since then, other diets for enteral feeding have been tested or developed. Pelleted horse feeds designed to supply the grain and forage components of the diet generally are used as the base for slurries.[154] These feeds have the advantage of being nutritionally balanced, less expensive, and widely available compared with the alfalfa-casein-dextrose mixture. The pelleted feed chosen should be age- and nutrient-appropriate for the horse. One also can tailor these diets to meet specific needs. To increase energy density, one may add vegetable oil. One may increase protein content by adding alfalfa meal, finely ground soybean meal, or a modular component used for human liquid diets. Adding raw eggs to increase protein is not advised because of the risk of salmonella contamination. As with the Naylor diet, large volumes of water are needed to reduce viscosity for hand pumping or funneling via tube.

5.6.2.2.1

BOX 5-3 FEATURES OF THE CLINICAL HISTORY SUGGESTING NUTRITIONAL RISK

1. Recent weight loss of 10% or more of usual body weight
2. Restricted oral intake of nutrients or intravenous infusions of simple solutions only for the preceding 10 days or more
3. Protracted nutrient losses resulting from the following:
    a. Diarrhea, malabsorption syndromes
    b. Surgical absence of portions of the gastrointestinal tract
    c. Draining fistulae, abscesses, wounds, burns
4. Increased metabolic needs because of the following:
    a. Extensive burns, infection, trauma
    b. Fever
    c. Lactation, recent pregnancy, recent surgery or trauma
5. Use of drugs with antinutrient or catabolic properties, such as the following:

   a. Corticosteroids
   b. Immunosuppressants
   c. Antitumor agents
   d. Miscellaneous (e.g., antibiotics, antacids, anticonvulsants)
6. Chronic disease or impaired function of any major organ system

Modified from Butterworth CE Jr: Some clinical manifestations of nutritional deficiency in hospitalized patients. In *Proceedings of the second Ross Conference on Medical Research: Nutritional Assessment—Present Status, Future Directions and Prospects,* Columbus, Ohio, 1981, Ross Laboratories.

Commercially available liquid diets fed to hospitalized human patients also have been used successfully as the sole source of nutritional support in a variety of clinical cases.[155,156,156a,156b] Proteins of high quality such as 256
casein, whey, or soy protein isolate are used most frequently as the protein source. The fat sources are all 257
vegetable. Many formulae contain a combination of one or more of the vegetable oils with medium-chain triglycerides, which are prepared from the fractionation of coconut oil and are 6 to 10 carbons long.[157] Medium-chain triglycerides are more water soluble, requiring little lipase activity or conjugation to bile salts for absorption to occur. Medium-chain triglycerides are hydrolyzed more rapidly than long-chain triglycerides and are transported directly into the blood via the portal system, making them a readily available energy source.[155] The carbohydrate sources are primarily starch hydrolysates (e.g., maltodextrins and oligosaccharides), which are absorbed rapidly, and because they are more complex, they help to reduce osmotic load. Because these formulae were designed to meet the nutrient needs of human beings, they do not meet all of the vitamin and mineral requirements of the horse and should not be used for long-term feeding. As a direct result of these efforts, an enteral formula designed exclusively for use in horses recently has become available (NutriPrime, KenVet, Ashland, Ohio). Though the cost per day for these liquid diets is higher than conventional slurries, the complete nutrient profile, consistent nutrient composition, ease of administration, and sterile packaging are noteworthy advantages. Tables 5-16, 5-17, and 5-18 give the ingredients and nutritional composition of some of the prepared formulae, including the equine diet.

TABLE 5-14 Some Reported Serum Chemistry Changes in Fed, Fasted, and Sick Horses

			SICK	
MEASUREMENT	FED	FASTED (88 HOURS)	ANORECTIC	HYPERLIPEMIC
Triglycerides, mg/dl	23 ± 7	160 ± 50	158 ± 66	1567 ± 972
Free fatty acids, mg/dl	0.5 ± 0.5	23 ± 10	—	23 ± 8
Total lipids, mg/dl	330 ± 10	510 ± 125	730 ± 860	1773 ± 769
Cholesterol, mg/dl	99 ± 9	84 ± 10	172 ± 134	242 ± 114
Blood urea nitrogen, mg/dl	26	18	23 ± 11	177 ± 179
Creatinine, mg/dl	1.6	No change	No change	10 ± 7
Total bilirubin, mg/dl	0.5	3.5–3.7	4.1 ± 2.7	1.8 ± 1.4

Data from Naylor JM, Kronfield DS, Acland H: Hyperlipemia in horses: Effects of undernutrition and disease, *Am J Vet Res* 41:899, 1980.

TABLE 5-15 Composition of and Feeding Schedule for the Naylor Diet

	DAY						
CONSTITUENTS	1	2	3	4	5	6	7
Electrolyte mixture, g[*†]	230	230	230	230	230	230	230
Water, L	21	21	21	21	21	21	21
Dextrose, g	300	400	500	600	800	800	900
Dehydrated cottage cheese, g[‡]	300	450	600	750	900	900	900
Dehydrated alfalfa meal, g	2000	2000	2000	2000	2000	2000	2000
Energy, Mcal[§]	7.4	8.4	9.4	10.4	11.8	11.8	12.2

From Naylor JM, Freeman DE, Kronfield DS: Alimentation of hypophagic horses, *Compend Cont Educ Pract Vet* 6:S93, 1984.

* These allowances should be divided and administered in three feedings daily. Maintenance requirements for a 450-kg horse are 13 Mcal of digestible energy and 580 mg crude protein.

† Ten g NaCl, 15 g NaHC03, 75 g KCl, 60 g K_2HPO_4, 45 g $CaCl_2 + 2H_20$, and 25 g MgO.

‡ Dehydrated cottage cheese: 82% crude protein with less than 2% lactose (American Nutritional Laboratory, St. Louis, Mo.) or casein (Sigma Chemical, St. Louis, Mo.).

§ Megacalories of digestible energy.

5.6.2.3 Nutrient Requirements

The purpose of enteral support is to provide the necessary nutrients for maintenance and healing until the horse can obtain sufficient quantities by voluntary oral intake. Though the specific nutrient needs of the sick horse are not known, data pooled from human and animal studies have provided reasonable estimates for use 257
in equine cases. The basal energy requirement rather than that for resting maintenance is the foundation for 258
calculating the energy expenditure in sick human beings and animals. Horses requiring medical attention are generally at stall rest in a controlled environment, reducing the energy need for physical activity and thermoregulation. One can calculate the resting energy requirement of horses in stalls from the formula 21 kcal(BM_{kg}) + 975 kcal.[15] This product is roughly 70% of that needed for field maintenance, equal to 11 Mcal/day for a 500-kg horse. The basal energy requirement is but one part of the resting energy value. Another significant component is the heat of fermentation produced by microbial action in the hindgut. When feed intake decreases, the substrate available for the microbes decreases, reducing substrate turnover. Microbial fermentation gradually decreases and less heat is produced. The energy for digestion, absorption, and product formation also have decreased, lowering energy losses even further. Therefore the actual energy requirement of the horse at rest may be closer to 55% to 60% of field maintenance. Until more data are available, the increases in energy expenditure resulting from illness or injury are applied to the resting energy estimate through the use of injury factors (Table 5-19). If more than one factor is involved, one should use the highest multiplier.

TABLE 5-16 Total Feed Required per Day to Meet Maintenance Requirements of a 500-kg Adult Horse

INGREDIENT	AMOUNT/RECIPE	×	NO. RECIPES NEEDED/DAY	=	TOTAL INGREDIENTS NEEDED/DAY
Alfalfa/casein/dextrose slurry					
Alfalfa meal	454 g	×	5.9	=	2679 g alfalfa meal
Casein	204 g	×	5.9	=	1204 g casein
Dextrose	204 g	×	5.9	=	1204 g dextrose
Electrolyte mixture	52 g	×	5.9	=	307 g electrolyte mixture
Water	5 L	×	5.9	=	30 L water
Pellet/vegetable oil slurry					
Complete pelleted horse feed	454 g	×	10.7	=	4860 g complete pellet
Corn oil	46 g	×	10.7	=	492 g (535 ml) corn oil
Water	3 L	×	10.7	=	32 L water

Nitrogen balance studies have been used to determine the protein needs of hospitalized human patients.[158,159] Protein catabolism increases dramatically, with nitrogen losses in burned and septic patients increasing by nearly 250%. Without sufficient protein or caloric intake, protein is metabolized for energy instead of being used for protein synthesis and repletion. One therefore uses the calories-to-nitrogen (Cal/N) or nonprotein calories- to-nitrogen (NPCal/N) ratio to estimate the protein requirement. A Cal/N ratio between 130:1 and 180:1 or an NPCal/N ratio between 100:1 and 150:1 is considered optimal for most stressed human patients.[159] The lower ratios are recommended for septic or severely traumatized patients. Until more data are available, these ratios seem reasonable for sick horses as well. For practical purposes, one should provide 5 g protein for every 100 kcal.

TABLE 5-17 Quantity of Diets Required per Day to Meet Daily Energy Requirement*

DIET	ENERGY REQUIRED	ENERGY PER RECIPE OR LITER OF DIET	RECIPES OR LITERS NEEDED PER DAY
Alfalfa/casein/dextrose slurry	16.4 Mcal	2.77 Mcal/recipe	16.4/2.77 = 5.9 recipes
Pellet/vegetable oil slurry	16.4 Mcal	1.53 Mcal/recipe	16.4/1.53 = 10.7 recipes
Osmolite HN	16.4 Mcal	1.06 Mcal/L	16.4/1.06 = 15.5 L
EquiCal	16.4 Mcal	1.00 Mcal/L	16.4/1.00 = 16.4 L

* Daily energy requirement for a 500-kg adult horse is 16.4 Mcal of digestible energy.

Increases in vitamin and mineral needs above those needed for maintenance do not appear to be necessary unless drug-nutrient interactions affect micronutrient status.

5.6.2.4 Method of Delivery

Feeding solutions generally are delivered by gravity flow or by hand pump. Nasogastric intubation or cervical esophagostomy are the preferred routes.[160] As with liquid diets, the soft pouches and gravity feeding sets developed for human hospitals have been relatively easy to adapt for veterinary use.[156] 258

TABLE 5-18 Nutrient Profile of Enteral Diets Supplying Daily Maintenance Energy Requirements for a 500-kg Adult Horse

NUTRIENT	REQUIREMENTS*	ALFALFA/CASEIN/DEXTROSE SLURRY	PELLET/VEGETABLE OIL SLURRY	OSMOLITE HN†	EQUICAL‡
Digestible energy, Mcal	16.4	16.4	16.4	16.4	16.4
Protein, g	656	1710	682	688	742
Calcium, g	20	81	32	11.7	32.8
Phosphorus, g	14	41	20	11.7	22.4
Sodium, g	8.2	16.2	10	14.4	25.9
Potassium, g	25	159	—	24.2	29.3
Magnesium, g	7.5	97	10	4.7	10.4
Copper, mg	82	35	122	23.6	120.8
Zinc, mg	328	100	438	264	414
Iron, mg	328	984	389	211	414
Selenium, mg	0.82	0.9	1.5	—	1.2

* From National Academy of Sciences: *Nutrient requirements of horses,* ed 5, Washington, DC, 1989, National Academy of Sciences, National Research Council.

† Ross Products Division, Abbott Laboratories, Columbus, Ohio.

‡ Marketed as NutriPrime, KenVet, Ashland, Ohio.

Nasogastric tubes for enteral support of mature horses are generally 200 to 250 cm (80 to 100 inches) long. The internal diameter should not be less than 14F to obtain a reasonable flow rate. One should place tubes in the stomach or in the distal esophagus. Gastric reflux is more likely to occur when large-bore tubes are passed into the stomach.[158] One should secure the tube to the halter or, in the case of esophagostomy, tape it to the neck. A muzzle may be necessary to prevent the horse from dislodging the tube. One should remove the tube before the reintroduction of solid food because it may double back into the oral cavity where it can be chewed in half.

Large-bore, thick-walled rubber and polyvinyl chloride (Tygon) stomach tubes used for drenching and gastric decompression commonly are used. One drawback, however, is that they become stiff and brittle when exposed to digestive fluids. Local irritation and ulceration of the nasal septum, larynx, and pharynx are also a major concern when these tubes are left in place. The smaller bore (6 to 8 mm internal diameter) versions of these tubes have been used for short-term support but suffer from excess pliability, making them difficult to place and subject to kinking.

TABLE 5-19 Activity and Injury Formulae Suggested for Use in Horses

ACTIVITY		INJURY	
Stall rest	1.2 × REE*	Minor trauma or surgery	1.3 × REE
Walking, grazing	1.7 × REE	Skeletal trauma	1.3–1.4 × REE
		Sepsis, cancer	1.5–1.7 × REE
		Major burn	1.7–2.0 × REE

Modified from Long CL, Schaffel N, Geiger JW et al: Metabolic response to injury and illness: estimation of energy and protein needs from indirect calorimetry and nitrogen balance, *JPEN J Parenter Enteral Nutr* 3:452, 1979.

* REE (resting energy expenditure) = 21 kcal (BM_{kg}) + 975 kcal.

Polyurethane and silicone tubes dominate the human enteral tube market. These materials offer several advantages in that they are softer, less irritating, and do not harden. Most are also radiopaque. Silicone stomach tubes designed for horses are available (BIVONA, Inc., Gary, Indiana) but are rather large and thick-walled, making flow rate difficult to control. A polyurethane, 18F, 98-inch enteral feeding tube for adult horses is currently available (KenVet).

5.6.2.5

Rate of Delivery

Bolus delivery, or the rapid administration of formula by hand pump or syringe, is used most often. Although this method certainly saves time, it can result in diarrhea, abdominal pain, and distention, especially during the period of diet introduction.

Continuous and intermittent feeding become more popular as the use of enteral support increases. In intermittent feeding, a prescribed volume is infused by gravity drip over a longer period of time (60 to 120 minutes) at set intervals (every 2 or 3 hours) rather than continuously. Many of the complications that occur with bolus feeding in human beings have been reduced by slower and more controlled rates of delivery.

5.6.2.6

Guidelines for Feeding the Sick Horse

The following are guidelines for feeding sick horses:

1. Hydrate the horse adequately and correct any electrolyte or acid-base abnormalities before initiating enteral feeding. 259

260

2. Estimate the energy needs of the horse using the *current* body weight rather than the ideal body weight. The *initial* goal is to prevent further weight loss. Estimate protein needs.
3. Select the formula or slurry to be used and calculate the volume needed to meet the energy needs. Extra protein, if needed, can be added with protein modules (i.e., ProMod, Ross Laboratories, Columbus, Ohio; Casec, Mead Johnson Nutritionals, Evansville, Indiana).

4. Design the feeding schedule such that the maximum volume per feeding when the full-volume goal is reached is 3 to 4 qt. A minimum of four to six feedings usually is required. Tolerance is best when small feedings are delivered frequently.

5. Place the stomach tube or indwelling nasogastric tube. Verify the proper position of the tube before each feeding. Horses must remain standing or in sternal recumbency during feeding to prevent reflux and aspiration.

6. Some horses may need to be brought up to full-volume feeding slowly. For young horses, postoperative cases, or horses that have been anorectic for 5 days or more, feed 25% of the full-feeding volume on the first day. Increase to 50% on the second or third day and then to 75% if tolerance has been acceptable. Though energy needs have been estimated, the actual calories needed to maintain body weight vary from horse to horse. Some may maintain their weight when fed at 75% of the calculated full-feeding volume. If this occurs, no further increases in formula volume may be needed. Otherwise, increase to 100% as dictated by body weight and tolerance.

7. Flush the tube with warm water *before feeding* to verify placement and patency and *after feeding* to rinse out formula and prevent clogging. If the tube cannot be flushed, formula exits from the nostril, or the horse is in distress, do not feed.

8. Feeding volumes of 3 to 4 qt have been delivered by bolus safely over 10 to 15 minutes. Slower rates (feeding over 30 to 60 minutes) are recommended during the adaptation period to reduce the risk of intolerance.

9. Between feedings, muzzle horses to prevent removal of the tube but allow for free-choice water consumption.

10. Feces production and volume varies depending on the diet used. Loose feces are not uncommon and are of little concern if not accompanied by other signs of organic disease. The liquid enteral preparations are low-residue diets, resulting in small, soft fecal balls with mucus of low frequency and volume. If the horse becomes depressed, febrile, colicky, or dehydrated, stop feeding.

11. Continuous or intermittent gravity drip of liquid enteral formula is strongly recommended for horses that may have reduced absorptive capability (i.e., prolonged inappetence or humane rescues). In some of these horses, a combination of enteral and parenteral support may be most effective until gastrointestinal function is restored.

5.6.2.7 Clinical Monitoring

One should review laboratory work as needed to monitor clinical status. No definitive indexes are available to assess the quality of nutritional intervention in horses. Serum triglycerides, cholesterol, and bilirubin generally respond quickly to refeeding, falling back to within normal ranges in 24 to 48 hours. Because of the peripheral insulin resistance that occurs in hypermetabolic states, one should monitor blood glucose concentration closely in the early stages for the development of hyperglycemia. In this instance, one should decrease formula volume to reduce glucose intake.

5.6.2.8 Reintroduction of Solid Feed

Once the disease process has been brought under control, most horses begin to show interest in hay and grain. Energy formerly used for battling disease is rechanneled to support increased voluntary activity, resulting in little if any change in estimates for energy need. The initial goal, however, is merely to substitute the enteral formula for solid feeds before implementing any increases in energy intake. As recommended for any feeding program, one should add these proprietary feeds gradually to allow the digestive tract time to adjust to the “new” substrates. Using feeds with which the horse is familiar further encourages eating. One should select hay that is leafy and has a fresh aroma to encourage consumption. The type of hay is not critical, though alfalfa tends to be higher in leaf content and therefore higher in energy and protein than most grass hays or clover hay. One should begin by offering 3 to 5 lb/day for the first 3 to 4 days, reducing the amount of enteral formula by roughly 1 qt for every pound of hay consumed. One should add grain only when hay alone cannot meet energy needs. If lean body mass has decreased, repletion by increasing energy intake above the requirement with no increase in muscular activity results in the deposition of fat instead of muscle. Repletion of all nutrients is accomplished best gradually.

5.6.2.9 Drug-Nutrient Interactions

Drugs commonly used in veterinary medicine can have a profound effect on the nutritional status of the animal through suppression of appetite or by a direct pharmacologic effect. This is not to suggest that one 260
should question the use of drugs thay may affect nutritional status, but a more thorough understanding of their 261
interactions will assist the clinician in addressing the nutritional needs of the patient more accurately.

Several drugs have been shown to affect the senses of taste and smell in human beings. If this also occurs in horses, many horses may refuse to consume feeds that are normal constituents of their diet. Table 5-20 lists the drugs commonly used in equine medicine and the effects that these drugs have been reported to have in human patients.

When should one administer medications to horses that are tube fed? Because most pharmacologic data suggest that absorption occurs most rapidly in the fasted state, the recommendation that drugs be given 2 hours before or 2 hours after a feeding seems appropriate. Using liquid preparations of certain drugs when available rather than grinding or crushing tablets for administration prevents clogging of the tube, necessitating tube removal and replacement.

TABLE 5-20 Drug-Nutrient Interactions Reported in Human Beings

DRUG	GASTROINTESTINAL INTERACTIONS	METABOLIC INTERACTIONS	LABORATORY VALUES*
Amikacin sulfate		Anorexia, polydipsia, salivation	↑ BUN, creatinine
Amoxicillin	Diarrhea	Anemia; affects taste	↑ AST
Ampicillin			↑ AST, ALT
Cimetidine (Tagamet)	Diarrhea, gastric secretion		↑ Creatinine, AST, ALT, ALP
Erythromycin	Diarrhea, cramps	Jaundice	↑ ALP, bilirubin, AST, ALT
Betamethasone valerate-gentamicin sulfate (Gentocin)		Anorexia, polydipsia	↑ BUN, AST, ALT, LDH, bilirubin, creatinine
Metoclopramide (Reglan)	Diarrhea		↓ Mg, Na, K, Ca
Metronidazole (Flagyl)	Diarrhea, epigastric distress	Anorexia; alters taste ↓ Bacterial synthesis of vitamin K	
Moxalactam			↑ AST, ALT, BUN, creatinine, ALP
Penicillin G procaine, penicillin G benzathine	Diarrhea, gastritis	Alters taste; anorexia	
Pyrimethamine (Daraprim)		Interferes with folate metabolism; anorexia	
Ranitidine (Zantac)	Constipation, abdominal pain		
Rifampin	Epigastric distress, cramps, diarrhea	Anorexia; alters taste	↑ AST, ALT, GGT, creatinine
Sucralfate (Carafate)	↓ Absorption of vitamins A, D, E, and K; constipation	Anorexia	↑ AST, ALP, BUN, bilirubin, uric acid
Sulfamethoxazole-trimethoprim (Bactrim)	Diarrhea, ↓ absorption of folate and vitamin K		↑ Creatinine, BUN, AST, ALT, bilirubin
Tetracycline	↓ Absorption of Ca, Fe, Mg, Zn, and amino acids; diarrhea	Anorexia, ↓ vitamin K synthesis by bacteria	↑ ALP, BUN, bilirubin, AST, ALT

* *AST,* Aspartate aminotransferase; *ALT,* alanine aminotransferase; *ALP,* alkaline phosphatase; *BUN,* blood urea nitrogen; *LDH,* lactate *GGT,* γ-glutamyltransferase.

5.6.2.10

Complications of Enteral Feeding

Aspiration of formula into the lungs can be one of the most serious complications of enteral feeding. Verification of tube placement before and after each feeding, checking for gastric residuals before feeding, and

ensuring that the head and neck are raised during administration of the diet are advisable. If one obtains a gastric residual volume equal to or greater than 50% of the normal feeding volume, one should withhold the feeding and should evaluate the cause of the decrease in gastric emptying. Gastric emptying rates are reduced in sepsis, peritonitis, or following abdominal surgery.[161] Certain drugs also may reduce the gastric emptying rate.

Because the composition of most enteral formulae makes them ideal growth media for bacteria, care in preparation and handling is essential. Though not a major cause of clinical problems in the human hospital setting, secondary infections attributable to bacterial contamination of formula have been reported.[162,163] 261
Many bacteria have been isolated, including *Escherichia coli, Enterobacter, Klebsiella,* and *Salmonella.* 262
Sterile commercial preparations can be contaminated at mixing or from the hospital environment and many home brews may be contaminated inherently. To reduce this risk, one should prepare the formula in a reasonably clean environment and administer it as soon as possible after preparation. One should prepare only enough formula for one feeding if feeding a bolus; one should not mix a 12- to 24-hour quantity, even if it is to be stored refigerated. One should thoroughly wash and rinse all utensils. One should rinse enteral feeding bags and administration sets until the wash solution is clear and should disposed of them after 24 to 48 hours.

Gastrointestinal complications such as bloating, colic, and diarrhea can occur during the first few days of enteral feeding and can have a variety of causes: an adaptation period that is too short; bolus feeding; rapid administration; large, infrequent feedings; carbohydrate intolerance; or a nondietary cause such as antibiotic therapy. One generally can avoid these complications by taking a conservative approach to enteral feeding, developing a reasonable adaptation schedule to guarantee compliance, feeding small volumes slowly and frequently, and returning to a previously tolerated rate and volume until tolerance is reestablished. The commonly encountered, antibiotic-induced diarrhea usually resolves quickly once the drugs are discontinued. However, if the microfloral population has been reduced significantly, additional time may be needed for these microbes to become reestablished.

5.6.2.11 Parenteral Support

Parenteral nutrition is the administration of nutrients by intravenous infusion. If desired, one can supply all of the nutritional needs of the horse by the intravenous route. The goals of parenteral support are the same as those for enteral support: providing energy and protein to prevent catabolism of body protein and to promote repair. This method of nutritional support is of value when use of the gastrointestinal tract is contraindicated for more than 5 to 7 days. Lack of access to the gastrointestinal tract (e.g., pharyngeal obstruction), severe diarrhea, malnutrition, massive small bowel resection, intestinal obstruction or prolonged ileus, continual gastric reflux, and a high risk of aspiration are situations when parenteral support is appropriate.[164] As with human patients, one must make the decision to use parenteral nutrition carefully case by case because of the expense, time, and expertise required for mixing, administering, and monitoring and because of the potential risks involved.[165] However, knowledge of the potential complications and their causes, careful monitoring, and prompt treatment by attentive, dedicated staff have made parenteral nutrition a successful adjunct to veterinary care.

5.6.2.11.1 Admixtures

Admixtures used in parenteral nutrition generally are composed of sterile solutions of dextrose, amino acids, and electrolytes. One also can add fat in the form of a lipid emulsion if greater caloric density is needed. Like enteral solutions, these mixtures are ideal growth media for bacteria and must be used within

12 hours or less after mixing. To prevent contamination, one should mix solutions using aseptic techniques in a designated area, preferably with a laminar flow hood. If one must store solutions after final mixing, one should refrigerate them immediately and use them within 12 to 24 hours.

Dextrose (glucose) is the carbohydrate used in most, if not all, parenteral mixtures. To increase the caloric density, several concentrations of dextrose are available, but one must take care because of the hypertonicity of such solutions. Fifty percent dextrose solutions are used most often in equine parenteral support, with an osmolarity of 2525 mOsm/L. For most standard formulae, the dextrose concentration should not exceed 30% of the final mixture or 60% of the total volume infused.

Crystalline amino acid solutions are the main source of nitrogen in parenteral mixtures. They contain essential and nonessential amino acids at various concentrations, with solutions containing 8.5% amino acids used most commonly. Again, as with concentrated dextrose solutions, these products are high in osmolarity, approximately 800 mOsm/L. Because of the lability of the essential amino acid tryptophan, one should store these solutions at room temperature and protect them from light. Amino acid solutions are available with added electrolytes in the free-base rather than chloride form, reducing the risk of hyperchloremia. Final amino acid concentrations of most standard admixtures should not exceed 4.5%. One must mix amino acid solutions first with the dextrose solution before adding them to the lipid solution. The high pH of these solutions breaks the emulsion if added directly to the lipid solution.

Lipid emulsions often are used to increase the energy density and nonprotein nitrogen calories of a solution and supply the essential fatty acids linoleate and linolenate. Though high in caloric density at 9 kcal/g, these solutions have the added advantage of being isotonic. All are composed of soybean oil or a combination of soybean and safflower oil, with egg yolk phospholipids and glycerin acting as the emulsifying agents. One can piggyback lipid emulsions into the main line below the filter or add them into premixed dextrose–amino acid solutions to provide an all-in-one mixture. Lipid emulsions are the most expensive component of the admixture. Lipid emulsions should not constitute more than 50% of the total calories to prevent the suppression of the immune response reported in vitro and in vivo.[166–168] Table 5-21 lists products available for parenteral admixtures. 262

TABLE 5-21 Products for Parenteral Alimentation

A. DEXTROSE SOLUTION CONCENTRATE (g/dl)	OSMOLARITY (mOsm/L)	CALORIES (kcal/L)	MAINTENANCE REQUIREMENT (L)[a]
2.5	126	85	71
5.0	253	170	35
10.0	505	340	18
20.0	1010	680	9
50.0	2520	1700	4

B. LIPID EMULSIONS

PRODUCT	CONCENTRATION (g/dl)	OSMOLARITY (mOsm/L)	CALORIES (kcal/L)
Intralipid[b]	10 (20)	280 (330)	1100 (2000)
Liposyn[c]	10 (20)	276 (340)	1100 (2000)

C. CRYSTALLINE AMINO ACIDS

PRODUCT (WITHOUT ELECTROLYTES)	CONCENTRATION (g/dl)	TOTAL NITROGEN	OSMOLARITY (mOsm/L)
Aminosyn[d]	8.5 (10.0)	13.4 (15.7)	850 (1000)
Travasol[e]	8.5 (10.0)	14.3 (16.8)	860 (1060)

D. SUPPLEMENTS

MULTIVITAMIN CONCENTRATE (M.V.C. 9 + 3)[f]				TRACE ELEMENTS (M.T.E. 5 [1-mlVIAL])[g]	
Ascorbic acid	100.0 mg	Niacin	40 mg	Zinc	5.0 mg
Vitamin A	3300.0 IU	Pantothenic acid	15 mg	Copper	1.0 mg
Vitamin D	200.0 IU	Vitamin E	101 IU	Manganese	0.5 mg
Thiamine (B_1)	3.0 mg	Biotin	60 μg	Chromium	10.0 g
Riboflavin (B_2)	3.6 mg	Folic acid	400 μg	Selenium	20.0 g
Pyridoxine (B_6)	4.0 mg	Vitamin B_{12}	5 μg		

a Maintenance for 50-kg foal is 120 kcal/kg per day or 6000 kcal.

b Intralipid, KabiVitrum Inc., Alameda, Calif.

c Liposyn, Abbott Laboratories, North Chicago, Ill.

d Aminosyn, Abbott Laboratories, North Chicago, Ill.

e Travasol, Baxter Healthcare Corp., Deerfield, Ill.

f Multivitamin concentrate, Lypho Med Inc., Melrose Park, Ill.

g Trace element additive, Lypho Med Inc., Melrose Park, Ill.

5.6.2.11.2

Guidelines for Delivery

As with enteral feeding, one must correct all fluid, electrolyte, and acid-base abnormalities before initiating any feeding. Energy goals for parenteral support are similar to those for enteral support. Table 5-22 lists some admixtures that have been used in partial and total parenteral support.

A central venous line (i.e., jugular) is preferred to using a peripheral vein because of the large volume of fluid required and the risk of thrombogenesis when hypertonic solutions are infused into small vessels. For mature horses, 16-gauge Silastic or polyurethane catheters 5 to 7 inches long have been recommended.[164,169] One should place catheters according to the guidelines of the manufacturer using strict aseptic techniques.[169] Once one establishes a line, dedicating this line for parenteral feeding only is best to reduce the risk of contamination. Dedication may not always be possible or practical. If a single line is necessary, aseptic technique is essential.[170] One should not use stopcocks so as to maintain a closed system. At no time should one draw blood through this line.

Gradual introduction of parenteral feeding solutions reduces the risk of complications. For adult horses, one should compute the desired goal, including injury factors, and prepare a mixture to infuse over the first 24 hours that will provide roughly half of the caloric needs of the patient. One can formulate most starter solutions using dextrose and amino acids. One can add lipids as an energy source when one cannot resolve hyperglycemia by decreases in rate alone. One adjusts the composition and rate of flow according to metabolic tolerance and nutrient need.

5.6.2.11.3

Complications of Parenteral Feeding

One may pattern monitoring procedures for the adult equine after those suggested for foals (Table 5-23). When lipid emulsions are included in the parenteral solution, one should check blood samples for evidence of lipemia. Serum triglycerides should be less than 150 mg/dl. If lipemia occurs, decreasing the rate of administration or administering heparin or insulin should improve clearance.

Hyperglycemia, glucosuria, and rebound hypoglycemia can occur. The insulin resistance and reduced 263
glucose use during sepsis, shock, and major trauma lead to the hyperglycemic state. Careful monitoring of 264
blood and urinary glucose concentrations and gradual weaning onto solutions over 24 to 36 hours can reduce the likelihood of occurrence. One should monitor serum and urine glucose concentrations at least 2 to 3 times per day and more often during introduction. When glucose concentrations greater than 200 mg/dl are present, one should decrease the flow rate until tolerance is achieved. Substituting some of the carbohydrate calories for fat calories also reduces the glucose load.

TABLE 5-22 Suggested Admixtures for Parenteral Nutrition of Horses

	MLS											
		AMINO ACIDS		LIPID			GRAMS/kg BODY MASS					
	DEXTROSE (50%)	(8.5%)	(10%)	(10%)	(20%)	KILOCALORIES (PER kg BODY MASS)	CHO*	PROTEIN	LIPID	NPNCal/N RATIO	CALORIES	
PARTIAL SUPPORT/STARTER REGIMEN												
Foals (45–50 kg)												
Protocol A												
Step 1	1000	—	1000	500	—	53	10	2	1	140:1	68	18
Step 2	1200	—	1500	1000	—	75	12	3	2	130:1	59	26
Protocol B	1500	1000	—	—	—	58	17	2	—	178:1	78	—
Horses (450 kg)	4500	3500	—	—	—	20	5	0.7	—	153:1	86	—
TOTAL PARENTERAL SUPPORT												
Foals (45–50 kg)												
Protocol A	1500	—	1500	1500	—	94	15	3	3	175:1	53	13
Protocol B	1000	1500	—	—	1000	90	11	3	4	172:1	42	45
Horses (450 kg)												
Protocol A	5000	4000	—	6000	—	34	5	0.75	1.3	255:1	35	56
Protocol B	4500	7000	—		3500	36	5	1.3	1.5	146:1	46	38

Data from Hansen TO: Nutritional support: parenteral feeding. In Koterba AM, Drummond WH, Kosch PC, editors: *Equine clinical neonatology,* Philadelphia, 1990, Lea & Febiger; Spurlock SL: *A practical approach to parenteral nutrition in the equine patient,* Deerfield, Ill, Baxter Healthcare, 1990; and Hansen TO, White NA, Kemp DT: Total parenteral nutrition in four healthy adult horses, *Am J Vet Res* 49:122, 1988.

1 g dextrose = 3.4 kcal; 1000 ml dextrose = 1700 kcal (500 g) 1 g amino acids = 4 kcal; 1000 ml 8.5% solution = 340 kcal (85 g) 1 g lipid emulsion = 11 kcal; 1000 ml 10% solution = 1100 kcal (100 g)

* *CHO,* Carbohydrates.

Rebound hypoglycemia occurs with a sudden cessation of glucose delivery without provision of an alternative source of enteral carbohydrate calories. One can prevent rebound hypoglycemia by slowly decreasing the rate of infusion by half for 12 hours while gradually increasing enteral support. One should take further decreases in accordance with patient response.

Azotemia and hyperammonemia are complications of protein metabolism that have been reported. Providing insufficient calories from nonprotein sources results in the amino acids being used for energy instead of protein synthesis. Assessing serum ammonia and blood urea nitrogen concentrations during total parenteral nutrition administration is recommended. The most common electrolyte abnormalities are

hypokalemia and hypomagnesemia.[164,171] Increased tissue synthesis increases the requirement for potassium and magnesium. Moreover, potassium also may be lost during glucose-induced osmotic diuresis.[172] One should follow supplementation with laboratory assessment to monitor status.

One of the common and serious mechanical complications that can develop in parenteral delivery is venous thrombosis.[169,173] One can reduce the risk of venous thrombosis by selecting catheters made of Silastic elastomers or the newer polyurethanes. Studies in human and veterinary medicine have documented the low thrombogenicity of these materials compared with polyethylene, polyvinyl chloride, or Teflon.[164,169,174]

5.6.2.11.4 Transition to Enteral Feeding

The transition to liquid or solid diets from parenteral delivery should gradual over 3 to 5 days to allow for
the stimulation of gut activity and digestive enzyme synthesis. Maintaining both systems of feeding for a 264
short period also guards against short-term nutrient deprivation should inappetence return. A survey of 265
transitional feeding practices in the human hospital setting indicated that parenteral nutrition most often was terminated when 50% to 75% of the calories were met orally.[175] This would appear reasonable for horses. The speed of progress is based largely on gastrointestinal tolerance. No transition protocols are established for horses. One may take a conservative approach by feeding 25% of caloric needs by the oral route in small, frequent meals and reducing the volume infused isocalorically at the expense of lipid. One can advance the transition daily in 25% increments as tolerance permits. Discontinuation of parenteral feeding when the horse has received 75% of its caloric needs enterally for at least 36 to 48 hours with no complications is probably reasonable.

TABLE 5-23 Monitoring Procedures for Parenteral Alimentation

OBSERVATION[*]	INITIAL MONITORING FREQUENCY	MONITORING AFTER STABILIZATION
Vital signs	q4h	q8h
Catheter, vein inspection	q8h	q8h
Intake-output	q8h	q8h
Weight	Daily	Daily
Urine glucose	q6–8h	q8–12h
Serum glucose	q6–12h	q24h
Serum electrolytes	q24h	1–2/week
Creatinine/BUN	q24h	2/week
Triglycerides, cholesterol	Baseline	Weekly
Liver enzymes (GGT, LDH, SAP)	Baseline	Weekly
Liver function tests (bilirubin)	Baseline	Weekly
Packed cell volume, total protein	q12–24h	q2–3d
White blood cell differential	Baseline	Weekly
Fibrinogen	Baseline	Weekly

From Vaala WE: Nutritional management of the critically ill neonate. In Robinson NE, editor: *Current therapy in equine medicine,* vol 3, Philadelphia, 1992, WB Saunders.

* *BUN,* Blood urea nitrogen; *GGT,* γ-glutamyltransferase; *LDH,* lactic dehydrogenase; *SAP,* serum alkaline phosphatase.

5.7 Nutritional Management of the Premature or Compromised Foal

Approximately half of the length and two thirds of fetal weight gain occur during the last 3 to 4 months of gestation.[28] The high metabolic rate and nutrient needs of the foal present many nutritional challenges. A premature or dysmature foal has been denied the benefit of intrauterine growth and nutrient transfer and therefore may not have the nutrient reserves of a full-term foal. In many cases, providing nutritional support can mean the difference between a positive and negative outcome.

5.7.1 NUTRIENT REQUIREMENTS

The nutritional requirements of the sick foal have not been defined, and lack of relevant data in the human pediatric literature provides little guidance. The issue of whether sick foals should or can be fed to support growth has not been resolved, though most would agree obtaining growth is a preferred goal. For healthy, preterm infants, growth rates similar to those of full-term infants during the last 30 days in utero are desired. In foals, growth rates comparable to those of equine fetuses during the last 30 days of gestation (0.5 to 0.7 kg/day) occur at 90 to 100 kcal DE per kilogram in healthy foals[28,176] and may be a reasonable starting point for

premature and sick foals. Additional increases may be needed for metabolic alterations induced by disease or trauma, but whether they are analogous to the injury factors applied to adults is not known. Caloric intakes between 120 and 160 kcal DE per kilogram have been reported to provide acceptable responses with minimal complications.[177]

Though only limited data were available, weight gains of foals receiving parenteral nutrition were found to be correlated negatively with the NPCal/N ratio, reemphasizing the need for a balance between protein and energy to achieve proper utilization of nutrients.[175] Nursing foals consume 6 to 7 g protein per kilogram of body mass during the first 2 weeks of life.[42] The NPCal/N ratio in mare's milk is approximately 105:1, slightly below that of several milk substitutes and much lower than that of most parenteral admixtures suggested for foals.[164,171] The current recommendations for the foal are higher than those for the human infant, especially considering their preciosity and rapid growth. Ratios less than 176:1,[164,176] and perhaps closer to the ratio of mare's milk, may be of greater benefit, but more studies are needed.

Between birth and 4 months of age, estimates of calcium and phosphorus accretion in foals appear to be 10 g/kg and 5 g/kg of gain, respectively.[2,50] If these values are taken as minimal and allowing for lower dietary availabilities from inorganic sources and reported endogenous losses, calcium and phosphorus requirements for neonates are at least 20 to 25 g/day and 10 to 16 g/day, respectively.[2] For foals gaining as little as 0.8 kg/day, extrapolation of these data suggests at least 18 g of calcium and 9 g of phosphorus are needed for neonates.

Again, one must estimate vitamin and trace mineral needs from the paucity of data in healthy foals and the nutrient content of mare's milk. Assuming that the foal would consume approximately 14 qt of milk per day 265
during the first week, the vitamin and trace mineral intake in Table 5-24 seems to be a reasonable starting place. 266

TABLE 5-24 Estimated Daily Trace Mineral and Vitamin Intake From Mare's Milk During First Week of Life

NUTRIENT		AMOUNT
Trace minerals	Iron	10.0 mg
	Copper	7.0 mg
	Manganese	17.0 mg
	Zinc	40.0 mg
	Selenium	0.09 mg
Vitamins	Thiamine	4.2 mg
	Riboflavin	4.9 mg
	Pyridoxine	5.4 mg
	Niacin	12.3 mg
	Pantothenate	117.5 mg
	Folic acid	1082.0 μg
	Biotin	116.0 μg
	Choline	964.0 mg
	B_{12}	40.9 μg
	Myo-inositol	984.0 μmg

5.7.2

ENTERAL SUPPORT

Generally accepted practice is to attempt enteral nutrition whenever possible. Lactase activity is present in the fetal small intestine during the last trimester, reaching its peak at 2 days post partum,[69] suggesting that a premature foal may have digestive capability. Intermittent enteral feeding most closely mimics the natural state, stimulating gastrointestinal enzyme and hormone activity and increasing small intestinal villous growth. Fewer complications are associated with enteral feeding (though some do exist), less monitoring is required, and enteral feeding is less expensive than providing nutrients parenterally.

5.7.2.1

Formulae

One should use mare's milk whenever possible. Because the goal is ultimately to return the foal to the mare, hand milking the mare provides food for the foal and helps to maintain lactation. Mare's milk has no discernible odor and has the appearance of 2% low-fat cow's milk. One should not use any milk that appears abnormal. One should strain the milk through a gauze sponge to remove hair, dirt, and other debris before storing and should keep it refrigerated.

Unfortunately, not all mares tolerate hand milking, and some stop lactating despite continual and frequent evacuation of the udder. Supplementation with other substitutes is usually necessary. Because of increased

endogenous losses, incomplete absorption, and the lower digestibility of nutrient sources compared with what nature provides, pediatric researchers have found that the concentration of most nutrients in infant formulae must be higher, especially those for the preterm infant.[178] This situation may well be applicable to compromised foals. The milk substitutes designed for the healthy orphan traditionally have been used for sick foals, but their efficacy has been highly variable, and many are too nutrient-dilute for sick foals when smaller feeding volumes are indicated.[179] A new nutrient-dense, equine neonatal formula is now available for such foals (NutriFoal, KenVet). This formula is similar in caloric distribution to goat's milk but has added protein to meet the needs of the young foal. (For the nutrient content, see Table 5-13). The casein-to-whey ratio and addition of the amino acid arginine results in an amino acid pattern similar to that of mare's milk protein. Minerals, vitamins, carnitine, and taurine have been added at levels to maintain nutrient balance.

5.7.2.2 Method of Delivery

For bottle feeding, most foals prefer the soft lamb nipples over the large, wide-bore calf nipples. When nasogastric delivery is needed, polypropylene enteral nutrition bags or flexible 1-L containers are ideal for the smaller volumes needed for foals. An attached in-line drip chamber and clamp permits control of the formula flow.

Stallion catheters (¼-inch outer diameter) and foal stomach tubes(⅜-inch outer diameter) of polyvinyl chloride or silicone have been used traditionally. Small-diameter (12F) polyurethane enteral feeding tubes adapt well for use in foals (Flexiflo Nasoenteric Feeding Tube #475, Ross Products Division, Abbott Laboratories, Columbus, Ohio). A stylet provides enough rigidity to prevent kinking during placement. A U tube placed at the nares helps to keep the tube in place. Foals have been fed for as long as 14 days with no signs of discomfort, irritation, or ulceration.[180] One should cap the Y connector with side port when not in use. One secures the tube by tape or by butterfly suture to the muzzle of the foal.

5.7.2.3 Rate of Delivery

For most foals in intensive care, intermittent or continuous drip results in the fewest complications. Foals that become colicky or bloated following even the smallest bolus feedings usually can tolerate slower, continuous infusions well. As the physical condition and gastrointestinal tolerance of the foal improve, one may attempt larger bolus feedings. Advancement to bolus feedings should be gradual by keeping the same per-feeding volume and slowly decreasing the time over which it is given. For example, if one feeds a foal intermittently
by infusing 1350 ml over 3 hours every 4 hours, one should advance the feeding to 1350 ml over 2 hours 266
every 4 hours. If the foal maintains tolerance for 24 to 48 hours, one should advance until attaining the desired 267
bolus schedule.

5.7.2.4 Guidelines for Enteral Feeding of the Compromised Foal

The following are guidelines for enteral feeding of compromised foals:

1. Correct the hydration, electrolyte, and acid-base status of the foal before initiating feeding.
2. Obtain the weight of the foal and estimate its energy needs. Start at 100 kcal/kg, with a goal of 120 to 150 kcal/kg.
3. Estimate the protein needs. Maintain an appropriate NPCal/N or Cal/N ratio.

4. Select the formula to be used and calculate the volume needed.
5. Place the indwelling nasogastric tube using the approved clinic procedure and following the guidelines of the manufacturer. If the tube becomes dislodged, do not attempt to reinsert the stylet into the dwelling tube. Remove the tube, reinsert the stylet, and replace the tube. As with adults, one must verify the proper placement of the tube, preferably by radiography or endoscopy.
6. Foals should be standing (if able) or in sternal recumbency during feeding. This helps to prevent gastric reflux and reduces the risk of aspiration pneumonia, a common and frequently fatal complication of sick foals.
7. The sicker the foal, the more intolerant it generally will be of feedings. In some cases, intolerance caused by early aggressive feeding is difficult to reverse. The conservative approach is best. Begin with half the desired volume and increase gradually after at least 24 hours, using tolerance as a guide. Intermittent or continuous feeding is recommended over bolus delivery. Start at 100 ml/hr and increase to 300 ml/hr as quickly as tolerance allows.
8. Check for gastric residuals before feeding. Gastric emptying is reduced in sepsis. If a volume greater than or equal to the designated feeding volume is present, do not feed and investigate possible causes.
9. Flush the tube before feeding to verify patency and placement. If resistance to flushing occurs, formula exits from the nostril(s) of the foal, or the foal is in distress, do not feed. Flush the tube after feeding to rinse out formula and prevent clogging.
10. After rinsing the tube, cap the feeding port to prevent air from entering the tube and formula from siphoning out.
11. Colic, constipation, and diarrhea are common problems that are not always diet related. Though one should discontinue feeding until the cause has been identified, prolonged gut rest (greater than 24 to 36 hours) may further compromise the foal. One should reverse parenteral support for those foals for which all attempts to feed enterally have been unsuccessful.
12. If the goal is to put the foal back on the mare, encourage suckling from the mare or a bottle to develop the suckle reflex as soon as the foal is able. If the foal is to be reared by hand, teach the foal to drink from a shallow container and raise the foal as suggested for an orphan.
13. Caution owners not to overfeed the foal in an effort to catch up with others of similar age. Premature and sick foals need time to continue recovery, even after release. Overfeeding will not be rewarding. One should feed foals according to their current age and weight rather than according to the weight of age-matched normal foals.

5.7.3 PARENTERAL SUPPORT

Table 5-22 summarizes suggested parenteral solutions for use in foals.[171,181] Initial caloric goals vary from 55 to 100 kcal/kg and are dictated primarily by the clinical status of the foal; for example, the sicker the foal, the lower the initial goal probably should be to minimize complications. Protein goals in current protocols range from 2 to 3 g/kg body mass. Fat intake from mare's milk early in lactation ranges from 5 to 6 g/kg body mass, slightly higher than the 2 to 4 mg/kg of most parenteral solutions. Other supplements added to the solution include

vitamins, trace minerals, and potassium chloride for foals not receiving enteral feedings. In human beings, potassium and protein coexist in body tissues in a constant ratio. Catabolic patients can lose as much as 3 mEq potassium in the urine for each gram of protein that is lost. Without adequate potassium, protein synthesis is depressed.[182] Osmotic diuresis that occurs during hyperglycemia and diuretic-induced diuresis also can increase potassium lost through urine.[162,183] To counter these losses, 40 to 60 mEq potassium has been added per liter of parenteral solution for foals. Calcium and phosphorus concentrations of admixtures fed to foals is not adequate to meet the needs of growing foals. One safely can add calcium gluconate (25 to 50 mEq/L) and potassium phosphate (5 to 15 mEq/L) to provide part of the needs until enteral feeding is possible.[183] Additional calcium supplementation to the admixture runs the risk of mineral precipitation.

One should take care to begin infusions slowly and increase it gradually. One may not achieve the goal infusion rate for 12 to 24 hours, depending on the foal. One can monitor patient tolerance to the parenteral mixture by using the schedule in Table 5-23.[184] One should wean foals from parenteral support along with the introduction 267
of enteral feeding. Rebound hypoglycemia should not be a problem as long as sufficient calories are provided 268
enterally. Parenteral support is best withdrawn over 2 to 3 days and followed with 1 to 2 L of 5% dextrose to aid in reestablishing glucose control mechanisms.[183]

One should base advances in volume or the inclusion of lipid emulsions on metabolic tolerance. As in human infants, lipid emulsions are not recommended for foals with hyperbilirubinemia, for this may further compromise bilirubin transport.

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6 CHAPTER 6 CRITICAL CARE

Joanne Hardy*

Equine intensive care units (ICUs) currently are integrating the following three critical components into many primary and referral hospitals: (1) the sickest patients; (2) highly technical and expensive equipment; and (3) staff with knowledge and experience to treat these patients. The goals of these units are to improve efficiency, improve efficacy, reduce cost, and ultimately improve patient outcomes.

One must base the decision to offer ICU services on the population needs, the consideration that emergency services should be offered also, and the economic environment of the hospital. Currently, few studies exist that describe the general case population, commonly performed procedures and treatments, and overall cost-benefit ratios of equine ICUs. One study described the overall case distribution of emergencies based on monthly admissions, the proportion of cases requiring surgery, and the most commonly performed treatments.[1] Because equine emergencies often are handled in ICUs, such information provides an initial database for understanding population dynamics. In that study, 20% of hospital admissions were received as after-hour emergencies. Approximately 60% of emergencies were for colic, of which approximately half required abdominal surgery. Other emergencies were distributed among the following categories: neonates (8%), orthopedics (7%), lacerations (5%), enteritis/colitis (4%), neurologic (4%), reproductive (4%), respiratory (4%), and others. In the miscellaneous category, the authors cited esophageal obstruction, ocular emergencies, rectal tears, hemorrhage, and undefined sickness. Although this case distribution probably reflects accurately the overall equine emergency caseload, reviewing the anticipated distribution of cases within a given area and the monthly distribution of cases is important. Such reviews ensure appropriate allocation of staff and equipment, tailored to the needs of the population. For example, in the previously quoted study the peak colic case admission was in August. In contrast, the peak neonatal case admission was in April and May. In general, in the northern hemisphere, peak foal season extends from February through June, whereas in the southern hemisphere, peak foal season is from September to January. Such distribution requires different staffing needs. During foal season, the ICU may need technicians trained to use mechanical ventilators, blood gas analyzers, glucometers, capnographs, or other specialty equipment. Addition of temporary unskilled staff (foal sitters) to help with managing of neonates is useful. Regular review of equipment and training on new equipment for staff, technicians, and clinicians is essential for smooth running of an equine ICU.

Commonly and anticipated procedures performed in ICUs include monitoring, fluid administration, and pain control. Monitoring includes not only manual techniques but also use of equipment. Fluid administration encompasses routine administration not only of crystalloids but also of colloids, blood, and blood substitutes. Providing appropriate analgesia can vary widely depending on the origin of pain (musculoskeletal versus abdominal) and the possible side effects of different medications. All of these considerations need to be based on the specific needs of the equine patient.

6.1 Organization of an Equine Intensive Care Unit

The design of the ICU should accommodate the care of horses with abdominal disorders, care of neonates, and care of horses with infectious diseases that require isolation. In addition, the design should include one or two stalls for easy unloading of down horses with the structure able to withstand hoisting. A central office facilitates oversight of the unit, organization, and issuing of directives. Sufficient storage space should be available to protect equipment not in use. The isolation unit must be separate from the ICU and have strict protocol. Ideally, the adult ICU should be separate from the foaling and neonatal ICU stalls. All stalls should have the equipment necessary for hanging

fluids. Large foaling stalls should be available. These stalls can have mobile separators for foals receiving intensive care. Locations with a large neonatal caseload should include a separate room to manage foals separately from their dams, which enables provision for increased ambient temperature and increased cleanliness and facilitation of monitoring and management.

Strict disinfection and isolation protocols should be in place to prevent the spread of infectious disease and to avoid nosocomial infections. One should encourage hand washing and use of foot baths between patients, particularly when dealing with infectious diseases, even when these are not contagious. Hand rubbing with an alcohol-based solution is more effective than hand washing with an antiseptic, probably because it does not require rinsing and drying of hands. Adoption of this practice can reduce cross-contamination between patients significantly,[2] which is also important when dealing with exudative wounds in which resistant organisms can grow. Growth of an oxacillin-resistant staphylococcus species should alert one to the presence of a methicillin-resistant organism. Guidelines for the judicious use of antibiotic regimens are available for human ICUs, and some of these guidelines are applicable to equine ICUs. Guidelines include the initial empirical selection of effective antibiotic regimens based on history, disease process, possibility of nosocomial infection, and knowledge of specific isolates for the hospital. One should base appropriate dosing and administration protocols on selection of time-dependent or concentration-dependent antibiotics, immune status of the patient, and cost. Once one obtains culture results, one should deescalate or modify therapy. Other methods that have been proposed to minimize antibiotic resistance and superinfections include cycling of antibiotics and restricted use of potent, broad-spectrum therapies.[3]

Salmonella organisms also can be a cause of nosocomial infection in the equine hospital.[4] Salmonella shedding is greater in horses with colic, and the use of common equipment such as stomach tubes and pumps may promote transmission.[5–7] Careful attention to hospital design and disinfection practices can help minimize the risk of hospital outbreaks.[8,9]

A separate food preparation bay should be available for preparation of enteral feeding. Staff members should pay attention to cleanliness in this area and particularly should avoid washing of hands or contaminated material in the same sink used for food preparation.

Technicians and staff available in the unit should be trained in techniques performed in the ICU and in early recognition of problems relating to equipment malfunction and invasive and noninvasive monitoring techniques. Technicians also need to be trained to indentify changes in patient status. When complex cases require continuous monitoring, a veterinarian may need to be present continuously.

6.1.1 EQUIPMENT

One should tailor the equipment used in an equine ICU to the anticipated case population and the maximal level of care anticipated for that population. Purchasing a ventilator would be a poor economic decision if mechanical 274

275 ventilation rarely is performed. Alternatively, one should investigate the possibility of renting medical equipment from suppliers in the area for such intermittent needs. Box 6-1 lists some equipment to consider for the anticipated needs of a new equine ICU.

6.1.1.1 BOX 6-1 EQUIPMENT LIST FOR DIFFERENT LEVELS OF EQUINE INTENSIVE CARE UNITS

6.1.1.1.1 Basic

Fluid administration system

Electrocardiogram

Centrifuge

Refractometer

Glucose strips

Urinalysis strips

Ultrasound

Oxygen tank and regulator

6.1.1.1.2 Intermediate

Blood pressure monitor

Cytologic exam/Gram stain

Glucometer

Intravenous pump delivery system

Sling and hoist for down horses

6.1.1.1.3 Advanced

Blood gas/electrolyte analyzer

Pulse oximeter

Mechanical ventilator

Colloid osmometer

Capnograph

Syringe infusion pumps

The most commonly used procedure in equine ICU is catheter placement, followed by intravenous fluid replacement. The section on fluid therapy covers catheter placement. For adult horses, one usually hangs intravenous fluids in several 3- to 5-L bags and administers them by gravity flow. A pulley system facilitates

hanging of fluids bags in the volumes needed for administration in horses.* Commercially available coil sets enable intravenous fluid administration without restraint. The drip chamber should be large enough to be visible from outside the stall. Having a drip chamber that allows administration of other medication (piggy-backing) is convenient. Smaller horses, ponies, miniature horses, and foals benefit from a more precise means of monitoring the volume of fluid administered. One can provide such monitoring by using volumetric fluid chambers or fluid pumps; however, commercially available pumps can deliver only up to 999 ml/hr, which may be insufficient for standard-size horses. For rapid administration of large volumes of fluids—for example, to treat shock—peristaltic pumps are available that allow delivery of 20 to 40 L of fluid in the first hour. Because of the pulsatile nature of the fluid flow, this method of fluid administration causes more damage to the venous endothelium. For administration of constant rate infusions of small quantities of drugs, syringe pumps are also available.

Standard monitoring equipment for use in ICU includes an electrocardiogram and blood pressure monitor, an ultrasound unit, a centrifuge for hematocrit determination, a refractometer for determining total protein and urine specific gravity, and urine test strips for urinalysis. Other equipment to consider includes glucometer, urine centrifuge, microscope with 100× magnification, equipment for cytologic examination and Gram stains, and a blood gas and electrolyte monitoring unit. A colloid osmometer is useful for determining colloid oncotic pressure in sick animals. If the ICU plans to provide mechanical ventilation, useful monitoring units include capnographs and pulse oximeters. Monitoring equipment should have quality control measures that must be performed regularly to ensure accuracy. Staff members should follow appropriate sampling procedures for each piece of equipment.

Oxygen ports for supplementation through nasal insufflation or for mechanical ventilation should be available. When installing a new ICU, one should consider ports for oxygen, vacuum, and air. A remote gas source and a pipeline system allow delivery of oxygen. The ICU can use compressed gas cylinders, but these must be stored and handled appropriately to avoid injury. For each cylinder type, knowledge of the capacity of the cylinder and the flow rate enables calculation of the amount of time provided. The small more portable E cylinders, containing 655 L of oxygen when full, provide oxygen for 260 minutes when set at a flow rate of 5 L/min. Adult horses require flow rates of 10 to 15 L/min to increase the FIo_2 to approximately 40%. Larger G or H cylinders containing 5290 or 6910 L respectively, allow oxygen supplementation for longer periods. Tight-fitting masks have been used satisfactorily in foals to manage hypoventilation, but in adults, nasal insufflation using an oxygen cannula is tolerated better. In adults with severe respiratory distress that require a greater FIo_2, the clinician can perform a tracheostomy using a cuffed silicone cannula* for administration of oxygen. Oxygen should be humidified, and the FIo_2 should not exceed 50% for prolonged periods.

An emergency (crash) cart should be available in the ICU. Table 6-1 lists emergency drugs and dosages used in adult horses. One can place the list in the crash cart for easy reference. In addition, one should place emergency tracheostomy packs at key areas, such as near the stall of patients with possible respiratory distress, near recovery stalls, and in the patient receiving area.

Ultrasonography also has become an essential component of ICUs. Ultrasound can be used for identifying and monitoring effusions, intestinal distention and motility, identifying umbilical structures, imaging of joint effusions, diagnosing the presence and location of urine in the abdomen, monitoring pregnancy, and imaging of ocular structures, among other things. To enable imaging of different structures, 3.5- and 7.5-MHz transducers and rectal probes are the minimal needed for these purposes.

* The authors acknowledge and appreciate the original contribution of Debra K. Rooney, whose work has been incorporated into this chapter.

* International Win, Ltd, Kennett Square, Pennsylvania.

* Bivona Medical Technologies, Gary, Indiana.

6.1.2 MONITORING

Patients in ICU require frequent monitoring. One should record all monitored parameters and preferably should display these on or near the patient's door for rapid review. More complete or extensive laboratory results can be stored in the patient's chart.

The remainder of this chapter describes aspects of fluid therapy in the adult and some important procedures performed in the ICU.

6.2 Fluid Therapy

Fluid administration for maintenance or replacement is one of the mainstays of equine critical care and should be 275
readily and easily available in any equine hospital. The availability of commercial materials and fluids for use in 276
large animals makes fluid administration easy and cost-effective in most situations. This section reviews materials available and principles to follow when planning fluid administration.

TABLE 6-1 Emergency Drug Chart for Adult Horses

DRUG	DOSE	DOSE PER 1000 lb	ROUTE*	COMMENT
Dobutamine (positive inotrope)	2–10 µg/kg/min (1 vial [250 mg] in 1000 ml = 250 µg/ml)	900–4500 µg/min	IV	Use diluted solutions within 24 hours. Is compatible with most IV fluids. Do not mix with alkaline solutions or calcium chloride/gluconate.
Doxapram (respiratory stimulant)	0.2 mg/kg	4.5 ml	IV or topical under tongue	Do not mix with alkaline drugs/fluids.
Epinephrine (for anaphylaxis or asystole)	0.01–0.02 mg/kg 0.1–0.5 mg/kg	4.5–9 ml	IV/IM/SQ Intratracheal	Do not give with bicarbonate, hypertonic saline, or aminophylline. Does not need to be diluted when given IV to adults.
Glycopyrrolate (for bronchodilation and bradycardia)	0.005–0.01 mg/kg	10–20 ml	IV/IM/SQ	Do not mix with alkaline drugs/fluids.
Lidocaine (treatment of ileus)	1.3 mg/kg loading slowly over 5 minutes 0.05 mg/kg/min infusion	Loading: 30 ml Infusion: 67 ml/hr	IV	Make sure product does not contain epinephrine.
Lidocaine (treatment of arrhythmias)	Bolus: 0.25–0.5 mg/kg (slowly) Infusion: 20–50 µg/kg/min	Bolus: 5–10 ml Infusion: 30–60 ml/hr	IV	Make sure product does not contain epinephrine.

* IV, Intravenous(ly); IM, intramuscularly; SQ, subcutaneously.

6.2.1 MATERIALS FOR FLUID ADMINISTRATION

6.2.1.1 Intravenous Catheters

Intravenous catheters are available in varying materials, construction, length, and diameter. In choosing a catheter, one should consider desired fluid rate, fluid viscosity, the length of time the catheter will remain in the vein, the severity of the systemic illness, and the size of the animal (Table 6-2). The rate of fluid flow is proportional to the diameter of the catheter and inversely proportional to the length of the catheter and the

viscosity of the fluid. Standard adult horse catheter sizes are usually 14 gauge in diameter and 5.25 inches in length. For more rapid administration rates (such as for shock), one should use a 12- or 10-gauge catheter. Plasma and blood products, because of their increased viscosity, also flow slower, so if the horse requires volume replacement, one can combine administration of these fluids with a balanced electrolyte solution. One should change Teflon catheters every 3 days, whereas polyurethane catheters can remain in the vein for up to 2 weeks. Horses that are very ill (bacteremic, septicemic, endotoxic) are more likely to encounter catheter problems and benefit from polyurethane or silicone catheters.

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One also must consider the catheter construction (Table 6-3). Through-the-needle catheters are most common for standard-size adult horses. An over-the-wire catheter is best used in foals and miniature horses or when catheterizing the lateral thoracic vein. Short and long extension sets are available, as well as small and large bore diameters. Using an extension that screws into the hub of the catheter is best, to avoid dislodgement. In horses with low central venous pressures, disconnection of the line can result in significant aspiration of air and cardiovascular collapse. Double extensions are also available for when the horse requires administration of other medication with the fluids.

TABLE 6-2 List of Available Catheter Materials

MATERIAL	EXAMPLE	COMMENT
Polypropylene	PE tubing, Medicut	Highly thrombogenic; not recommended
Teflon	Angiocath	Less thrombogenic
Polyurethane	Mila	Much less thrombogenic
Silastic	Centrasil	Least thrombogenic

TABLE 6-3 List of Commercially Available Catheter Constructions

TYPE	DESCRIPTION	ADVANTAGE	DISADVANTAGE
Butterfly	Needle attached to tubing	Ease of use	Laceration of vessel; vessel puncture and extravascular administration
Over-the-needle	Stylet inside catheter for venipuncture	Available in large diameter	Limited length of catheter
		Ease of insertion	Not flexible; break at catheter and hub junction
Through-the-needle	Short needle is inserted; catheter is threaded through needle	All lengths available Flexible Peel-away needle	Technically more difficult to insert
Over the wire	Needle serves as guide to insert wire, which serves as guide for catheter	Flexible; long catheters available; ensures proper catheter placement	More technical expertise required to place catheter; expensive

6.2.1.2 Catheter Maintenance

In adult horses, one usually does not cover a catheter with a bandage, so that one can identify any problem quickly. One may need to apply bandages in foals if they are tampering with the catheter. A triple antibiotic ointment may be applied at the insertion site on the skin to decrease infection. One should flush catheters with heparinized saline (10 IU/ml) 4 times a day if they are not used for fluid administration. When administering a medication, one should wipe the injection cap with alcohol before inserting the needle and should change the injection cap daily. One should culture all infected catheters for identification of the causative organism and for possible nosocomial infection.

6.2.1.3 Coil Sets and Administration Sets

Coil sets are used for in-stall fluid administration and are essential because they allow the horse to move around, lie down, and eat without restraint. An overhead pulley system with a rotating hook prevents fluid lines from getting tangled.

Administration sets are used for short-term fluid or drug administration and are available at 10 drops/ml and 60 drops/ml. When using a calibrated fluid pump, one should take care to use the appropriate set calibrated for the brand of pump. One then can use long coiled extension sets to connect fluids to the horse. Foal coil sets are also available that deliver 15 drops/ml.

Special foal fluid administration sets are available as pressurized bags that allow delivery of fluids at 250 ml/hr. These bags can be placed in a special harness on the back of the foal, thereby avoiding entanglement with the mare.

6.2.1.4 Pump Delivery

Calibrated pumps are available that allow delivery at various rates. These pumps have alarms that signal air in the line, an empty fluid bag, or catheter problems. The maximal fluid rate these pumps can deliver is 999 ml/hr, which is not enough for an adult horse. The pumps are useful for recumbent foals or for combined drug infusions. For large volume fluid delivery, peristaltic pumps are available that can deliver up to 40 L/hr. One must supervise these pumps constantly when in use because they continue to run even if fluids run out. One should use large-bore catheters to avoid trauma from the jet effect on the endothelium of the vein.

6.2.1.5 Sites for Intravenous Catheterization

Common sites for insertion of intravenous catheters in horses include the jugular, lateral thoracic, cephalic, and saphenous veins. The lateral thoracic vein makes an acute angle as it enters the chest at the fifth intercostal space; therefore an over-the-wire catheter is best to use when catheterizing this vein. Catheters placed in any location other than the jugular require more frequent flushings (every 4 hours), because they tend to clot more easily. Leg catheters usually are bandaged because they are more prone to dislodgment than jugular catheters.

6.2.1.6 Oral Feeding Tubes

Oral fluid administration offers a good alternative to intravenous fluid therapy in animals that require 277
maintenance fluids because of inability to swallow or in horses with impaction colic. One also can administer 278

complete or partial enteral nutrition for foals and adults. Commercially available feeding tubes for foals, weanlings, and adults* enable fluid or liquid diet supplementation while allowing the horse to continue to nurse or eat (Figure 6-1).

Figure 6-1 An adult horse with a nasogastric feeding tube in place for administration of fluids or enteral feeding solutions.

* Mila International, Florence, Kentucky, www.milaint.com

6.2.2

DESIGNING A FLUID THERAPY REGIMEN

Administration of fluid therapy can be for maintenance or replacement. Horses usually receive maintenance regimens orally, and oral electrolyte formulations are available for this purpose. Intravenous maintenance fluids

are lower in sodium and higher in calcium, potassium, and even magnesium than replacement fluids. Replacement regimens replace fluids lost through dehydration and ongoing losses.

When designing a fluid therapy regimen, one must answer three questions:

- What volume of fluid must be given?
- What type of fluid will be given?
- What will be the rate of administration?

The volume of fluids to give equals the maintenance requirements plus the correction for dehydration and for ongoing losses.

6.2.2.1 Maintenance

In the adult horse, maintenance fluid requirements have been estimated at 60 ml/kg/day. This figure probably overestimates the actual needs of a resting, fasted animal but appears to be safe in most situations. In horses with renal failure, where elimination of excess fluids is problematic, monitoring of body weight and central venous pressures is indicated. If one notes weight gain, edema, or increased central venous pressure, one should decrease the fluid administration rate.

6.2.2.2 Dehydration

Evaluation of dehydration is at best a subjective estimate, and the clinician must understand that this estimate will need to be adjusted based on monitoring parameters. Table 6-4 lists useful parameters for evaluating acute, extracellular dehydration.

Once one obtains an estimate of dehydration, one can calculate the amount of fluids to be given as follows:

$$\text{Correction of dehydration} = \text{Estimate of dehydration (\%)} \times \text{body mass (kg)}$$

6.2.2.3 Ongoing Losses

Sometimes one can measure and record ongoing losses—for example, for nasogastric reflux—but most commonly one must estimate ongoing losses. One monitors the patient to determine whether the calculated fluid volume is meeting the ongoing losses. One should monitor patients given fluids intravenously at least twice a day, including heart rate and measurement of packed cell volume and total protein, but one should monitor more frequently (every 2, 4, or 6 hours) depending on the severity of cardiovascular compromise. One should also monitor creatinine concentration once daily when initially elevated to ensure adequate return to normal. Additional means of monitoring adequate fluid delivery may include measurement of central venous pressure, arterial blood pressure, and urine output.

6.2.2.4 Type of Fluid

The type of fluid to give depends on evaluation of the chemistry profile and disease state. The first step is to decide on the baseline fluid (saline or balanced electrolyte solution), and the second step is to decide on which

additives to add to the baseline fluid, depending on specific deficits or excesses, such as hypo- or hypernatremia, hypo- or hyperkalemia, hypo- or hypercalcemia, hypoglycemia, or acid-base disorders.

Two categories of fluids commonly are used for fluid replacement: 0.09% saline and balanced electrolyte solutions (BESs). Table 6-5 lists the composition of various commercially available fluids. In general, one chooses BESs when serum electrolytes are close to normal. All BESs contain some potassium. Saline is higher in sodium and much higher in chloride than serum concentrations and is used when sodium is lower than 125 mEq/L. Saline also is used in disease processes associated with high potassium such as hyperkalemic periodic paralysis or with renal failure, in which a potassium-free solution is preferable. In cases of long-term fluid maintenance therapy (greater than 4 to 5 days), if the oral route is not available, one should consider half-strength basic fluids to which potassium and calcium are added. Long-term fluid therapy with routine BESs results in hypernatremia, hypokalemia, hypomagnesemia, and hypocalcemia. 278 279

TABLE 6-4 Parameters Used for Estimation of Dehydration in the Horse

% DEHYDRATION	HEART RATE (BEATS PER MINUTE)	CRT (SECONDS)*	PCV/TP (%/g/L)*	CREATININE (mg/dl)
6	40–60	2	40/7	1.5–2
8	61–80	3	45/7.5	2–3
10	81–100	4	50/8	3–4
12	>100	>4	>50/>8	>4

* CRT, Capillary refill time; PCV, packed cell volume; TP, total protein.

In horses, routine fluid replacement includes calcium and potassium supplementation, in particular when the horse receives no oral intake because of gastrointestinal disease. Both electrolytes are important for smooth muscle function and vascular tone. Recently, magnesium supplementation also has received interest, particularly with fasting and ileus.[10,11]

Horses with metabolic acidosis also may require bicarbonate supplementation. Because the most common cause of nonrespiratory acidosis is lactic acidemia resulting from poor perfusion, providing fluid replacement should be the first and principal means of correcting this problem. Rules of thumb for bicarbonate supplementation in *acute* metabolic acidosis are as follows:

- Normal respiratory function: If the horse is unable to exhale the generated CO_2 because of a respiratory problem, the acidosis will worsen.
- pH <7.2: In acute acidosis associated with dehydration, fluid replacement results in restoration of urine output, and renal compensation follows and usually is complete if the pH is greater that 7.2.
- Administration of half of the calculated amount rapidly, followed by the rest over 12 to 24 hours.
- Intravenously administered bicarbonate and calcium-containing solutions are incompatible.

In chronic metabolic acidosis, particularly with ongoing losses of bicarbonate (e.g., diarrhea), the horse usually requires the full calculated amount, partly because the bicarbonate loss is distributed over all fluid

compartments, not just the extracellular fluid. Orally administered bicarbonate is a good means of dealing with ongoing losses in horses with diarrhea.

TABLE 6-5 Crystalloid Solutions Available for Fluid Therapy

PRODUCT	APPROXIMATE pH	mOsm/L	Na (mEq/L)	K (mEq/L)	Ca (mEq/L)	Mg (mEq/L)	Cl (mEq/L)	BUFFER (mEq/L)
Lactated Ringers	6.5	273	130	4	3	—	109	Lactate 28
Lactated Ringers and 5% dextrose	5	525	130	4	3	—	109	Lactate 28
PlasmaLyte A	7.4	294	140	5	—	3	98	Acetate 27
								Gluconate 23
Plasmalyte 148	7.4	294	140	5	—	3	98	Acetate 27
								Gluconate 23
Plasmalyte 148	5.5	294	140	5	—	3	98	Acetate 27
								Gluconate 23
Plasmalyte 56 and 5% dextrose	5	362	40	13	—	3	40	Acetate 16
5% Dextrose	4	252	—	—	—	—	—	—
0.9% NaCl	5	308	154	—	—	—	154	—
7% NaCl	—	2400	1196	—	—	—	1196	—
5% Dextrose and 0.9% NaCl	4	560	154	—	—	—	154	—
5% Dextrose and 0.45% NaCl	4	280	77	—	—	—	77	—
5% $NaHCO_3$	8	1190	595	—	—	—	595	—
8.4% $NaHCO_3$	—	2000	1000	—	—	—	1000	—
1.3% $NaHCO_3$ (must be mixed)	—	308	154	—	—	—	1541	—

Bicarbonate can be given orally as a powder, where 279

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$$1\ \mathrm{g}\ NaHCO_3 = 12\ \mathrm{mEq\ of}\ HCO_3$$

6.2.2.5 Rate of Administration

In severe shock, one should give a shock dose of fluids in the first hour (60 to 80 ml/kg), which can be done only with pressurized bags or a pump.

In other situations, one calculates the rate of administration based on 24-hour requirements and estimates as a volume per hour. Keeping tally of the fluids given is important to ensure that the correct amount is given.

6.2.2.6 Orally Administered Fluids

One can provide orally administered fluid therapy using the following fluid composition for every 21 L of water:

$NaCl$	10 g
$NaHCO_3$	15 g
KCl	75 g
K_2HPO_4	60 g

One should administer calcium separately because it causes precipitation of the solution. This electrolyte solution meets daily needs for an adult horse and can be given by a small, preplaced nasogastric feeding tube or by intermittent intubation.

6.2.3 FLUIDS USED TO EXPAND CIRCULATING BLOOD VOLUME

6.2.3.1 Isotonic Crystalloids

Intravenous administration of isotonic crystalloids immediately reconstitutes circulating volume. However, because the fluids are crystalloids, they distribute to the entire extracellular compartment within a matter of minutes. Because the extracellular fluid compartment is 3 times the volume of blood, one must administer 3 times as much isotonic crystalloid to gain the desired amount of volume expansions.

The dose is 60 to 90 ml/kg/hr, and the fluid types usually are BESs such as lactated Ringer's solution.

6.2.3.2 Hypertonic Crystalloids (7.2% NaCl)

Hypertonic crystalloids are approximately 8 times the tonicity of plasma and extracellular fluid; their immediate effect is to expand the vascular volume by redistribution of fluid from the interstitial and intracellular spaces. However, this effect is short lived. As the electrolytes redistribute across the extracellular fluid, fluids shift again and the patient becomes hypovolemic again. Because the principal effect is fluid redistribution, a total body deficit still exists that must be replaced. The duration of effect of hypertonic solutions is directly proportional to the distribution constant, which is the indexed cardiac output.

The dose is 4 ml/kg, administered as rapidly as possible, and the fluid is 5% or 7% saline.

Because of its short duration of effect, one must follow hypertonic saline administration with isotonic volume replacement.

6.2.3.3

Colloids

Colloids are fluids that contain a molecule that can exert oncotic pressure. These molecules do redistribute to the extracellular fluid but at a much slower rate than crystalloids, so that the duration of effect is prolonged compared with crystalloids. Table 6-6 describes the different colloids available.

Natural colloids (plasma or albumin) have the disadvantage that they are more antigenic and can cause allergic reactions. Synthetic colloids have a much lower antigenicity, but they can cause bleeding disorders by their tendency to coat platelets or by causing a decrease in coagulation factor. In the horse, Dextran 40 can cause anaphylactoid reactions. Hetastarch administration can cause a decrease in coagulation factors and prolong clotting times, particularly at high doses (20 ml/kg).[12]

The dose is 10 ml/kg of 6% solutions.

Synthetic colloids do not register on a refractometer. One can evaluate oncotic pressure accurately only by using a colloid osmometer. If one is not available, one must use clinical evaluation (presence of edema and poor circulatory volume and pressure).

6.2.3.4

Blood Substitutes

Blood substitutes are hemoglobin solutions. Currently, only one commercial hemoglobin solution (oxyglobin, which is made from bovine hemoglobin) is available. The major advantage of oxyglobin is that it does not depend on 2,3-diphosphoglycerate for oxygen-carrying capacity, such that it can be stored and is immediately able to transport oxygen. The duration of effect is approximately 18 hours in horses, after which one must consider another dose or a blood transfusion.[13,14] Unfortunately, cost limits the usefulness of oxyglobin in horses.

6.2.3.5

Whole Blood

Whole blood is the ideal fluid for blood loss or platelet loss, providing it is fresh blood and has been cross-matched. One must remember that stored blood loses its oxygen-carrying capacity, and that it can take several hours to restore it after administration.

Ideally, the ICU should maintain blood donors that are free of antigenic determinants, particularly Aa and Qa and of isoantibodies. One can perform a major and minor cross-match to select an appropriate donor, providing complement is added to the test for hemolysin detection. Interpretation of the minor cross-match can be difficult if autoagglutination is present. If unavailable, one can use a non-Thoroughbred gelding. One can 280
collect a volume of 20 ml/kg safely every 3 weeks in adult horses[12] and can collect whole blood in sodium 281
citrate using sterile technique. Commercial blood collection kits are also available. Complications of blood transfusion include acute anaphylactic reactions, allergic reactions, hemolysis, fever, tachypnea, and hypocalcemia caused by citrate chelation.

TABLE 6-6 Characteristics of Colloids Solutions Available for Fluid Therapy

CHARACTERISTICS	5% ALBUMIN	25% ALBUMIN	OXYPOLYGELATIN	DEXTRAN 40	DEXTRAN 70	HETASTARCH
Molecular weight (d)						
Average	69,000	69,000	30,000	40,000	70,000	450,000
Number average	69,000	69,000	22,000–24,000	25,000	39,000	70,000
Range	—	—	5,600–100,000	10,000–80,000	15,000–160,000	10,000–3,400,000
Solvent	—	—	Balanced electrolyte solution	0.9% saline or 5% dextrose	0.9% saline or 5% dextrose	0.9% saline or balanced electrolyte solution
Maximum water binding (ml/g)	18	18	39	37	29	20
Concentration (%)	5	25	5.6	10	6	6
Half-life	14–16 days	14–16 days	2–4 hours	2.5 hours	6 hours	25 hours
Plasma percentage (after 24 hours)	—	—	12	18	29	38
Extravascular percentage (after 24 hours)	—	—	—	22	33	39
Overall survival in blood	—	—	168 hours	44 hours	4–6 weeks	17–26 weeks
Colloid oncotic pressure (mm Hg)	20	100	45–47	40	—	30

6.3 Monitoring Techniques in the Adult Equine ICU

6.3.1 ARTERIAL BLOOD PRESSURE

One can measure *arterial blood pressure* by direct catheterization of a peripheral artery or by indirect measurements that depend on a cuff placed over an artery and cuff inflation until blood flow is occluded. Measurement of arterial blood pressure is one of the indirect estimates of tissue perfusion, using the mean pressure as the driving pressure.

One can measure mean arterial blood pressure electronically by integrating the area under the pressure waveform and dividing this by the duration of the cardiac cycle.

Pulse pressure is the difference between systolic and diastolic pressure and is responsible for the palpable pulse. A bounding pulse pressure results from an increased systolic pressure or a depressed diastolic pressure.

Ultimately, the clinician is interested in oxygen delivery to tissues, which depends on adequate perfusion of tissues, which in turn depends on functional capillary density and blood flow in capillaries. In the clinical arena,

measurement of tissue perfusion or of tissue blood flow is impractical. Therefore clinicians use blood pressure as an estimate of adequate blood flow and tissue perfusion. The problems with this assumption are these:

- Tissue blood flow is regulated locally; an adequate systemic blood pressure may not reflect local blood flow if vasoconstriction, shunting, poor capillary recruitment, edema, or thromboembolism exist. An example is the central redistribution of blood flow in shock, with preferential shunting of abdominal organs.
- Blood flow depends on pressure differential. If vasoconstriction is generalized, blood pressure may be normal but flow may be poor.
- Blood flow through a vessel depends on the viscosity of the fluid (blood) and the radius of the vessel. At high viscosity, blood flow may be impaired. Severe vasoconstriction, although maintaining blood pressure, may impair flow.

6.3.2 DIRECT OR INVASIVE BLOOD PRESSURE MEASUREMENT

An over-the-needle catheter configuration is preferable for direct or invasive blood pressure measurement to avoid bleeding at the site of puncture. Preferably, one uses a small (20- or 22-gauge) catheter to minimize hematoma formation on catheter removal. The radial artery over-the-needle catheter* with a wire guide is suitable for arterial catheterization of peripheral vessels in the horse. As an alternative, one can use an over-the-wire catheter sheath† that uses a Seldinger technique for insertion. The catheter is connected to noncompliant 281
tubing filled with heparinized saline, which is linked to a pressure transducer. 282

Figure 6-2 An adult horse with an arterial catheter in the transverse facial artery for direct blood pressure monitoring.

Suitable arteries for arterial catheterization in the horse include the transverse facial, facial, and greater metatarsal arteries. In the standing horse, the transverse facial or the facial artery are most practical (Figure 6-2).

The reference unit for blood pressure is millimeters of mercury (mm Hg), meaning the force exerted by the blood against an area of the vessel wall to raise a column of mercury by a certain number of millimeters. Occasionally, centimeters of water (cm H_2O) are used. One mm Hg equals 1.36 cm H_2O. The mercury manometer is too slow, however, to record changes in blood pressure rapidly. Therefore for clinical use, a continuous method of pressure recording is preferable. A transducer transforms the pressure signal to an electronic signal that can be displayed continuously. One then can use the transducer and flush device* for continuous blood pressure measurement. One should place the transducer at the level of the heart base, as estimated by the point of the shoulder.

In addition to blood pressure measurement, invasive blood pressure measurement enables evaluation of the pressure waveform, which in turn can provide insight as to the status of the stroke volume and peripheral vascular tone.

* Catheter-over-needle, Arrow International, Reading, Pennsylvania.

† Seldinger technique transradial aretery catheter, Arrow International.

* Pressure Monitoring Kit with Truwave disposable pressure transducer, Edwards Lifescience, Irvine, California.

6.3.3 INDIRECT MEASUREMENT

Indirect blood pressure measurements depend on a cuff placed around the tail or the metatarsus. The diameter of the cuff influences the accuracy of the measurement, with cuffs that are too wide resulting in underestimation of the blood pressure. An ideal cuff width-to-circumference ratio of 0.25 to 0.35 has been recommended for use on the tail or limbs of horses. Cuff widths are available for neonate, pediatric, and adult horses. Once the cuff is in place, one measures blood pressure by recording the signal emitted by the changing blood frequency during the pulse wave. The following is a list of noninvasive blood pressure measurement methods.

6.3.3.1 Doppler

Doppler uses a small ultrasound probe placed on a peripheral artery, and a piezoelectric crystal within the probe converts the pulse wave into an audible signal. One must place the probe over a shaved area (usually under the tail of the horse) after application of a coupling gel. This method only allows measurement of systolic pressure and is therefore not as useful.

6.3.3.2 Oscillometric Sphygmomanometry

Oscillometric sphygmomanometry relies on the recording of the change in oscillations generated by the change in blood flow during deflation of the cuff. Oscillations start as the cuff pressure reaches systolic pressure, are maximal at mean pressure, and disappear when the cuff reaches diastolic pressure. The meter records and displays systolic, diastolic, and mean pressures. When placing the meter on the tail, one can use tape or a bandage below the cuff to prevent its slipping, but one should not restrain the cuff itself (Figure 6-3).

6.3.3.3 Photoplethysmography

Photoplethysmography relies on the detection of arterial volume by attenuation of infrared radiation. Photoplethysmography originally was designed for use in human fingers and has been validated for use in small dogs and cats but has not been evaluated for use in horses.

6.3.3.4 Transesophageal Ultrasound

Transesophageal ultrasound is a method of hemodynamic monitoring that uses a transesophageal probe with two ultrasound transducers.* An M-mode transducer continuously measures the aortic diameter, while a pulsed doppler measures the flow velocity, providing hemodynamic measurements including mean arterial pressure. Although transesophageal doppler† echocardiography has been evaluated for determination of 282
cardiac output in horses, the accuracy and repeatability of the combined probes for blood pressure 283
determination has not been evaluated.

Figure 6-3 Indirect oscillometric blood pressure measurement in a horse using a cuff placed on the tail.

* HemoSonic, Arrow International, Reading, Pennsylvania.

† Vingmed Sound, Horten, Norway.

6.3.4 GOALS OF BLOOD PRESSURE MEASUREMENT

The goal of blood pressure measurement is to identify hypotension and to follow the response to therapy after interventions. Blood pressures of greater than 70 mm Hg are targeted in the adult and greater than 60 mm Hg are targeted in neonates. In horses, hypertension rarely is identified other than after giving high doses of pressor agents such as phenylephrine.

6.3.5 PITFALLS

When using direct pressure monitoring, one should ensure that a good waveform is visible on the oscilloscope. Dampening of the pressure waveform by an air bubble or improper catheter placement leads to flattening of the pressure waveform and falsely low pressure measurements. Alternatively, long connector tubing lengths (>4 feet) can lead to a resonant system that records a falsely elevated systolic pressure. The flush test can help determine whether the recording system is distorting the pressure waveform.

6.3.6 BLOOD GAS ANALYSIS AND ELECTROLYTE AND LACTATE ANALYSIS

One can perform blood gas analysis easily using one of several point-of-care units. * These systems provide blood gas and electrolyte analysis. Depending on the system, ionized calcium can be part of the standard output or may require a special cartridge. Some units also report hemoglobin and hematocrit, but this is unreliable for horses because values are based on analysis of human hemoglobin. Two of the units (I-Stat and Nova) also provide lactate analysis, but this will be available shortly on the Diametrics as well. Alternatively, one can use a handheld lactate analyzer.* This unit measures plasma lactate and calculates an algorithm based on human values for whole blood. The best performance of the unit on equine plasma is measurement of lactate using the plasma mode. For all these units, carefully observing the manufacturer's instructions for sample handling and use of cartridges is essential.

* IRMA Blood Analysis System; I-Stat, I-Stat Corporation; NOVA Stat Analyzer, Nova Biomedical, Waltham, Massachusetts, www.Novabiomedical.com

* Accusport Portable Lactate Analyzer.

6.3.7 GLUCOSE

Glucose concentration is measurable in serum or whole blood. Automated chemistry analyzers use serum, whereas point-of-care glucometers† use whole blood with or without anticoagulants. Following the directives of the manufacturer is important to avoid errors. One also should keep in mind that glucose measurement made on a serum sample always will be higher than those on whole blood for the same sampling time.

† Accucheck, Roche Diagnostics, Indianapolis, Indiana, www.Roche.com; SureStep Pro glucose module, Diametrics Medical, St. Paul, Minneapolis, www.Diametrics.com

6.4 Important Procedures in Adult Critical Care

Critical care for adult horses varies depending on the underlying problem being addressed. Expertise with a variety of technical procedures is advisable to facilitate diagnostic and therapeutic efforts.

6.4.1 NASOGASTRIC INTUBATION

6.4.1.1 Procedure

Nasogastric intubation is an essential and possibly lifesaving procedure performed in cases of equine colic. One should restrain the horse adequately, with a twitch and sedation if needed. The clinician should stand on the side of the horse, with the hand closest to the horse placed on the nose, with the thumb in the nostril. With the other hand, the clinician passes the tube in the ventral meatus, using the thumb to keep it directed correctly. If one encounters a hard structure, it is the ethmoidal area, and one should redirect the tube more ventrally. Once one reaches the pharynx, one feels a soft resistance. One can turn the tube 180 degrees to direct its curvature dorsally. The clinican stimulates the horse to swallow by gentle to-and-fro movement or by 283
blowing in the tube. Keeping the head of the horse flexed at the poll is helpful. Once the horse swallows, the 284
clinician pushes the tube into the esophagus. Blowing into the tube to dilate the esophagus facilitates insertion. If the horse coughs, the clinician withdraws the tube and repeats the procedure until the tube is positioned correctly. One can determine that the tube is placed correctly in the esophagus by three ways: gentle suction should elicit a negative pressure, shaking of the trachea should not elicit a rattle, or visual confirmation of correct placement. Direct observation is the safest method. One advances the tube until it is in the stomach (fourteenth rib). If one encounters difficulty in passing the cardia, one can inject 60 ml of mepivacaine into the tube. Once in place, if reflux is not spontaneous, one must cause the horse to reflux. One should never administer medication by nasogastric tube to a horse with colic without checking first for reflux. For this, one fills the tube with water using a pump and directs the end of the tube downward to verify the presence of gastric contents. Subtracting the amount pumped in from the amount obtained is useful to get net amount of reflux.

One removes the tube by first occluding it (thumb on the end or folding it) to prevent its contents from spilling out in the pharynx and possibly the trachea as it is withdrawn. One then applies gentle traction in a direction parallel to the nose. If one encounters bleeding, one can place a towel over the nose of the horse. The bleeding, even if severe, is self-limiting.

6.4.1.2 Interpretation

Nasogastric reflux is not normal. Occasionally, one obtains a small amount of reflux (1 L or less) if a horse had a tube in place for a long time. When one obtains reflux, one should note the amount, character, and timing in relation to the onset of colic. In addition, one should note the response to gastric decompression.

Typically, reflux refers to small intestinal ileus, functional or mechanical. Lesions of the proximal small intestine produce large amounts of reflux early in the onset of the colic. With lesions of the distal small intestine (ileum), one initially obtains no reflux, and as the condition persists, one obtains reflux, but usually several hours after the onset of the colic. Occasionally, large colon disease can be associated with reflux, if the colonic distention exerts pressure on the duodenum as it curves over the base of the cecum.

Foul-smelling, fermented, or bloody, copious reflux is associated with anterior enteritis. With intestinal obstruction, the reflux usually is composed of fresh feed material and intestinal secretions. Reflux originating from the small intestine is alkaline, whereas reflux composed of gastric secretions is acidic. Because outflow obstruction is rare in horses, one usually does not measure pH.

Response to gastric decompression is important information. Horses with functional ileus show relief of pain, and the heart rate decreases in response to decompression. Horses with a mechanical obstruction usually remain painful, although some horses also respond. One then must consider the rest of the examination, deciding whether functional or mechanical ileus is present. One should note the amount of reflux obtained because this factors into ongoing losses, and the volume of fluids given intravenously must be adjusted accordingly.

Horses with functional ileus need gastric decompression usually every 4 hours, although if the condition is severe, they may require decompression every 2 hours.

One should leave the nasogastric tube in place only as long as required, because some horses develop pharyngeal and laryngeal irritation associated with its presence.[15] These horses then have pain when swallowing when they resume feeding.

6.4.2 ABDOMINOCENTESIS

Abdominocentesis is important for evaluating abdominal disease, whether it is colic, weight loss, or postoperative problems. Box 6-2 provides the materials needed to perform this procedure.

6.4.2.1 Procedure

The clinician clips a 2- by 2-inch area approximately 3 cm caudal to the xyphoid, and 1 to 2 cm to the right of midline. With sterile gloved hands, one inserts an 18-gauge needle through the skin and gently advances it into the abdomen. If one does not obtain fluid, one can insert another needle next to the first one. If one obtains no fluid, one can try a bitch catheter, cannula, or dialysis catheter. Obtaining fluid from very dehydrated horses can be difficult.

To insert a teat cannula, bitch catheter, or dialysis catheter, one places a small bleb of local anesthetic at the site, punctures the skin and abdominal wall with a 15-gauge scalpel blade using sterile technique, and then 284
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inserts the chosen device in the abdomen. One will encounter two points of resistance: as the device goes through the abdominal wall and as it goes through the peritoneum. One collects the fluid in EDTA (shaken out of the tube to avoid excessive amount in the sample) and, if the fluid is cloudy, in a culture tube.

6.4.2.1.1 BOX 6-2 MATERIALS NEEDED FOR ABDOMINOCENTESIS

18-gauge, 1½-inch needle

Teat cannula

Bitch catheter

Dialysis catheter

Sterile gloves

EDTA and culture tubes

6.4.2.2
Interpretation

Normal values for abdominocentesis are as follows: total protein, less than 2.5 g/dl, and white blood cells, less than 5000 cells/μl. On cytologic examination, neutrophils comprise approximately 40% of cells, the rest being lymphocytes, macrophages, and peritoneal cells.

With intestinal strangulation, the protein increases first (in the first 1 to 2 hours) such that the fluid is clear but more yellow. After 3 to 4 hours of strangulation, red blood cells also leak, and the fluid takes is more orange. After 6 hours or more, white blood cells increase gradually, with the progression of intestinal necrosis.

6.4.2.3
Common Problems

6.4.2.3.1
Differentiating Enterocentesis From Intestinal Rupture

Enterocentesis sometimes occurs and must be differentiated from intestinal rupture. With enterocentesis, a cytologic examination reveals plant material, bacteria, and debris, but no cells. The clinical condition of the horse is not consistent with rupture, although in early rupture, clinical signs may not reflect rupture (2 to 4 hours are necessary for manifestation of signs). Cytologic examination of abdominal fluid with intestinal rupture shows neutrophils, bacteria, and bacteria that have been phagocytized by neutrophils.

6.4.2.3.2
Differentiating Blood Contamination From Internal Hemorrhage

Blood contamination can occur from the procedure and must be differentiated from internal hemorrhage or severely devitalized bowel. Blood from skin vessels usually swirls in the sample and spins down when centrifuged, leaving the sample clear. If an abdominal vessel is punctured, blood also spins down. All fresh blood contamination shows platelets, which are not present with blood older than 12 hours. If the spleen is punctured accidentally, centrifugation reveals a packed cell volume higher than the peripheral packed cell volume. In internal hemorrhage, blood is hemolyzed such that the supernatant is reddish after centrifugation; the sample has no platelets and shows erythrophagocytosis. Ultrasonography also reveals fluid swirling in the abdomen.

Excess EDTA in the sample falsely elevates the total protein. When performing an abdominocentesis, shaking out the EDTA from the tube to avoid this sampling error is useful.

6.4.2.3.3
Differentiating Peritonitis From Normal Postoperative Changes

Abdominal surgery increases the total protein and white blood cell count for some time after surgery. Typically, if no enterotomy occurred, the white blood cell count increases greatly for 4 to 7 days and returns to normal by 14 days. The total protein may remain elevated for 3 to 4 weeks after surgery. Neutrophils appear to be nondegenerate. After an enterotomy or an anastomosis, one may see degenerate neutrophils and occasional bacteria in the first 12 to 24 hours. Subsequently, the white blood cell count remains elevated for approximately 2 weeks, but on cytologic examination the neutrophils appear to be nondegenerate and no bacteria are apparent. The total protein remains elevated for 1 month after surgery. If septic peritonitis is present, clinical signs are consistent with bacterial infection (fever, depression, anorexia, ileus, pain, endotoxemia). The white blood cell count and total protein are elevated greatly. On cytologic

examination, greater than 90% of cells are neutrophils, and they appear to be degenerate. Free and phagocytized bacteria are visible.

6.4.3 TROCARIZATION

Trocarization is useful to decompress the abdomen for abdominal compartment syndrome (severe distention associated with pain and dyspnea). Box 6-3 lists the materials needed for this procedure.

6.4.3.1 Procedure

One should perform trocarization only for large colon distention and never to decompress the small intestine. Before one decides to trocarize, identifying the segment of intestine involved is important. In adult horses, one can make such indentification by rectal palpation, and in foals or small horses, one can use radiographs or ultrasound. The distended segment of large colon also must be close to the body wall so it can be reached safely.

6.4.3.2 Method

The most common site for trocarization is the right upper flank area, just cranial to the greater trochanter at the location of the cecal base. The clinician clips, prepares, and infiltrates with a local anesthetic a 4- by 4-cm area 285
of skin. With gloved hand, one inserts a 14-g catheter with an extension tube perpendicular to the skin. One 286
places the end of the extension in water so that gas bubbles are visible when the tip of the catheter is positioned correctly. When one obtains gas, one withdraws the trocar part of the catheter slightly to avoid laceration of the bowel. The catheter may need to be repositioned several times when gas is not obtained. After decompression, one removes the trocar and infuses an antibiotic (usually gentamicin) while withdrawing the catheter.

6.4.3.2.1 BOX 6-3 MATERIALS NEEDED FOR TROCARIZATION

14-gauge, 5¼-inch catheter

Local anesthetic

Gloves

Extension tubing

Small water container (syringe case works)

Peritonitis and local abscessation are the two most common problems encountered after trocarization. One observes the horse for 24 hours for signs of peritonitis. If one suspects peritonitis, confirmation is with abdominocentesis, and one administers systemic broad-spectrum antibiotics to the horse until the condition resolves. If a local abscess develops, one can drain it externally.

6.4.4 TRACHEOSTOMY

Box 6-4 lists the materials needed for an emergency tracheostomy pack.

6.4.4.1
Preparation

If possible, one should clip, prepare, and infiltrate with local anesthetic the planned incision site. In acute respiratory distress, this may not be possible, and one makes the tracheostomy.

6.4.4.2
Procedure

One makes an 8- to 10-cm longitudinal incision at the junction of the proximal and middle third of the neck, just above the V made by the junction of the sternothyrohyoideus muscles. One must take care to stay on midline to favor drainage, separating the sternothyrohyoideus muscles on the midline and exposing the trachea. One makes a transverse incision between two tracheal rings, taking care to avoid damaging the tracheal cartilages. If the head of the horse is supported in elevation during the procedure, one should make the tracheal incision distal in relationship to the skin incision, to avoid covering the incision when the head is lowered. In emergency situations one uses a J-type tracheostomy tube because of ease of insertion. When the horse is calmed down, or if the situation is not critical, a self-retaining tube is preferable for maintenance because J-tubes tend to fall out. If the animal is to be ventilated, a silicone-cuffed tube is preferable to allow for closed-system ventilation.

6.4.4.2.1
BOX 6-4 MATERIALS NEEDED FOR AN EMERGENCY TRACHEOSTOMY PACK

Local anesthetic

Needle and syringe

Disposal blade

Scissors

Hemostats

J-type tracheostomy tube

6.4.4.3
Maintenance and Removal

One should clean the tracheostomy tube daily and change it as needed. Petroleum gel applied around the incision prevents skin scalding. In general but particularly in foals, one should remove tracheostomy tubes as early as possible to avoid permanent tracheal deformity. To help decide when to remove the tube, one can occlude the tube temporarily to see if the horse can breathe without it. Once removed, one cleans the site of exudate twice daily and allows it to heal by second intention. The wound generally closes down in 10 to 14 days and heals in 3 weeks.

6.4.5
PLACEMENT OF A CHEST TROCAR

Thoracic drainage is an essential part of managing pleural effusion and can be lifesaving in cases of severe effusion. Pleural effusion is identified by typical increases in area of cardiac auscultation, dullness in the ventral area of auscultation, and if unilateral, a discrepancy in auscultation between the two hemithoraxes. One can use percussion, although typically ultrasound provides the best method of identifying fluid in the chest. One can

drain pleural fluid through a cannula or by placement of a chest drain. Chest drains are placed in the seventh intercostal space approximately 2 inches above the elbow joint. The clinician clips, prepares, and infiltrates the site with a local anesthetic. One makes an incision in the skin cranial to the proposed point of entry and inserts the trocar through the skin. One moves the incision in a caudal direction to a point between two ribs and using pressure pushes the trocar into the chest. One hand serves as a stop to control depth of entry. Once one obtains fluid, one places a Heimlich valve on the end of the tube and sutures the tube in place using a Chinese finger trap pattern.

6.4.6 PNEUMOTHORAX

Pneumothorax (air in the pleural cavity) is classified as open (external wound) or closed. The pleural pressure equilibrates with atmospheric pressure, resulting in lung collapse. Tension pneumothorax develops when air continuously enters the chest without evacuation. The pleural pressure can reach supraatmospheric levels and can be life-threatening. 286

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In open pneumothorax, sealing of the chest must occur, followed by evaluation of air. One can seal the chest with sheets of plastic wrapped around the site of entry or close the wound if possible. One evacuates the chest by placing a small trocar, or a 14-gauge catheter, in the dorsal twelfth intercostal space, removing the trocar once the air has been evacuated satisfactorily.

In closed pneumothorax or tension pneumothorax, one must leave the catheter in place until the source of air entry can be sealed.

6.4.7 URINARY CATHETERIZATION

Measurement of urine output in adult horses is an infrequent procedure, compared with foals, but may be useful in instances of oliguric renal failure or when 24-hour volumetric measurements are needed. Male horses with neurologic disorders that make them unable to express their bladder may require bladder decompression.

In the mare, one achieves urine collection easily by placing a Foley catheter that is connected to collection tubing. One can make a closed system by using a solution administration set and empty fluid bag. In geldings, one can insert a male urinary catheter and suture it in place using a Chinese finger trap. If the horse is recumbent and thrashing, leaving as little of the catheter protruding to avoid it being pulled out is important. Normal horses produce 1 to 2 ml/kg/hr of urine.

6.4.8 CARE OF THE DOWN HORSE

Nursing care is an essential component of successful recovery of down horses. For a favorable outcome, identification of a disease process that has a potential for recovery in a short period (days to weeks) is important. Care of down horses is time consuming and is more difficult if the horse is heavy. Mechanical ventilation of down adult horses has a high failure rate because of complications. The down horse requires fluid and nutritional support, evacuation of the intestinal and urinary tracts, prevention of eye trauma, and management of decubital ulcers.

Recumbent horses usually have decreased maintenance fluid requirements, because they are for the most part inactive, are kept at a reasonable ambient temperature, and usually have decreased feed intake. A lower maintenance fluid rate (40 ml/kg) suffices in these horses and can be provided by allowing the horse access to water (if the horse remains sternal for short periods), by using a preplaced feeding tube, or by using intravenous

fluids. For the short term, intravenous fluids are convenient, but the risk of thrombophlebitis is higher with prolonged catheterization.

One can feed the recumbent horse by allowing the horse to eat (this is possible if mental status is minimally affected), by nasogastric intubation, or by partial parenteral nutrition. If one uses nasogastric intubation, one can prepare a slurry of pelleted feed to facilitate administration. Importantly, one must ensure correct placement of the tube before feed administration, particularly in dysphagic horses. Partial parenteral nutrition of glucosed-based solutions is well tolerated in adult horses and obviates the need for oral feeding. One must ensure strict asepsis of all lines, because these solutions are rich in glucose.

Figure 6-4 Recumbent horse with a head bandage to protect the eyes from injury.

Recumbent horses are often reluctant to urinate or defecate, at least initially. Colic caused by impaction or tympany is a common complication. To obviate this, one should empty the rectum regularly and administer mineral oil to facilitate gastrointestinal transit. Most horses learn to urinate while recumbent, but initially, one must empty the bladder 2 to 3 times daily. Horses that cannot urinate require placement of a urinary catheter.

Decubital ulcers at pressure points (elbow, point of the shoulder, tuber coxae, hip, hocks, zygomatic arch) are difficult to avoid in recumbent horses. Adequate padding, frequent (every 4 hours) turning, and changing from sternal to lateral recumbency can be helpful in delaying the onset of decubital ulcers. Bandaging of the lower legs can be useful in thrashing horses to minimize injury.

Adequate eye cleaning and lubrication are essential in the recumbent horse. Debris often accumulates in the dependent eye, which can cause ulceration. One should clean the eye frequently using ophthalmic solution and should apply lubrication. In horses that are recumbent for prolonged periods, bandaging of the head is useful to prevent eye injury (Figure 6-4).

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7 CHAPTER 7 DISORDERS OF THE RESPIRATORY SYSTEM

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Disorders of the respiratory system are second in importance only to those of the musculoskeletal system in limiting the athletic performance of the horse. Owners sustain major economic losses when respiratory diseases interrupt the training programs of horses or when horses must be retired because of lung damage sustained from respiratory disease. Thus early detection and treatment of respiratory problems is essential for the rapid return of athleticism to performance animals.

7.1 Diagnostic Approach to Respiratory Disorders

7.1.1 HISTORY

The clinician should direct questions to the person most familiar with the performance and medical history of the horse. Accurately defining the problem, devoid of subjective impressions, can be the most difficult part of taking the history.

7.1.1.1 Age and Breed

The age and breed of the animal exhibiting respiratory-related signs may provide clues as to the problem. Congenital defects (nasal septal deviations, choanal atresia, subepiglottic cysts, and hypoplastic lungs) are typically evident at birth, whereas other conditions, such as chronic bacterial pneumonia *(Rhodococcus equi),* may not be evident until the foal is older (1 to 3 months of age). Viral and bacterial upper respiratory tract infections tend to occur in the weanling and yearling, whereas conditions such as inflammatory airway disease, pleuropneumonia, or exercise-induced epistaxis are found more commonly in performance horses 2 years or older. In contrast, recurrent airway disease (heaves) or neoplasia of the respiratory tract are diagnosed primarily in the middle-aged or older horse.

Considering the breed of the horse is also important in investigating respiratory disorders. For example, one should evaluate Arabian foals with chronic infections for combined immunodeficiency syndrome, a heritable condition in which cell-mediated and humoral limbs of the immune system are deficient. In addition, solitary defects in the humoral immune system also predispose horses to develop chronic respiratory and enteric infections. Selective immunoglobulin M (IgM) deficiency tends to occur more frequently in Arabians and Quarter Horses, whereas agammaglobulinemia has been documented in Thoroughbreds and Standardbreds.[1,2]

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7.1.1.2 Environment

One should ascertain the environment to which the horse was or presently is exposed. For example, is the horse stabled at a racetrack where population turnover is high and the potential for viral respiratory outbreaks is increased, or is the horse a sole inhabitant of a small pasture, seldom exposed to other horses? Does the farm have a history of endemic infections, as often occur with *Streptococcus equi* outbreaks? Has the horse been exposed to pastures grazed by donkeys? This, along with information regarding the diet (hay, pelleted rations, or pasture), the nature of the bedding materials (straw, peat, or shavings), and the amount of time the horse is

stabled are important considerations in establishing the risk factors for some respiratory disorders such as recurrent airway obstruction or lungworm infections. One also should obtain the deworming and vaccination schedules. Young horses are at risk for developing verminous pneumonia resulting from *Parascaris equorum* migration. Nematode eggs can survive for prolonged periods on a pasture. Thus establishing whether the foal was exposed to pastures grazed by yearlings or 2-year-olds may help in establishing the diagnosis and treating the problems. If the horse is a performance animal and is at an increased risk for developing upper respiratory tract infections, one should determine how frequently equine influenza and equine herpesvirus type 1 (EHV1) and type 4 (EHV4) vaccinations are administered.

7.1.1.3 Prior Medical Problems

Has the horse had a previous history of illness or trauma that might be related to the present complaint? Viral respiratory conditions often precede the development of bacterial pneumonia. Sequelae to *S. equi* infections include internal abscessation, guttural pouch empyema, retropharyngeal abscesses, and purpura hemorrhagica, which ultimately may affect the respiratory and cardiovascular systems. Trauma may be implicated in the development of diaphragmatic herniae, pneumothorax, or tracheal injury and subsequent stricture formation.

7.1.1.4 Present Medical Problem

The clinician should direct questions to define the exact problem, establishing the chronicity of the disorder and the rapidity of its development. Is the problem insidious in onset or an acute disorder of less than 2 weeks' duration? Is the onset associated with a stressful event such as racing or a prolonged van ride? Does the farm have new arrivals that have not been quarantined? One should also determine the effect of the respiratory complaint on the expected athletic performance: Are clinical signs evident during eupneic (resting) breathing or only noticeable during the hyperpnea of exercise? Has the horse received any medication and did the clinical condition improve? Amelioration of signs during therapy suggests that the previous treatment protocols were not of sufficient duration or that an underlying immunodeficiency exists.

7.1.2 PHYSICAL EXAMINATION

Before examining the horse, simply stepping back and evaluating the demeanor and mental status (alert or depressed), posture, and manner of movement of the horse is helpful. Has the horse adopted a particular stance (extended head and neck), or is it reluctant to move because of pain (pleurodynia)? Are changes in the pattern of breathing from the normal eupneic state obvious (Box 7-1)? Is the breathing pattern rapid and shallow? Does nostril flaring accompany a pronounced expiratory effort? The normal respiratory rate of the adult horse varies from 8 to 15 breaths per minute, with a slightly noticeable abdominal component during expiration (an active process in the horse).

Abnormalities of the respiratory system are evident also by the production of unusual sounds associated with respiration; the presence of a cough; a nasal or ocular discharge; lymphadenopathy; epistaxis; facial, pharyngeal, or cervical swellings; and cyanotic mucous membranes. Ataxia or reluctance to move, the presence of ventral thoracic or limb edema, foul odors associated with breathing, and a history of weight loss may occur with respiratory disorders.

7.1.2.1 BOX 7-1 BREATHING PATTERNS

7.1.2.1.1 Eupnea

The normal quiet and seemingly effortless breathing pattern adopted by the healthy horse at rest. Inspiration and expiration in the horse are active processes.

7.1.2.1.2 Tachypnea

A breathing pattern characterized by rapid frequency and shallow depth or small tidal volume.

7.1.2.1.3 Hyperpnea

A breathing pattern characterized by an increase in the depth and rate of breathing, as might be found during exercise.

7.1.2.1.4 Apnea

A period of time in which no discernible respiratory effort is made and air flow has ceased. Apnea may accompany sleep-related disorders or excessive ventilation (hypocapnia-induced apnea).

7.1.2.1.5 Hypoventilation

A pattern of breathing that alters gas exchange sufficiently to cause hypercapnia or elevations of arterial carbon dioxide tension.

7.1.2.1.6 Hyperventilation

A pattern of breathing that increases alveolar ventilation and results in arterial hypocapnia.

7.1.2.1.7 Dyspnea

A breathing pattern that appears to reflect difficulty in breathing. The animal appears to be distressed, and increased work of breathing is obvious. 290

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The clinician should assess the airflow from both nostrils to rule out potential obstructions or masses within the nasal cavity. One can detect atheromata, which may restrict airflow during exercise, by palpating the false nostrils. Any peculiar breath odors are detectable at this time. Percussion of the frontal and maxillary sinuses, performed by gently tapping over the sinuses while one holds the mouth of the horse slightly open, may reveal a dullness because of accumulations of fluid or inflammatory products, but the absence of a percussable change does not rule out a sinus disorder. One also should determine evidence of swelling in the submandibular space (lymphadenopathy) or in the pharyngeal (guttural pouch or retropharyngeal disorders) and cervical areas (accessory lungs[3]). Palpation of the larynx and trachea is routine and should not elicit coughing episodes in the normal horse. One also can detect evidence of muscle atrophy or prior surgeries (laryngoplasty, laryngotomy, myotomy) during the physical examination of the upper respiratory tract. One should check for patency of the jugular veins (thrombophlebitis and secondary pulmonary abscessation) or for evidence of perivascular

injections that may contribute to upper respiratory tract obstructions by involving the recurrent laryngeal nerve or vagosympathetic trunks.

The clinician then should conduct a complete physical examination, paying attention to all organ systems (and not simply focusing on the respiratory system). In respiratory emergencies, one conducts an abbreviated initial examination and directs efforts at patient stabilization until that time when a more thorough physical examination can be conducted.

7.1.2.2 Auscultation of the Lung Fields

The clinician should examine the horse during eupneic and hyperpneic (by use of a rebreathing bag) breathing patterns. Normal *breath sounds* are those produced by turbulent air movement through the tracheobronchial tree and vary in intensity and quality depending on the portion of the lung field auscultated. The *vesicular sounds,* over the middle and diaphragmatic lung lobes, are the quietest sounds; the *bronchial sounds,* over the trachea and the base of the lung, are the loudest.[4–6] In the normal horse one can hear breath sounds more easily on the right side than on the left. Considerable variation exists between normal patients in the intensity of the breath sounds. For example, vesicular sounds are often barely audible during eupneic (normal) breathing in the obese patient and are perceived as soft rustling sounds. However, one may hear breath sounds easily in the thin or young animal because of less attenuation of lung sounds by the chest wall. The intensity of breath sounds also increases with increased airflow. Thus breath sounds are accentuated in febrile or excited animals or in animals hyperpneic from a variety of causes (exercise, hypoxia, pain). However, auscultatory findings do not always correlate well with the degree of alveolar ventilation.[5] For example, in horses with lung consolidation the transmission of breath sounds from adjacent areas gives the false impression that that region is well ventilated. Breath sounds also may become more difficult to hear in cases of (1) alveolar overinflation in which the aerated tissue of the lung is a poor conduction medium of sound or (2) pneumothorax and pleural effusions in which the sound is reflected at the pleural surface (acoustic impedance).[5]

Adventitious lung sounds are abnormal sounds superimposed on the normal breath sounds and have been described as crackles or wheezes. Crackles are short, explosive, discontinuous sounds that have been likened to the sound of salt thrown in a hot frying pan or the sound of cellophane being crumpled. They are usually of low intensity and are audible during the inspiratory phase of respiration. Their production has been attributed to the sudden equalization of pressure in two compartments after airways have reopened. Crackles are audible in cases of interstitial pneumonia and pulmonary edema (restrictive pulmonary diseases) and in cases of bronchopneumonia or airway diseases (obstructive disorders). Breathing 100% oxygen also may produce crackles because the nitrogen stent maintaining alveolar distention is eliminated.[7] Crackles are also audible in cases of subcutaneous emphysema. Wheezes are musical sounds thought to arise from the vibration of airway walls or tissue masses in close contact with the airway walls and may be audible during inspiration or expiration. Wheezes may be monophonic or polyphonic, the latter indicative of multiple sites of airway obstruction. Pleuritic friction rubs have been described as sandpaper-like sounds generated by the movement of the visceral and parietal pleurae across each other. One may detect them (infrequently) in the early stages of “dry” pleuritis, before the effusive stage.

7.1.2.3 Percussion of the Thorax

One percusses by methodically tapping the intercostal spaces of the thorax using a plexor and pleximeter (foals) or a large spoon and neurologic hammer (adults) and evaluating the nature of the sound produced. Aerated tissues produce a resonant sound, whereas fluid-filled structures (bowel, heart, lung abscesses,

consolidated lung) produce a dull sound. One should identify the transitional site where sound quality changes during percussion by using a piece of tape. One then compares the limits of the percussed field with those of the normal horse. The cranial limit is the shoulder musculature, the dorsal limit is the back musculature, and the caudoventral limits are the seventeenth intercostal space at the level of the tuber coxae, the sixteenth intercostal space at the level of the tuber ischii, the thirteenth intercostal space at the midthorax, the eleventh 291
intercostal space at the level of the scapulohumeral articulation, and the sixth intercostal space at the 292
olecranon.[8] Thoracic percussion should not be painful: resentment by the horse may indicate pleuritis or rib fractures. A ventral dullness suggests pleural effusion, pleural thickening, lung consolidation, or pericardial effusion. Occasionally, caudal borders may be expanded, suggesting alveolar overinflation, which may accompany recurrent airway disease.

7.1.2.4 Endoscopy

Fiberoptic endoscopy provides an invaluable method for assessing the equine respiratory tract. Endoscopy is helpful in establishing (1) the origin of respiratory noises that accompany laryngeal hemiplegia, dorsal displacement of the soft palate, epiglottic entrapment, rostral displacement of the palatopharyngeal arches, arytenoid chondritis, tracheal collapse or stenosis, and pharyngeal narrowing; (2) the existence of congenital defects such as subepiglottic cysts, cleft palate, or choanal atresia; (3) the source of exudate or hemorrhage occurring with guttural pouch mycosis or empyema, ethmoidal hematoma, pulmonary epistaxis, retropharyngeal abscessation, and chronic pulmonary disease; and (4) in extracting foreign bodies from the tracheobronchial tree. Videoendoscopic examination of the upper respiratory tract during maximal treadmill exercise (12 to 14 m/sec) is a routine diagnostic modality at many referral centers, allowing visualization of dynamic collapse of the upper airway structures during exercise in certain pathologic conditions. Accumulations of mucopurulent exudate within the pharynx or the trachea following exercise are compatible with a diagnosis of inflammatory airway disease. Endoscopy is a potential means of obtaining tracheobronchial aspirates (guarded swabs or catheters).[9] A number of different fiberoptic endoscope models are available but an 11-mm (outer diameter) endoscope usually is used in adult horses, and a smaller, 7.8-mm (outer diameter) pediatric scope is recommended for foals.

7.1.2.5 Sinuscopy

Sinuscopy, direct examination of the interior of the paranasal sinuses using an endoscope or an arthroscope that is introduced through a small trephine opening, is useful for diagnosing or treating sinus disease.[10,11] In selected cases, sinuscopy may be a diagnostic or therapeutic alternative to flap sinusotomy. One can access the caudal maxillary sinus at a point 2 cm rostral to the midpoint of a perpendicular line drawn from the medial canthus of the eye to the facial crest. One can access the rostral maxillary sinus 2 cm caudal to the midpoint of a line drawn from the rostral end of the facial crest to the infraorbital foramen. The frontoconchal sinus is accessible through a portal placed 40% of the distance of a perpendicular line drawn from 0.5 cm caudal to the medial canthus from the midline. The frontoconchal site affords excellent visualization of the frontoconchal sinus and, via the large frontomaxillary opening, the caudal maxillary sinus. Following sedation of the horse and surgical preparation and local anesthesia of the selected site, the clinician makes a small skin incision and opens the bone with either a ¼-inch Steinman pin (for a 4-mm diameter arthroscope) or a 10-mm trephine (for a 9-mm endosocope). The endoscope provides better illumination and a greater field of view. In addition to direct examination of the sinus interior for diagnosis (Figure 7-1), sinuscopy allows the examiner to biopsy tissues under direct visualization and to apply therapeutic procedures such as formalin injection of mass

lesions (Figure 7-2), cyst removal, and removal of sequestered bone fragments. Following examination, the clinician may close the portal with cutaneous sutures or leave it open for therapeutic lavage.

7.1.2.6 Computed Tomography

Interpretation of conventional radiographs of the equine head is notoriously difficult because of the complex anatomy and the spectrum of radiographic densities: air, soft tissue, bone, and tooth. Thus radiographs and clinical examination may not define accurately the location and extent of lesions in the head region.[12] 292
Computed tomography uses a rotating, highly columnated x-ray beam to generate digital cross-sectional 293
images.[13,14] These cross-sectional images afford a much better evaluation of normal and pathologic anatomy than can be achieved through conventional radiographs (Figure 7-3). Additionally, the digital format enables three-dimensional reconstruction of structures and manipulation of images to optimize interpretation (Figure 7-4). Constraints for this technique are its expense, limited availability, and the need for general anesthesia of the horse.

Figure 7-1 Sinuscopic view of the caudal maxillary sinus in 16-year-old Thoroughbred gelding with a history of epistaxis. A large fungal colony is on the infraorbital canal, and several small blood clots are visible. (Courtesy R.P. Hackett, Ithaca, New York, 2001.)

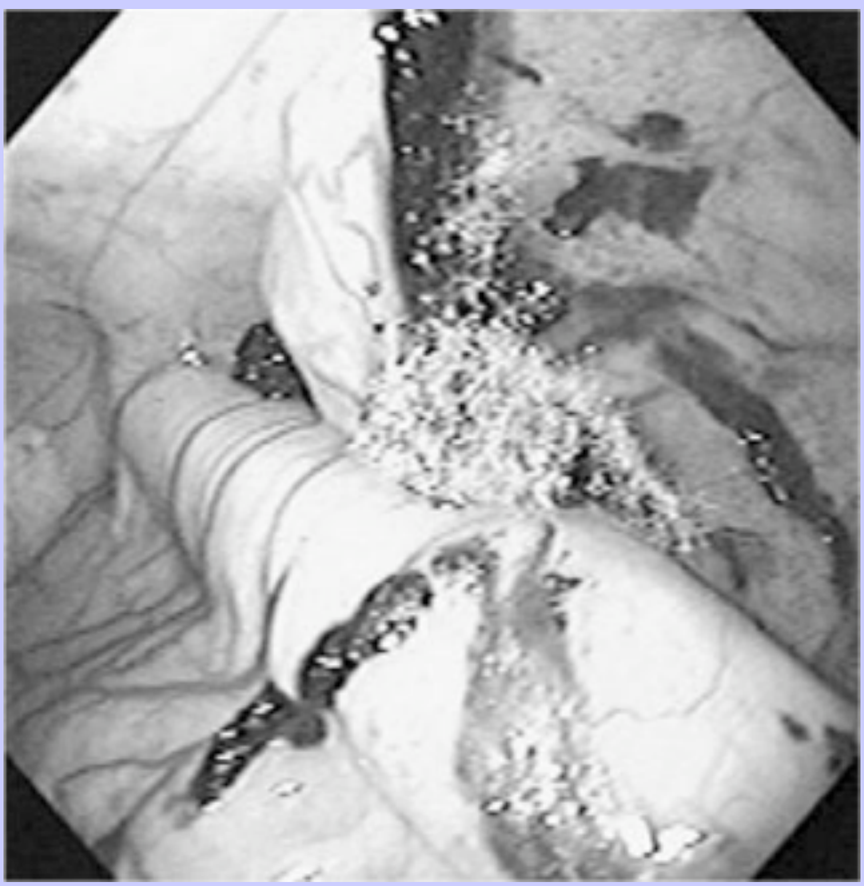
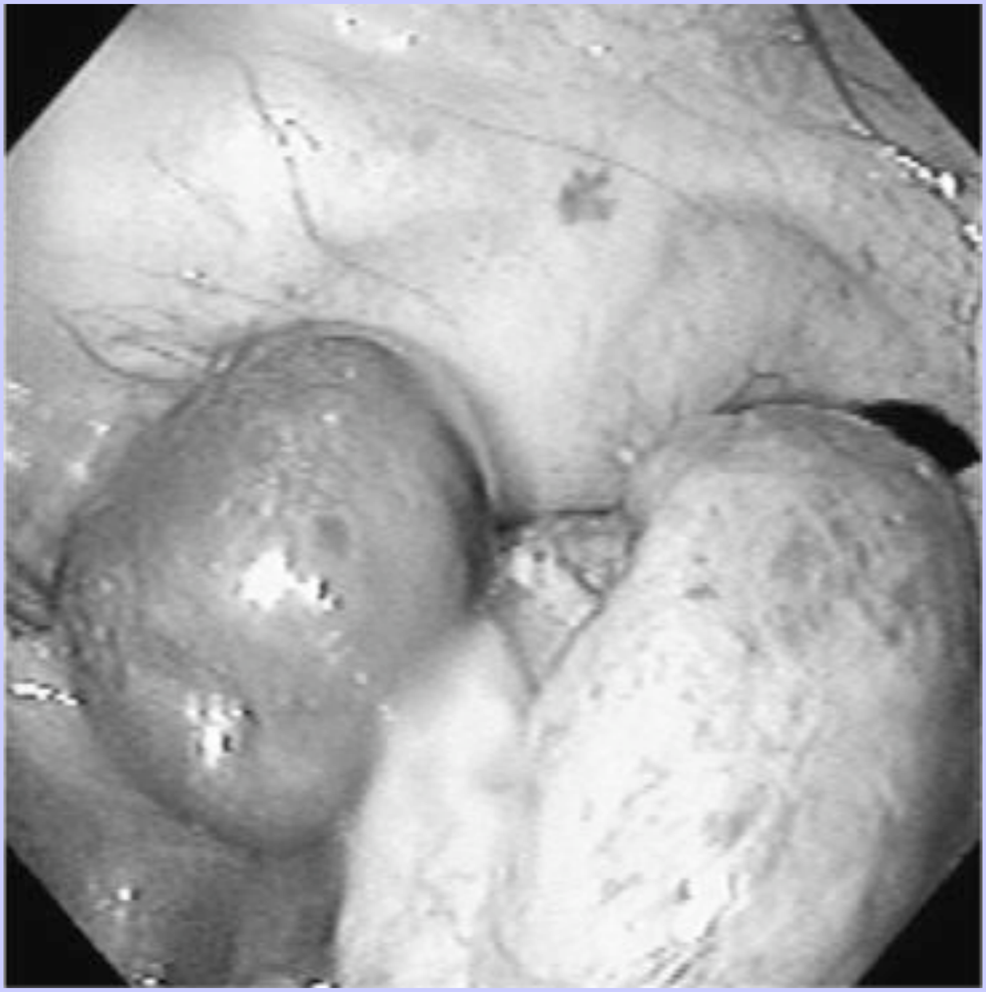


Figure 7-2 Sinuscopic view of small ethmoidal hematoma in 12-year-old Thoroughbred gelding. This lesion was injected with formalin transendoscopically. (Courtesy R.P. Hackett, Ithaca, New York, 2001.)



Computed tomography has proved particularly useful for examining the anatomically complicated structures such as the nasal turbinates, paranasal sinuses, teeth, nasopharynx, and guttural pouches and for evaluating areas obscured by overlap of adjacent structures on conventional radiographs (Figure 7-5). Improved evaluation of these structures has enhanced considerably the diagnosis, the selection of surgical approaches, and the ability to render an appropriate prognosis.

Computed tomography has been of limited usefulness in evaluating the lower respiratory tract structures in adult horses because of the size constraints of the gantry. However, computed tomography is an excellent diagnostic modality for detecting and defining the extent of pulmonary or mediastinal masses in the thorax of foals, ponies, and miniature horses.[15]

Figure 7-3 Cross-sectional image of an amelioblastoma involving the upper left premolars of a 4-year-old Warmblood gelding. A large soft issue mass is encroaching on the nasal passages, and significant osseous destruction of the hard palate, ventral concha, and premaxilla is visible. (Courtesy R.P. Hackett, Ithaca, New York, 2001.)

7.1.2.7 Sampling of Respiratory Tract Secretions

7.1.2.7.1 Centesis of the Paranasal Sinuses

The clinician can perform centesis of the paranasal sinuses when radiographic or computed tomographic examination reveals fluid lines or soft tissue densities in the sinuses. One performs the technique aseptically using local anesthesia on the sedated horse, using a Steinmann pin for the initial puncture of the sinus. One enters the rostral maxillary sinus at a site 2.5 cm dorsal to the facial crest and 2.5 cm caudal to the infraorbital foramen. One enters the caudal maxillary sinus (which communicates with the frontal sinus) at a site 2.5 cm dorsal to the facial crest and 2.5 cm rostral to the medial canthus. The clinician should submit aspirates for cytologic examination and bacterial culture.

7.1.2.7.2 Guttural Pouch Catheterization and Culture of the Exudate

One performs guttural pouch catheterization in cases of empyema, chondroids, or distention of the pouches. With the horse sedated, the clinician passes a fiberoptic endoscope into the guttural pouch using the biopsy instrument or a Chambers catheter as a guide for the endoscope. One obtains a sample of the exudate with a triple-guarded catheter or protected swab. A technique for obtaining percutaneous aspirates of the guttural

pouches recently has been described, but the authors prefer to use endoscopic visualization for sample collection.[16] Cytologic examination of guttural pouch aspirates from normal horses demonstrates the presence of mucus; a predominance of ciliated columnar epithelial cells; neutrophils; and a few (<1%) macrophages, lymphocytes, and eosinophils. Aspirates are not normally sterile: common bacterial isolates include α-hemolytic *Streptococcus, Staphylococcus,* and *Moraxella* species.[17] *Streptococcus equi* is not a normal inhabitant of the guttural pouch. 293 294

Figure 7-4 Three-dimensional reconstruction of the premaxilla in Figure 7-3. The extent of osseous destruction of the hard palate can be delineated clearly, as can separation of the second and third premolars. (Courtesy R.P. Hackett, Ithaca, New York, 2001.)

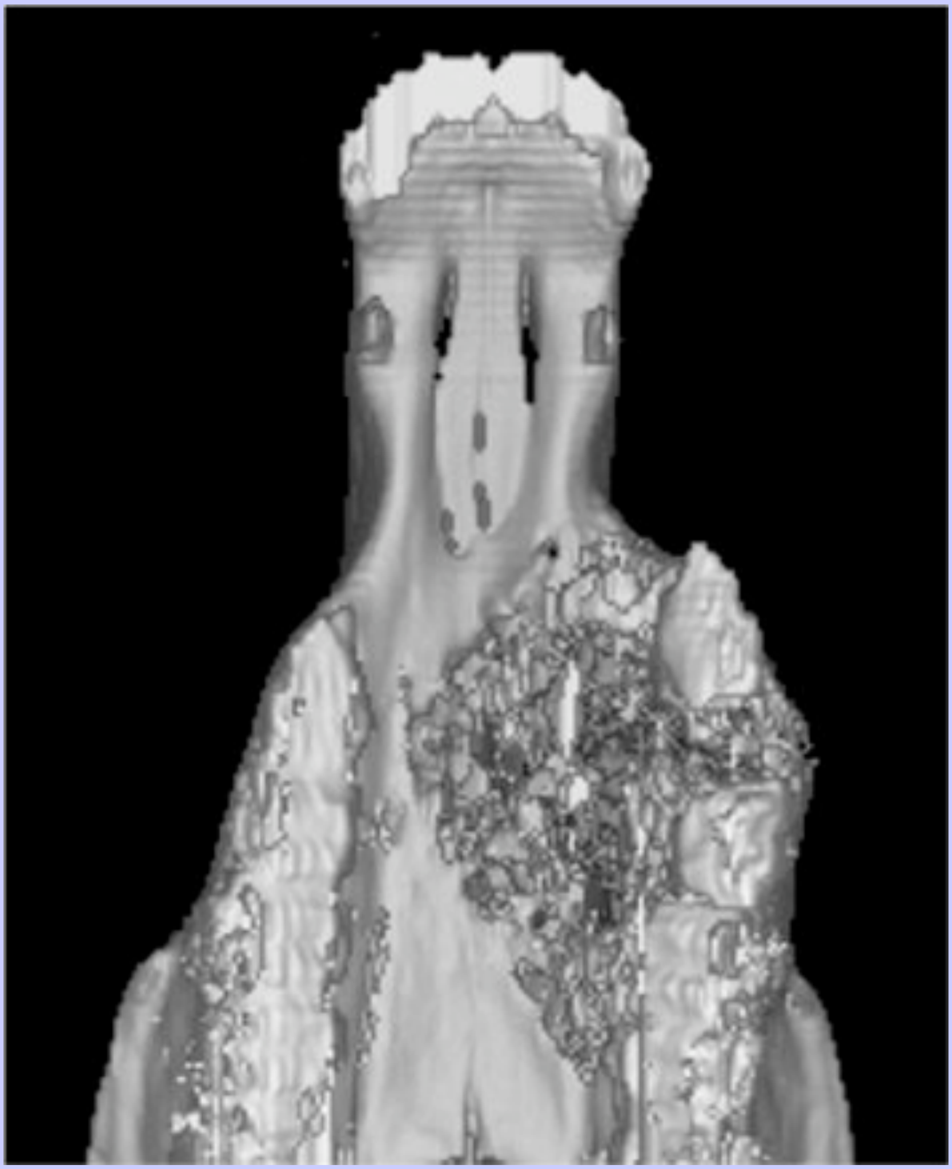
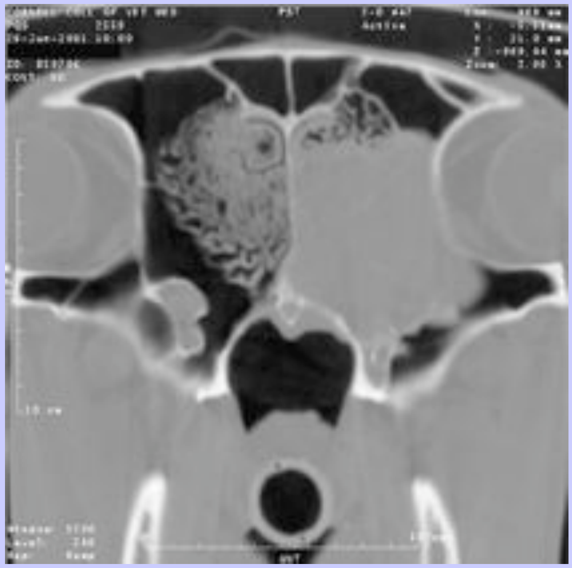


Figure 7-5 Cross-sectional image of an adenocarcinoma affecting the ethmoid turbinate in a 23-year-old hinny. (Courtesy R.P. Hackett, Ithaca, New York, 2001.)



In horses suspected of having guttural pouch epistaxis, one should exercise caution regarding catheterization or endoscopic examination of the pouch. The procedure may dislodge a blood clot and cause fatal hemorrhage.

7.1.2.7.3 Sampling of Tracheobronchial Secretions

Several techniques have been advocated for obtaining tracheobronchial samples. The site of the collection sample (tracheal versus bronchoalveolar) depends on the nature of the respiratory disorder. The appropriateness of using tracheal samples to evaluate chronic inflammatory disorders has been challenged, because little correlation exists between tracheal and bronchoalveolar lavage cytologic findings or between tracheal and pulmonary histopathologic findings.[18,19] In contrast, a good correlation exists between bronchoalveolar lavage cytologic findings and pulmonary histopathologic findings.[20] Bronchoalveolar lavage is indicated in the investigation of chronic *inflammatory* diseases but may be performed with collection of transtracheal aspirates if one cannot dismiss an infectious process. Table 7-1 shows representative cytologic findings from bronchoalveolar lavage studies of normal horses. Sedation of horses before bronchoalveolar lavage is recommended. Using a fiberoptic endoscope (permitting direct visualization of the lung segment to be lavaged) or using a thick-walled flexible tube with a cuffed end (which is passed blindly into the distal airways), the clinician instills 100 to 500 ml of physiologic saline solution within the pulmonary segment. One can retrieve 50% to 75% of the fluid and examine it cytologically. Before lavage, one may instill 20 to 40 ml of 2% lidocaine to desensitize the airways.

In the absence of a suitable method for collecting bronchoalveolar lavage fluid aseptically, tracheobronchial aspirates remain the method of choice for investigation of *infectious* lower respiratory tract disorders.

Collection of culture samples by fiberoptic endoscopy simplifies the procedure and eliminates some of the complications formerly associated with the transtracheal technique, such as cellulitis and pneumomediastinum. Guarded tracheal swabs[21] and the telescoping plugged catheter of Darien[9] are convenient techniques for obtaining representative samples. However, oropharyngeal contamination still may occur when one obtains tracheobronchial aspirates endoscopically using telescoping plugged catheters.[22,23] The authors prefer to obtain tracheobronchial aspirates percutaneously.

Characterization of the normal bacterial isolates from tracheobronchial aspirates in healthy horses has been 294
well documented. When examining a horse with suspected respiratory disease, one must evaluate culture 295
results in light of the cytologic findings and clinical examination. The tracheobronchial aspirates of approximately 8% of normal horses (pastured or stabled) were found to be culture-positive for *Klebsiella,* β-hemolytic streptococci, *Pasteurella* species, and *Pseudomonas aeruginosa*. For a microorganism to be implicated in a lower airway disorder, one would expect (1) to obtain a moderate to heavy growth of the organism on culture ($\geq 1 \times 10^3$ colony-forming units); (2) to identify organisms within phagocytic cells; and (3) to have evidence of degenerative neutrophils. In contrast, anaerobes, which are a normal component of the oropharyngeal flora, normally are not isolated from aspirates of healthy horses, emphasizing their importance in diseased processes when recovered from respiratory cases. Based on their studies, Sweeney, Beech, and Roby[24] also have described a group of transient bacterial flora of questionable pathogenicity such as *Enterobacter, Bacillus, Acinetobacter*, α-hemolytic streptococci (except for *Streptococcus pneumoniae* type 3), and *Staphylococcus epidermidis,* which may be isolated from tracheobronchial aspirates. In contrast, *S. pneumoniae* now is recognized as a pathogen of the respiratory tract in horses.[25–27] Fungal hyphae may be found free or engulfed within mononuclear cells in normal horses.

TABLE 7-1 Differential Counts in Bronchoalveolar Lavage Fluid[*]

NEUTROPHILS	MACROPHAGES	LYMPHOCYTES	EOSINOPHILS	MAST CELLS	EPITHELIAL	REFERENCE
8.9 ±1.2	45.0 ±2.8	43.0 ±2.7	<1.0	1.2 ±0.3	3.5 ±0.7	18
5.0 ±4.0	72 ±10	18 ±3.0	2.0 ±4.0	1.0 ±1.4	—	20
6.2 ±5.0	70.3 ±15.2	7.6 ±3.9	1.0 ±1.4	0.6 ±1.4	14.3 ±13.4	516
6.2 ±2.4	48.5 ±2.5	35.3 ±2.5	2.5 ±0.9	5.2 ±0.8	2.3 ±1.4	517

* Percent of total white blood cell count plus-or-minus standard error or standard deviation.

7.1.3 RADIOGRAPHY

Radiographs may be helpful (1) in detecting soft tissue masses (abscesses, granulomata, neoplasms, hematomas, polyps) or fluid accumulations within the paranasal sinuses, the nasal cavity proper, the guttural pouches, and the retropharyngeal areas; and (2) in evaluating orofacial deformations or fractures following trauma. Radiography also allows assessment of the anatomic dimensions of the pharyngeal and laryngeal structures (thickened soft palate, hypoplastic epiglottis, hyoid bone fractures). When one suspects nasal or sinus disorders, one should take lateral, dorsoventral, and oblique views. The clinician usually can achieve proper restraint of the horse for positioning of the cassette with xylazine, detomidine, or butorphanol sedation. In the horse that one can anesthetize safely, one may obtain a more thorough definition of the extent of nasal and upper respiratory tract diseases using computed tomography scans (see the previous discussion).

Radiographic evaluation of the equine thorax remains preferable to ultrasonography for detecting diffuse parenchymal diseases such as interstitial pneumonia, pulmonary edema, and chronic airway disorders or for detecting mediastinal or deep parenchymal abscesses.[28] Imaging the thorax of the standing horse requires three to four overlapping lateral radiographs. However, compared with human or small animal medicine, in which correlations between the pulmonary disorders and the radiographic findings are well-established, the radiographic changes in equine respiratory disorders tend to be rather nonspecific. In addition, many pulmonary diseases such as inflammatory airway disease, exercise-induced pulmonary hemorrhage, lungworm infections, and recurrent airway obstruction may be associated with normal radiographs.[29]

Four types of radiographic patterns have been described: an alveolar (airspace), an interstitial, a bronchial, and a vascular pattern. In the alveolar pattern, opaque areas coalesce and completely obliterate the vessels and bronchi. Air bronchograms may be notable. This pattern occurs with pulmonary edema, hemorrhage, lung consolidation, or neoplastic infiltration. Interstitial patterns are found most commonly and are associated with a variety of conditions. This pattern causes a blurring of the edges of the pulmonary vessels, a diffuse increase in lung opacity, and variable reticular, linear, or nodular opacities.[29,30] A reticular pattern occurs with viral, bacterial, or parasitic pneumonia; pulmonary edema; interstitial pneumonia; and pulmonary fibrosis. An irregular linear pattern occurs with resolving bronchopneumonia, and a nodular pattern occurs with abscesses, granulomata, or neoplasms. Bronchial patterns alone are not found commonly but usually occur in association with interstitial patterns. Paired linear opacities or numerous small circular opacities represent thickening of the large- and medium-sized airways or of the septa around the lobules. This pattern occurs in cases of equine bronchitis and 295
bronchiolitis. Variations in the size, shape, and number of the pulmonary vessels cause a vascular pattern and 296
may be visible in horses following exercise or in horses with left-to-right cardiac shunts.

Extraparenchymal disorders that are evident radiographically include the presence of free pleural fluid or of free gas (pneumothorax) represented by the separation of the right or left or both caudal lung lobes from the dorsal and dorsolateral body wall by a free-air density.

7.1.4 ULTRASONOGRAPHY

Thoracic ultrasonography is useful for diagnostic, therapeutic, and prognostic evaluation of peripheral parenchymal lung or pleural disorders. Unlike thoracic radiography, which requires technology limited to specialty practices or veterinary medical teaching hospitals, ultrasonography is a method readily available to the practicing veterinarian. Ultrasonography is considered to be superior to thoracic radiography for detecting pleural effusion, pulmonary consolidation, pulmonary or mediastinal abscesses, tumors, or granulomata[28] and should be performed when clinical examination or thoracic percussion reveals pain and areas of dullness within the thorax.

Normal lung tissue reflects the ultrasound beam, producing an echogenic pulmonary periphery (thin white line) and reverberation artifacts or concentric equidistant echoes. Normal pleural fluid appears as an anechoic (black) area separating the parietal pleura from the lung tissue, and one commonly detects a small amount of pleural fluid in the ventral thorax of racehorses.[31] In respiratory disorders, one may detect accentuated amounts of anechoic or hypoechoic (gray) pleural fluid. The clinician can determine the character of the pleural fluid, the presence of fibrin or gas, the degree of loculation, and the existence of pleural adhesions during the examination. Pulmonary abscesses appear in ultrasonography as encapsulated cavitated areas filled with fluid or echogenic (white) material, whereas areas of pulmonary consolidation appear as dense patterns of homogeneous internal echoes with a gray tone.[32] Depending on the degree of consolidation, one may visualize bronchial and vascular

structures more easily on the sonogram, as well as mediastinal masses. Detection of caudal mediastinal masses improves when pleural effusion is concurrent, because the aerated caudal lungs impair examination. One may visualize masses located in the cranial mediastinum at the third right intercostal space in the absence of pleural effusions.

Diagnostically, certain limitations are inherent in ultrasonography. One may not detect a deep parenchymal lesion if the overlying aerated lung reflects the ultrasound beam. In addition, cases of pneumothorax may be difficult to identify because the free air in the dorsal thorax and the aerated ventral lung appear similar with ultrasound.[28] One also may use ultrasonography prognostically. The detection of free gas echoes (associated with anaerobic bacterial infections or bronchopleural fistulae), extensive fibrinous tags, or areas of loculations within the pleural fluid are associated with a poorer prognosis requiring a more extensive therapeutic regimen.[33]

7.1.5 THORACOCENTESIS

Sampling of the pleural fluid by means of thoracocentesis is beneficial for diagnostic, prognostic, and therapeutic purposes. Abnormal pleural fluid accompanies numerous respiratory disorders, including pulmonary abscessation (pleuropneumonia), chronic pneumonia, systemic mycoses, neoplasia, pulmonary granulomata, and equine infectious anemia. One may perform the technique easily at the sixth or seventh intercostal space approximately 10 cm dorsal to the olecranon by aseptically inserting a teat cannula through an anesthetized site in the intercostal space, just cranial to the border of the rib. To reduce the amount of air aspirated into the pleural cavity, one attaches a three-way stopcock to the cannula. The clinician should take samples from both sides of the thorax and submit the aspirate for cytologic and microbiologic examination. One may obtain up to 100 ml of pleural fluid, although smaller amounts (10 to 30 ml) are more routine.[34] Normal pleural aspirates contain less than 10,000 nucleated cells per µl (60% of which are neutrophils) and less than 2.5 g/dl of protein. Samples should be cultured aerobically and anaerobically. Fluid with a putrid odor is associated with anaerobic bacteria and carries a less favorable prognosis for the horse.[35]

7.1.6 NUCLEAR MEDICINE IMAGING

Scintigraphy, or nuclear medicine imaging, is a specialized technique available at some university and practice facilities. Using γ-emitting radioisotopes such as krypton-81m or technetium-99m, the clinician can assess pulmonary ventilation and perfusion in the horse.[36,37] The horse, breathing through a closed circuit, inhales aerosolized technetium particles generated by a nebulizer. Aerosolized particles are of sufficiently small diameter to be deposited in the alveoli and small airways. Thus their distribution mirrors ventilation. A gamma camera records the sites of deposition within the lung fields. For the perfusion scan, one injects technetium-labeled macroaggregated albumin intravenously. The large protein particles lodge in the blood capillaries of the lung, enabling imaging of the pulmonary perfusion by the gamma camera. Hence lung scintigraphy permits evaluation of the ratio of regional ventilation to perfusion ($\dot{V}$ / $\dot{Q}$) not possible by radiography or ultrasonography. Scintigraphic images may provide additional insights into the diagnosis and pathogenesis of such disorders as chronic obstructive pulmonary disease or exercise-induced pulmonary hemorrhage (EIPH) or 296
in the evaluation of the horse with poor performance.[38,39] For example, horses with EIPH appears to have a 297
perfusion deficit in the caudodorsal lung lobe that results in a high V/Q area.[38] Horses with chronic obstructive pulmonary disease may produce several patterns, including V/Q deficits in the costophrenic angle (caudoventral diaphragmatic margin), ventilation deficits in the middorsal lung area, or patterns similar to those seen in EIPH.

[40] In addition to assessment of pulmonary ventilation and perfusion, scintigraphy can show tracheal mucus transport after intratracheal injection of technetium. One performs the technique by timing the movement of the radioactive bolus over a given tracheal distance.[41,42] Normal values for the unsedated horse range from 16.6 to 20.7 mm/min. Further studies are needed to examine alterations in tracheal mucus transport during disease. For further information, the reader is referred to additional reviews.[40]

7.1.7 PULMONARY FUNCTION TESTING

Measurements of lung volumes, pleural (esophageal) pressure changes, and airflow, coupled with nitrogen washout studies and arterial blood gas determinations, have been used to assess horses with pulmonary disease. Pulmonary function testing requires that the horse wear a breathing apparatus to which an airflow meter has been attached. Pleural pressure changes occurring during each breath are estimated by catheters placed in the midesophagus, exteriorized through the nares, and attached to pressure transducers. By integrating airflow relative to time, one obtains the inspiratory and expiratory volumes. Additional parameters obtained from measurements of airflow include inspiratory and expiratory times, breathing frequency, and peak airflows. In general, the simple measurement of tidal volume, breathing frequency, or minute volume (the product of tidal volume and breathing frequency) provides limited information regarding the functionality of the lung, because these values tend to be maintained near normal limits until the respiratory disease is advanced.[43]

Measures of lung distensibility (dynamic compliance) and airway obstruction (pulmonary resistance) provide more meaningful information regarding pulmonary health. One measures dynamic compliance (C_{dyn}) by dividing the tidal volume by the change in pleural pressure occurring between the start and end of inhalation. One measures pulmonary resistance by several different techniques, depending on whether one measures the resistance at peak airflow or at specific ventilatory volumes (e.g., 50% tidal volume), and calculates it by dividing airflow by the change in pleural (esophageal) pressure. Alterations in these two values can provide information on the nature of the lung disorder. For example, in obstructive disorders of the tracheobronchial tree, dynamic compliance decreases and pulmonary resistance increases. A decrease in dynamic compliance in the absence of a change in pulmonary resistance suggests that the lung parenchyma has been stiffened by alveolar disease or by obstruction of the peripheral bronchioles. (One may recall that peripheral bronchioles, because of their immense cross-sectional area, contribute little to the resistance of breathing until the disorder is well advanced.) Conversely, an increase in pulmonary resistance in the absence of a change in dynamic compliance suggests that the obstruction exists in the upper airway, trachea, or bronchus.[44]

Currently, measures of airway hyperreactivity in horses are becoming more routine at referral centers.[45–47] In this technique, one determines the dosage of a nebulized bronchoconstrictor agent, such as histamine or methacholine, that causes a 35% increase in baseline respiratory resistance or a 35% decrease in baseline lung compliance. As might be predicted, the dosage to achieve this in a horse with inflammatory airway disease or recurrent airway disease is much smaller than that needed to achieve a similar effect in a healthy horse. Measurement of airway hyperreactivity requires that the horse be sedated and outfitted with an airtight breathing mask. Laboratory assistants should wear protective masks to prevent inhalation of the bronchoconstrictor agent.

Pulmonary function testing of *exercising* horses and its use in assessing the poor performer is a new diagnostic approach offered at select referral centers. With the availability of high-speed treadmills and the ability to measure airflow during exercise,[48] analysis of tidal volume, breathing frequency, dynamic compliance, lung resistance, end-expiratory lung volume, and flow-volume and pressure-volume loops may provide additional

insights into the cause of the poor performance, the recognition of expiratory flow limitation, and the documentation of EIPH on respiratory mechanics.

7.1.8

LUNG BIOPSY

A histopathologic diagnosis may prove useful in the therapeutic management of certain lung disorders. Percutaneous lung biopsy has been used to investigate disorders (1) characterized radiographically by a pulmonary miliary pattern and (2) disorders for which radiographic or ultrasonographic results are compatible with pulmonary neoplasia or granuloma.[49] Raphel and Gunson originally described the methodology and application of this technique in equine medicine.[50] The clinician inserts the biopsy instrument aseptically through the seventh or eighth intercostal space, approximately 8 cm above a horizontal line through the scapulohumeral articulation. The technique is not recommended in patients that are tachypneic, are in respiratory distress, exhibit uncontrollable coughing, or have bleeding disorders. The technique is not indicated in cases of 297
pulmonary abscessation, pleuropneumonia, or pneumonia.[49] The most common complications observed with 298
lung biopsy are epistaxis, pulmonary hemorrhage, tachypnea, and respiratory distress. Hemothorax also may develop following lung biopsy.[51]

7.2

Disorders of the Upper Respiratory Tract

One may encounter a variety of disorders of the upper respiratory tract in horses. Presenting complaints for upper airway disorders may include dyspnea (especially inspiratory), nasal discharge, dysphagia, lymphadenopathy, swelling or pain in the throatlatch region, or decreased exercise tolerance. Endoscopic or radiographic examination of the head and upper airway facilitate a diagnosis of most such disorders.

7.2.1

SINUS DISORDERS

7.2.1.1

Sinusitis

7.2.1.1.1

Anatomic Considerations

Six pairs of sinuses communicate with the nasal cavity in the horse: the dorsal, middle, and ventral conchal and the maxillary, frontal, and sphenopalatine.[52] The conchal sinuses, extensions of the turbinates, communicate with the frontal (dorsal conchal) or maxillary sinuses (middle and ventral conchal). However, most clinically important conditions involve the maxillary and frontal sinuses. The sinuses are lined by a respiratory epithelium—pseudostratified ciliated columnar—interspersed with goblet cells and underlying serous glands.[53]

The maxillary sinus is divided into a rostral and caudal compartment by a bony oblique septum. In most horses the division is complete so that no communication exists between the two compartments. Each compartment communicates with the middle nasal meatus by the nasomaxillary opening, a narrow slit that is occluded easily during inflammation of the mucosa. This process leads to retention of exudate within the sinuses. In horses less than 5 years of age, the last three cheek teeth—the first, second, and third molars—occupy most of the maxillary sinus. As the horse ages and the residual root decreases, the sinus cavity enlarges. The larger frontal sinus communicates with the caudal compartment of the maxillary sinus via the

frontomaxillary opening, thus establishing a natural drainage route of the frontal sinus with the nasal cavity. [54]

7.2.1.1.2
Causes

Primary sinusitis or empyema reflects accumulation of exudate within the sinus cavities and is a sequela of viral or bacterial upper respiratory tract infections. Streptococcal (and rarely staphylococcal) organisms are the usual bacterial isolates. Secondary sinusitis (empyema) of the maxillary sinus usually is associated with dental disorders such as fractured teeth, patent infundibula, and alveolar periostitis. The first molar is the most commonly involved tooth. However, secondary sinusitis may follow traumatic head injuries or the development of congenital paranasal cysts. The latter usually are found in the maxillary sinus but also have been identified in the frontal sinus.[55,56] Neoplasms associated with secondary sinusitis include squamous cell carcinoma (most common), osteogenic sarcoma, lymphosarcoma, myxoma, osteoma, sarcoids, neurofibroma, and mast cell tumor.[57,58] Although rare, fungal granulomata induced by *Cryptococcus neoformans* and *Coccidioides immitis* may cause secondary sinusitis.[59,60] Progressive hematomas also may occur in the maxillary sinus.[57]

7.2.1.1.3
Clinical Signs

Clinical signs depend on the inciting agent, its location, and the chronicity of the disorder. A unilateral nasal discharge suggests unilateral sinus involvement; a bilateral nasal exudate suggests that the right and left sinuses are involved. Sinusitis following dental disease or invasive neoplastic masses is characterized by a purulent foul-smelling and persistent nasal discharge, whereas a serosanguineous exudate is more typical of sinus cysts, slowly growing neoplasms, and certain stages of mycotic granulomata and hematomas. Inflammatory reactions of the skin, subcutaneous tissues, teeth, and bones may produce facial asymmetry. When sinus inflammation extends into the periorbital region, exophthalmos may ensue. Other clinical signs associated with sinusitis include breathing difficulties, epistaxis, epiphora, headshaking, weight loss, and neurologic signs if the sinusitis extends through the cribriform plate and causes meningoencephalitis.

7.2.1.1.4
Diagnosis

Diagnosis is based on the history of the disorder, the age of the animal, and the nature of the clinical signs. Percussion of the sinuses may reveal dullness. Endoscopic examination helps to eliminate other potential sources of nasal discharge and demonstrates the presence of an exudate from the nasomaxillary opening, visualized from the middle meatus just proximal to the ethmoids. This visualization confirms that the exudate originates from the sinus compartment. One may further define the sinus disorder by sinuscopy or computed tomography scan. The clinician always should conduct a thorough oral examination of the upper dental arcade even if it requires short-acting anesthesia. The presence of fractured or displaced teeth, receding gum lines, exudate around a specific tooth, or patent infundibula suggests that dental disease is the cause of the disorder. Lateral and dorsoventral radiographs are helpful in diagnosing sinusitis and may demonstrate single or multiple horizontal fluid-air interfaces, abnormalities of the teeth, lysis of alveolar bone, or a combination of these (Figures 7-6 and 7-7). Nuclear scintigraphy may indicate increased uptake of radioisotope around an infected tooth root.[61] Neoplastic lesions may be visible radiographically as loculated or diffuse soft tissue densities. Culture and cytologic examination of the sinus fluid or biopsy of 298
the tissue mass is helpful in the diagnosis, although differentiating between a neoplastic or dysplastic 299

process or inflammatory reaction may be difficult. Tumors may vary in consistency but usually have gelatinous areas mixed with combinations of cysts and solid tissue (fibrous tissue).[62]

Figure 7-6 Five-year-old Thoroughbred with 7-month history of left-sided nasal discharge.**A,** Air-fluid interface *(arrows)* in the caudal maxillary sinus on the lateral radiograph resulting from dental disease and consistent with maxillary sinusitis. **B,** Increased soft tissue opacity in the left *(L)* maxillary sinus on the dorsoventral radiograph is notable. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)

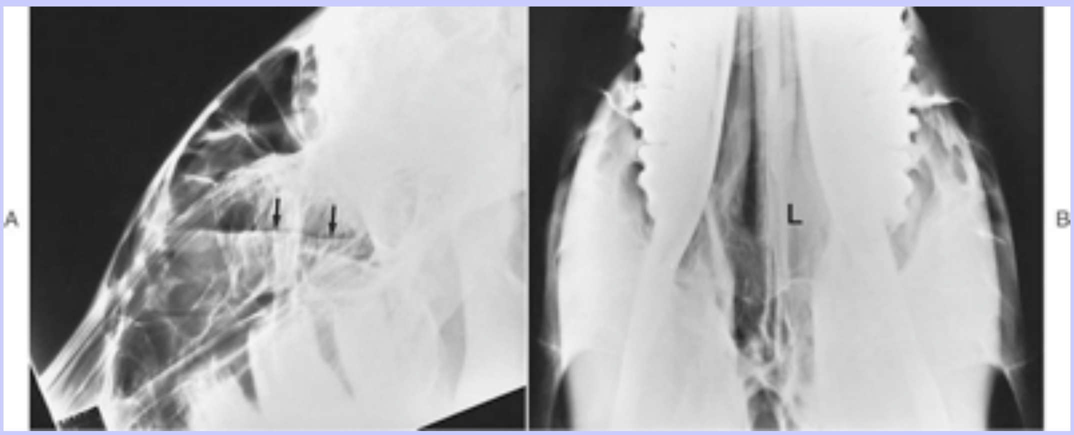
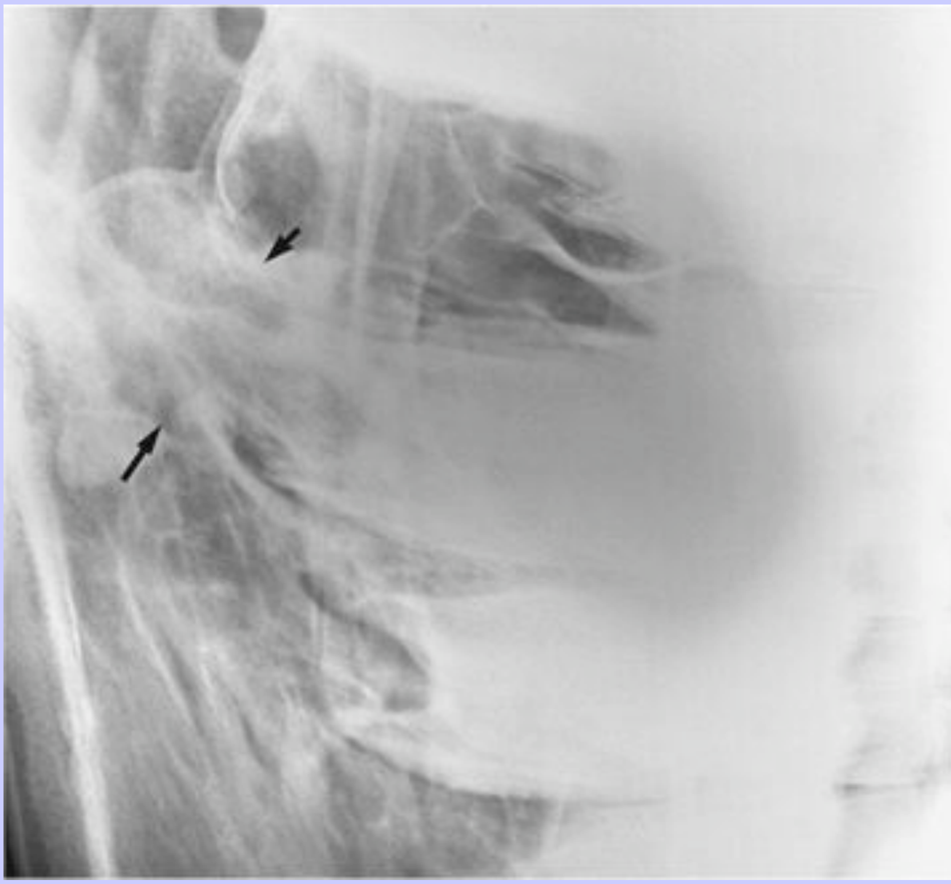


Figure 7-7 Lateral oblique radiograph of midmaxillary cheek teeth. The fourth cheek tooth had been removed previously. Sclerotic bony reaction *(arrows)* surrounds the root of the third cheek tooth. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



7.2.1.1.5

Treatment

Treatment requires surgical removal of the affected teeth or tumorous or granulomatous tissue and establishment of adequate drainage. Extensive flushing of the sinuses has been recommended until the trephine sites have healed. Surgical removal of paranasal cysts has been associated with a favorable prognosis,[56] whereas fungal granulomata and neoplasms have a poor prognosis. With neoplastic lesions, surgical resection or ablation apparently achieves a low rate of success because of extensive infiltration of the neoplasm or recurrence of the tumor.[58,62]

7.2.1.2
Ethmoid Hematomas

7.2.1.2.1
Definition

Ethmoid hematomas are encapsulated, expansive angiomatous masses that appear to develop from the mucosal lining of the ethmoid conchae but also may originate from the walls of the maxillary and frontal sinus. The inciting factor in their development is not known. Some have speculated that ethmoid hematomas develop following chronic infection, repeated episodes of hemorrhage, or congenital or neoplastic conditions.[63] They appear bilaterally in 50% of the cases and are more prevalent in older horses.[64]

7.2.1.2.2
Clinical Signs

Clinical signs include intermittent unilateral discharge consisting of frank blood or serous or mucopurulent exudates. Stertorous breathing because of obstruction of nasal air flow also may occur.[63] Clinical signs also may include facial swelling, exophthalmos, malodorous breath, headshaking, and coughing.[59] 299

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7.2.1.2.3
Diagnosis

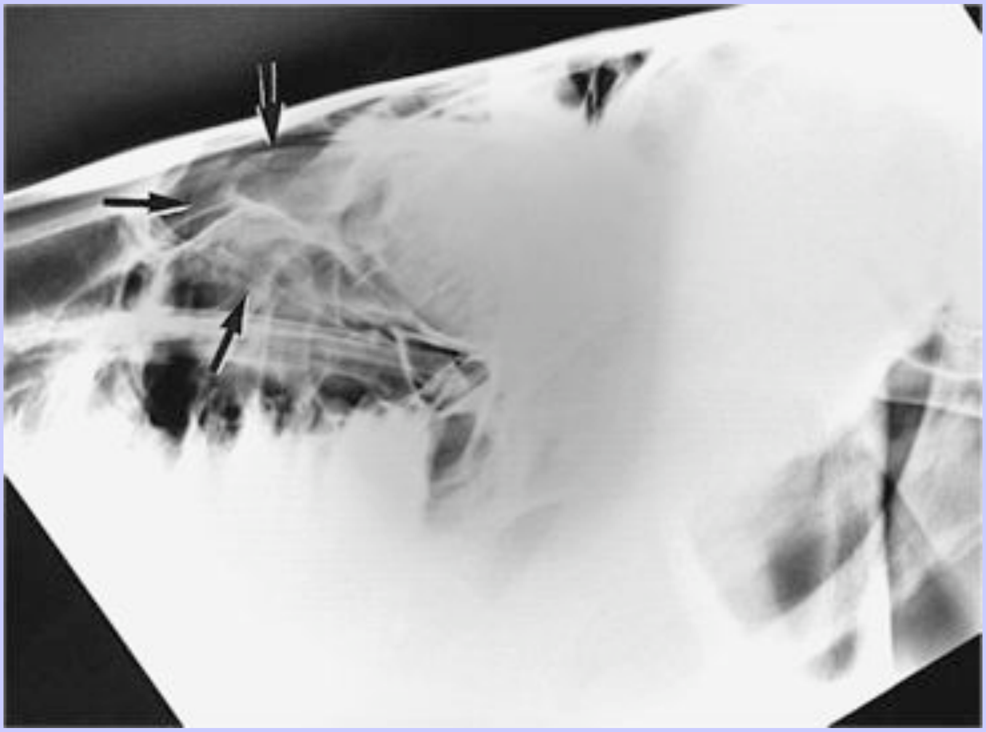
Diagnosis is based on the clinical signs, endoscopic examination, radiographic evaluation, and in some cases computed tomography scan. Confirmation of a diagnosis is by histopathologic study of the removed tissue. Endoscopy (see Figure 7-2) reveals a yellow, yellow-green, yellow-gray, red to red-purple smooth glistening mass originating from the ethmoid region.[63] One also may note petechial hemorrhages or surface erosions. The mass may protrude beyond the nasal septum (and in those cases may cause a bilateral nasal discharge). Radiographs reveal a space-occupying soft tissue density with smooth margins. Single or multilobular rounded opacities may be visible radiographically in the ventral aspect of the caudal maxillary sinus superimposed on the ethmoid turbinates. The ethmoid hematoma in some cases may extend dorsally into the frontal sinus (Figure 7-8).

7.2.1.2.4
Treatment

Treatment options include surgical resection, cryosurgery, laser ablation, and formalin injection. Surgical removal is associated with extensive hemorrhage, and surgery may require blood transfusions. A preoperative crossmatch is warranted, and the horse may require a postoperative tracheotomy if one uses extensive packing of the nasal cavity to effect hemostasis. Antimicrobials and antiinflammatory agents are indicated. Following surgical removal, approximately 20% to 50% of the hematomas recur.[63,64] Postoperative complications may include facial wound dehiscence, suture periostitis, facial bone sequestration, persistent nasal discharges, fungal sinus plaque formation, and encephalitis.[65]

Transendoscopic injection of lesions with formalin is an alternative to surgical resection, particularly for smaller lesions. Most cases require multiple injections.[66] Severe complications also may ensue if the cribriform plate is fenestrated, allowing communication of the ethmoidal hematoma with the cranial vault.[67]

Figure 7-8 Ethmoidal hematoma. Focal increased soft issue opacity *(arrows)* adjacent to the ethmoids is notable. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



7.2.2 GUTTURAL POUCH DISORDERS

7.2.2.1 Anatomic Considerations

The guttural pouches are caudoventral diverticula of the auditory tubes the functions of which remain undefined, although some investigators have suggested that the pouches play a role in cooling the arterial supply to the brain.[68] Each pouch has a capacity of 300 ml and is divided into a medial and lateral compartment by the invagination of the stylohyoid bone. The mucosal lining of each pouch is secretory, being covered by ciliated pseudostratified epithelium with goblet cells and glands.[69] The mucosal lining is generally thinner than that found in the nasopharynx and also contains small aggregates of subepithelial lymphocytic tissue.[53]

Disorders of the guttural pouches often induce dysfunctions of the surrounding neural structures—cranial nerves VII, IX, X, XI, and XII and the sympathetic trunk, or cause erosion of the vascular structures—the internal carotid, the external carotid, and the maxillary arteries.

7.2.2.2 Tympany

7.2.2.2.1 Definition

Tympany is a nonpainful distention of the guttural pouch by air that may produce an external swelling in the parotid region. Congenital tympany occurs in young foals (predominantly fillies), and acquired tympany usually affects older horses.[70]

7.2.2.2.2 Pathogenesis

The cause of congenital guttural pouch tympany is unknown. An abnormal mucosal flap at the pharyngeal orifice has been proposed to function as a unidirectional valve, trapping air or fluid within the pouch. Tympany is more likely a functional defect rather than an anatomic defect because no abnormality is visible endoscopically or during surgical exploration. Acquired tympany typically is associated with upper respiratory infection and is thought to be caused by swelling of tissues around the pharyngeal orifice, causing a one-way valve effect. This problem is transient, and rarely does pouch distention become severe.

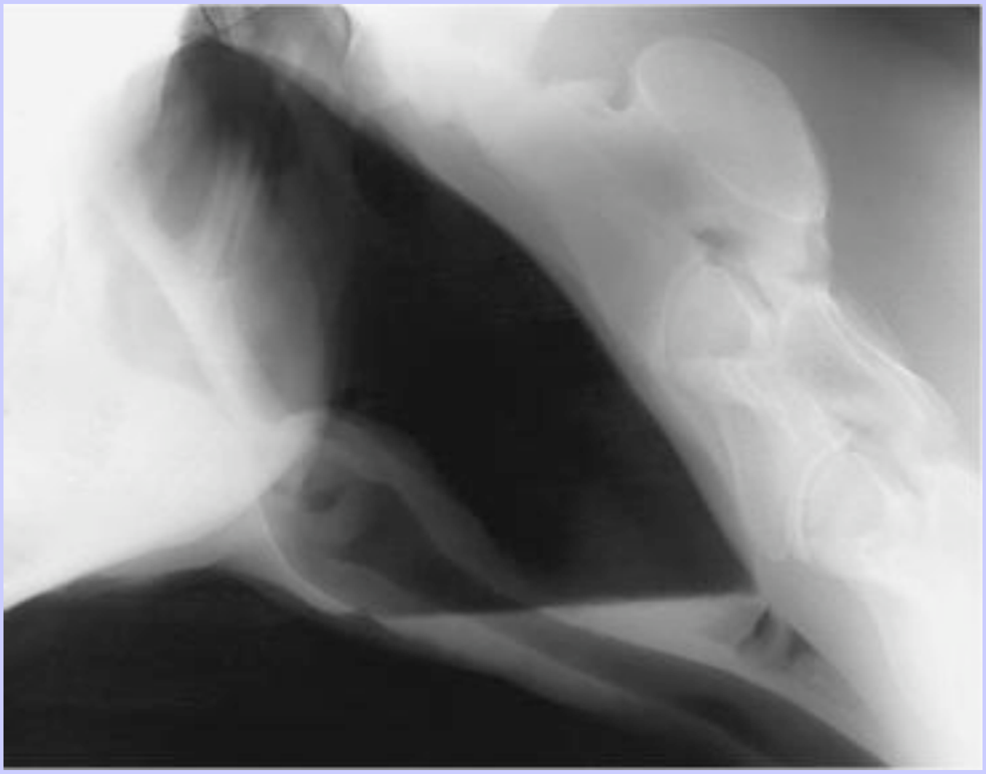
7.2.2.2.3 Clinical Signs

Clinical signs depend on the degree of pouch distention and hence the degree of compression of the nasopharynx. If distention is significant, the foal may exhibit stertorous breathing, respiratory distress, dysphagia, nasal discharge, or evidence of pneumonia caused by aspiration. Regurgitation of milk from the nostrils also may be evident.[71] Endoscopic examination may reveal significant compression of the nasopharyngeal area because of distention of the pouches.

7.2.2.2.4 Diagnosis

Confirmation of diagnosis is by the presence of tympanitic swelling in the Viborg's pouch area.
Radiographs reveal a large, air-filled guttural pouch with or without fluid accumulation (Figure 7-9). The 300
distinction as to whether the problem is unilateral or bilateral can be difficult. Catheterization of one 301
guttural pouch should correct the problem if a unilateral tympany exists. Dorsoventral radiographic views may help in diagnosing bilateral involvement.

Figure 7-9 Guttural pouch tympany. Lateral radiograph of 5-month-old Arabian colt demonstrating a greatly distended gas-filled pouch. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



7.2.2.2.5
Treatment

Guttural pouch tympany requires surgical correction. For unilateral tympany, one performs fenestration of the median septum separating the two guttural pouches by conventional surgery or transendoscopic laser surgery. Bilateral involvement may necessitate resection of the excessive plica salpingopharyngeal flap.[72] Because many cultures of the pouches yield β-hemolytic streptococci and the potential for the development of aspiration pneumonia exists, administration of antimicrobials is justified.[70] The prognosis for uncomplicated cases of guttural pouch tympany is favorable.

7.2.2.3
Empyema

7.2.2.3.1
Definition

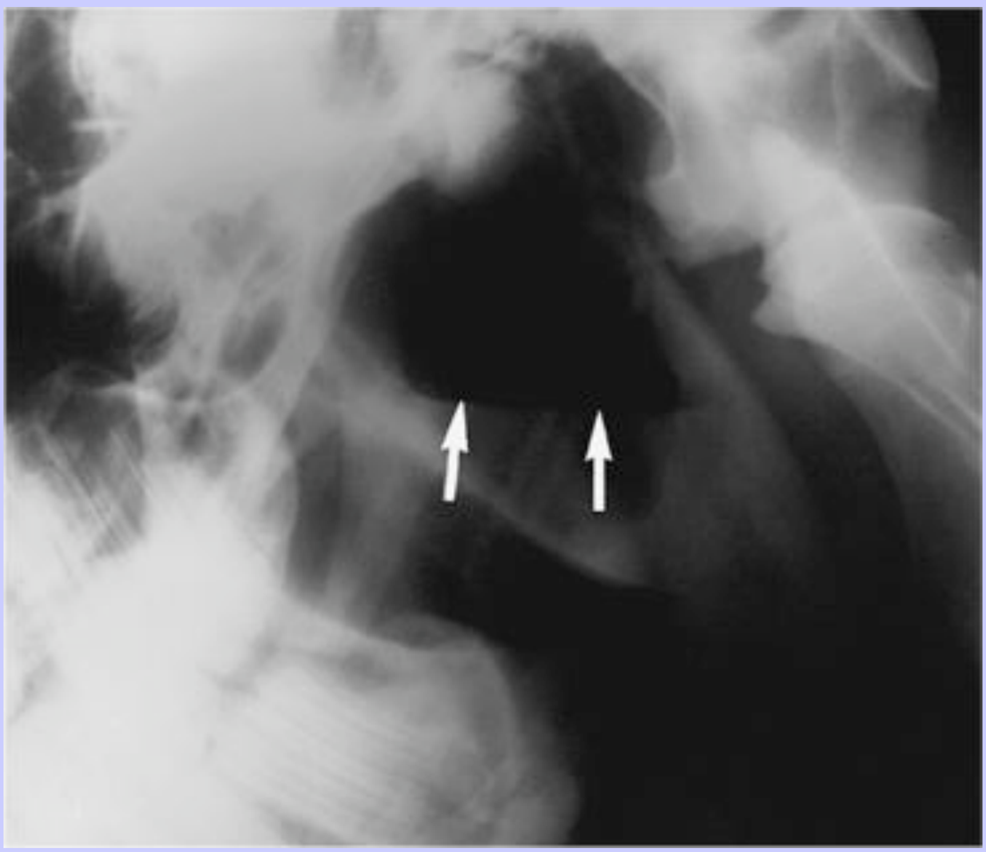
Empyema is an accumulation of exudate within the guttural pouches and is usually a sequela of upper respiratory tract infections (*Streptococcal* spp.). In a recent survey, *Streptococcus equi* was isolated from 32% of the cases evaluated for empyema.[73] Empyema may also result from the rupture of abscessed

retropharyngeal lymph nodes into the pouches[71] or may accompany cases of guttural pouch tympany. The condition may be unilateral or bilateral.

7.2.2.3.2 Clinical Signs

Clinical signs include a white nonodorous nasal discharge (unilateral or bilateral), lymphadenopathy, painful distention in the parotid region, stertorous breathing, dysphagia, and occasionally epistaxis.[71] Inspissation of the material may occur with chronic infections, forming masses called chondroids.

Figure 7-10 Guttural pouch empyema. Lateral radiograph shows a fluid line *(arrows)* or air-fluid interface within the guttural pouch. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)

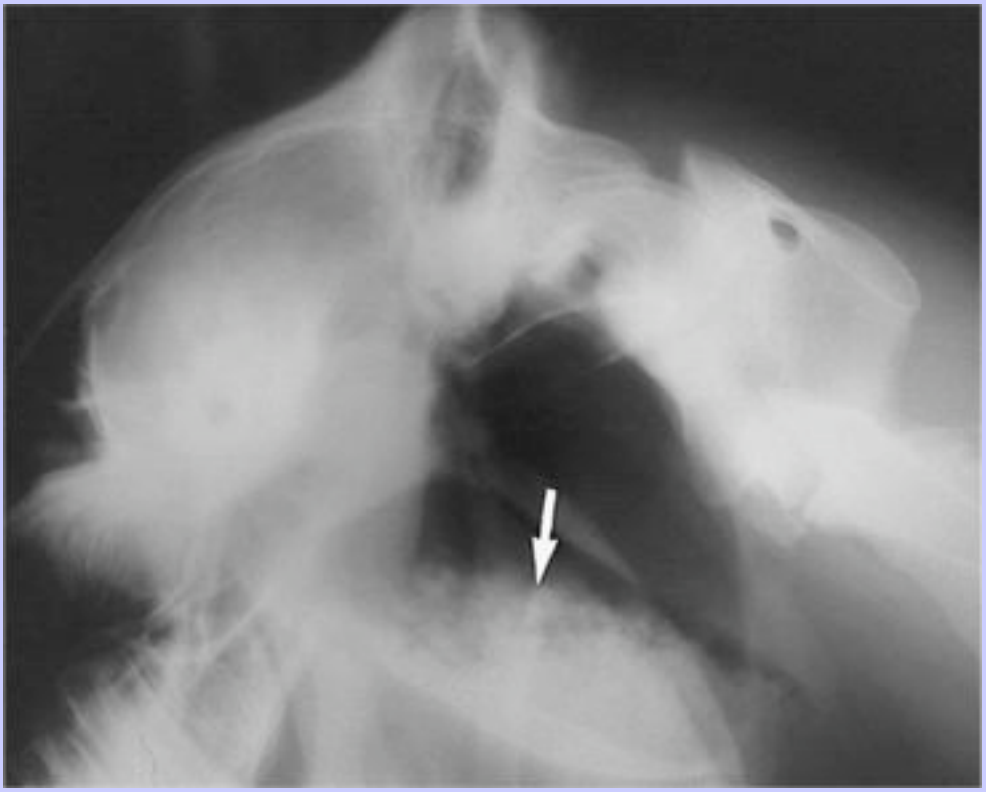


7.2.2.3.3 Diagnosis

Confirmation of diagnosis is by radiographic examination or endoscopy. Radiographs demonstrate a fluid line or an opacity in the pouch (Figure 7-10). Inspissated material also may be evident radiographically 301
(Figure 7-11). Endoscopic examination may reveal a purulent material at the pharyngeal orifice of the 302
auditory tubes and within the medial or lateral compartments of the guttural pouches. Small pebblelike

structures, chondroids, also may be visible endoscopically. Distortion of the pharynx may occur if distention is significant.

Figure 7-11 Guttural pouch chondroids. Lateral radiograph demonstrates irregular soft tissue opacities in the ventral guttural pouch outlined by gas *(arrow)*. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



7.2.2.3.4

Treatment

Because *S. equi* may be involved, the clinician should isolate affected horses and take precautions to avoid spread of the bacteria (see the following discussion). Treatment may entail medical and surgical modalities. Aggressive lavage of the guttural pouch with saline solutions and the administration of systemic antimicrobials is a first step in therapeutic management.[73] One may attempt removal of chondoids with an endoscopic snare, avoiding the complications or risk of surgery and minimizing the cost of treatment.[74] Surgery may be necessary if medical therapy is unsuccessful.[71]

7.2.2.4
Mycosis

7.2.2.4.1
Definition

Guttural pouch mycosis is characterized by development of fungal plaques on the mucosal walls of the guttural pouches. The plaques usually are found at two sites, with the majority of them located on the roof of the medial compartment and less frequently on the lateral wall of the lateral compartment of the pouch. The plaques are associated closely with the underlying vascular structures, the internal and external carotid arteries, or the external maxillary artery.[75]

7.2.2.4.2
Pathogenesis

The events leading to the formation of fungal plaques are not known. Some workers have suggested that aneurysmal dilations of the vasculature, visualized radiographically,[76] provide a suitable environment for fungal organisms to proliferate. Fungal colonization leads to erosion of the underlying mucosa and vascular structures or inflammatory injury to the adjacent nerves.

7.2.2.4.3
Clinical Signs

Clinical signs depend on the integrity of the neural and vascular structures surrounding the guttural pouch. Horses may exhibit episodes of spontaneous epistaxis, dysphagia, nasal catarrh, laryngeal hemiplegia, Horner's syndrome, abnormal head extension, swelling in the parotid region, facial paralysis, mycotic encephalitis, and atlantooccipital joint infections.[77] The stress of being handled may precipitate fatal epistaxis in the horse.

Although guttural pouch mycosis is uncommon in the young horse, it has been reported in foals less than 6 months of age.[78,79]

7.2.2.4.4
Diagnosis

Confirmation of diagnosis is by endoscopic observation of a fungal plaque in the guttural pouch. Shortly after an acute bout of epistaxis, endoscopic examination can confirm that blood is exiting the pouch, but attempts to visualize the interior of the pouch may be unsuccessful if blood obscures the visual field of the endoscope. Some risk exists of dislodging a blood clot if one performs endoscopic examination of the pouch.[75] Endoscopic confirmation of secondary neuropathies such as pharyngeal paralysis or laryngeal hemiplegia supports the diagnosis of guttural pouch mycoses.

Radiographs of the guttural pouch may show evidence of fluid accumulation or osteolytic changes in the stylohyoid bone or may suggest mycotic plaque formation. Angiographic demonstration of aneurysms of the internal or external carotid artery supports the diagnosis and may aid in surgical planning.

7.2.2.4.5

Treatment

Treatment depends on surgical occlusion of the affected vessels. Several different techniques have been advocated.[75,80–83] Current therapies of choice use an intravascular balloon or coil to allow obliteration of arterial flow proximal and distal to the fungal lesion. Complications, including ischemic optic neuropathy and those associated with aberrant vasculature, have been reported.[84,85] Lane[75] has recommended that surgical treatment be combined with topical therapy (natamycin irrigation of the guttural pouch) and supportive care. Horses that have dysphagia may need enteral support via nasogastric intubation or esophagostomy. Medical treatment alone, with topical or parenteral antimycotic drugs, is not efficacious in eliminating the mycotic plaques.

7.2.3

PHARYNGEAL AND LARYNGEAL DISORDERS

7.2.3.1

Lymphoid Hyperplasia

7.2.3.1.1

Definition

Acute inflammation of the lymphoid (and surrounding) tissues in the pharynx is termed *pharyngitis*. The condition occurs with equine influenza, EHV1, EHV2, and EHV4 and with *Streptococcus equi* infections. Acute pharyngitis also may develop with prolonged nasogastric intubation. Chronic inflammation of the pharynx, also termed *pharyngeal lymphoid hyperplasia, lymphoid follicular hyperplasia,* and *pharyngeal folliculitis,* is a condition frequently observed in weanlings to performance horses 2 to 3 years of age.[86]

7.2.3.1.2

Causes

The cause of chronic pharyngitis is not known but probably is multifactorial. Many horses have a history of upper respiratory tract infections, causing some clinicians to speculate that lymphoid hyperplasia is a sequela of chronic antigenic stimulation. The condition has been reproduced experimentally with inoculation of EHV1 and EHV2.[87,88] The severity of the pharyngeal lymphoid hyperplasia does not correlate with EHV1 titers or with the isolation of EHV2.[89]

Streptococcus zooepidemicus, Bordetella bronchiseptica, and *Moraxella* species have been isolated from nasopharyngeal swabs of horses with lymphoid hyperplasia, but their role in the development of the lymphoid hyperplasia is also unknown. In one study the pharyngeal flora was similar in normal control horses and in those exhibiting grade I lymphoid hyperplasia. As the severity of the pharyngitis increased, the number of bacterial organisms that were isolated (colony-forming units per gram of swab material) also increased.[90] 302

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7.2.3.1.2.1

BOX 7-2 GRADING SCHEME FOR LYMPHOID HYPERPLASIA

Grade I: A small number of inactive, white follicles scattered over the dorsal pharyngeal wall. The follicles are small and inactive, a normal finding in horses of all ages.

Grade II: Many small, white inactive follicles over the dorsal and lateral walls of the pharynx to the level of the guttural pouch. Numerous follicles that are larger, pink, and edematous are interspersed throughout.

Grade III: Many large pink follicles and some shrunken white follicles are distributed over the dorsal and lateral walls of the pharynx, in some cases extending onto the dorsal surface of the soft palate and into the pharyngeal diverticula.

Grade IV: More numerous pink and edematous follicles packed close together covering the entire pharynx, the dorsal surface of the soft palate and epiglottis, and the lining of the guttural pouches. Large accumulations appear as polyps.

From Raker CW: The nasopharynx. In Mansmann RA, McAllister ES, editors: *Equine medicine and surgery,* Santa Barbara, Calif, 1982, Veterinary Publications.

The contribution of husbandry factors to the development or maintenance of lymphoid hyperplasia also has been examined. Young horses that are stabled indoors have a more severe pharyngitis than when kept outdoors with access to a three-sided shelter.[91]

7.2.3.1.3 Epidemiology

An inverse relationship appears to exist between the age of the horse and the prevalence of pharyngeal lymphoid hyperplasia. Approximately 60% to 90% of 2-year-olds exhibit a grade II or more (Box 7-2). Between 35% and 65% of 3- and 4-year-olds and 10% to 20% of 5-year-olds still show grade II or greater lymphoid hyperplasia. With aging, lymphoid follicles regress and tend to disappear.

7.2.3.1.4 Clinical Signs

Some horses may show a nasal discharge and mild submandibular lymphadenopathy. Manipulation of the larynx may induce a cough. Unless severe, lymphoid hyperplasia has not been associated with poor performance or alterations in arterial blood gases.[92]

7.2.3.1.5 Diagnosis

Endoscopically, one observes raised hyperemic and edematous follicles distributed throughout the nasopharyngeal walls. Some follicles may have an ulcerated edge to them; others may appear thickened and fibrotic. Raker[93] proposed a gradation scheme based on the severity of the nodule formation, as outlined in Box 7-2. Histopathologic examination reveals lymphocytic proliferation and necrosis, the degree of which does not correlate with endoscopic findings.

7.2.3.1.6 Treatment

In the absence of a clear understanding of the pathogenesis of this disorder, the most appropriate therapy has not been determined. One treatment that has been tried is nebulization (30 min/day) using an antiinflammatory solution (350 ml nitrofurazone, 125 ml dimethyl sulfoxide, and 500 mg prednisolone acetate). Stabling changes to reduce dust and mold exposure may be helpful.

7.2.3.2 Dorsal Displacement of the Soft Palate

7.2.3.2.1 Definition

During respiration, the caudal free border of the soft palate normally occupies a position ventral to the epiglottis. This position reverses abruptly during swallowing as the palate moves dorsally and the epiglottis covers the adducted arytenoid cartilages and vocal folds. These motor activities ensure that food or saliva is directed dorsally into the esophagus and not into the trachea.[94] The positioning of the palate is complex, controlled in part by the palatine, the tensor, and the levator muscles, which are innervated by the trigeminal, vagus, and glossopharyngeal nerves, respectively. Intermittent or persistent malpositioning of the soft palate dorsal to the epiglottis is termed *dorsal displacement of the soft palate* (DDSP).[95]

7.2.3.2.2 Causes

On occasion, persistent displacement is evident endoscopically at rest. This finding may be associated with pharyngeal paralysis of numerous causes, but in the absence of signs of dysphagia, the condition often is caused by mechanical or inflammatory mechanisms that obliterate the subepiglottic space (subepiglottic cyst, epiglottitis, epiglottic entrapment, subepiglottic fibrosis, etc.). In most cases of DDSP, palate position is normal on resting and displacement occurs only during strenuous exercise. The cause of displacement in such cases is unclear. Formerly suggested mechanisms including excessively negative intrapharyngeal pressures,[96] excessive poll flexion,[97] epiglottic shortening,[98] and caudal retraction of the larynx by the sternothyrohyoideus[94] now appear largely unfounded. Recent studies have focused on palatal innervation and on factors controlling the relative positions of the larynx and hyoid apparatus as potential causes of palate displacement. Bilateral blockade of the pharyngeal branch of the vagus nerve with local anesthetic has been shown to cause DDSP, implicating a dysfunction of the palate musculature or its innervation as the cause.[99] Furthermore, the observation that some horses with displacement have lymphadenopathy of retropharyngeal nodes that lie in close proximity to the pharyngeal nerves supports the hypothesis that a neuropathy is contributory.[100] Other investigators have evaluated the effects of altering the relationships between the larynx and hyoid apparatus through denervation or transection of various muscles inserting on 303
those structures. Selective transection of the thyrohyoideus muscle causes intermittent dorsal displacement 304
of the palate in some horses, leading to speculation that neuromuscular disorders of this muscle may predispose to displacement.[101] Recent observations that DDSP and nasopharyngeal collapse were observed in horses following desensitization of the laryngeal mucosa with local anesthetic suggests that receptors within the laryngeal mucosa may be important in maintaining upper airway patency in exercising horses.[102]

7.2.3.2.3 Pathogenesis

Along with dorsal displacement of the soft palate is a reduction in the cross-sectional area of the nasopharynx. This increases the resistance to airflow and may occlude the passageway completely, temporarily inducing asphyxia (choking down). The displacement creates a stertorous noise most obvious during expiration as the soft tissues vibrate. Because of the obstruction, airflow may be diverted through the mouth during expiration. A study of 10 horses exercising on a high-speed treadmill found that the time of displacement relative to exercise intensity varied, occurring in two horses at peak speed, in two horses before obtaining peak speed, and in six horses as they started to slow down during the exercise protocol.[96]

The time of displacement relative to the breathing-swallowing cycle also varied, with displacement occurring during inspiration in three horses, during expiration in three horses, and being associated with a swallow in four horses.[96]

7.2.3.2.4

Clinical Signs

The cardinal signs of DDSP are respiratory noise and decreased exercise tolerance. Signs are often dramatic, leading to the descriptor “choking down.” Affected horses typically make an inspiratory and expiratory noise, but the latter is much more prominent. The noise often is described as snoring, rattling, or gurgling. Occasionally, a horse may show an expiratory noise at rest or while eating. Clinical signs are ordinarily apparent only during strenuous exercise. In racehorses, a history of acute onset of noise and respiratory embarrassment is frequent. Normal respiratory function returns immediately when the horse swallows.

7.2.3.2.5

Diagnosis

Given the intermittent nature of the displacement, diagnosis is often difficult to confirm. Definitive diagnosis is by observation of displacement during endoscopic examination while the horse is exercising rigorously on a high-speed treadmill. With displacement the caudal border of the palate moves dorsal to the epiglottis, obscuring its shape. The caudal free border of the palate is evident, as is dorsal billowing of the palate on expiration. Before the actual displacement, a lowering of the pharyngeal roof, an elevation of the rostral portion of the soft palate, and a caudal retraction of the larynx are apparent.

In the absence of treadmill access, diagnosis is based subjectively on typical historical findings and resting endoscopic examination. An important note is that most horses examined at rest have no evidence of DDSP.This examination primarily enables the clinician to rule out other causes of upper respiratory obstruction. In some horses, endoscopic findings such as ready displacement by nasal occlusion, ulceration of the free border of the soft palate, or epiglottic hypoplasia or deformity may support a diagnosis of DDSP. Normal horses often exhibit palate displacement during resting endoscopy, especially if sedated, excessively restrained, or subjected to tracheoscopy.

Radiographs of the larynx may demonstrate a hypoplastic epiglottis, that is, one less than 7 cm in length. The clinician should perform a thorough examination of the lower respiratory tract (e.g., auscultation and endoscopy) on these horses to rule out concomitant pulmonary disease.

7.2.3.2.6

Treatment

Treatment varies and includes medical and surgical approaches. Medical therapy includes tying the tongue, use of a cavesson or figure-eight noseband to keep the mouth closed and treatment of concurrent upper respiratory disorders. One uses the tongue tie in an attempt to prevent caudal retraction of the larynx and subsequent displacement of the soft palate. Keeping the mouth closed is thought to protect the laryngopalatal seal. A short course of nebulization therapy using a solution of 350 ml of liquid nitrofurazone (Furacin), 125 ml of dimethyl sulfoxide, and 500 mg of prednisolone acetate (30 min/day for 7 to 10 days) may be helpful in horses with guttural pouch or pharyngeal inflammation.

The variety of surgical options for treating DDSP reflects the limited understanding of the cause of this disease. Described surgical treatments include resection of the caudal margin of the soft palate

(staphylectomy) to increase the size of the ostium intrapharyngeum,[95] partial sternothyrohyoideus myectomy to prevent caudal retraction of the larynx, and epiglottic augmentation by injection of Teflon into the epiglottal submucosa.[103] The current procedure of choice in North America is a combination of conservative staphylectomy and resection of the musculotendinous junction of sternothyroideus muscle, the Llewellyn procedure.[104] Dorsal displacement of the soft palate is a difficult condition to treat: the success rate for the myectomy and staphylectomy approaches 50% to 60%.[105]

7.2.3.3 Rostral Displacement of the Palatopharyngeal Arch

7.2.3.3.1 Definition

In the horse the soft palate terminates caudally by the confluence of the caudal pillars to form the palatopharyngeal arch that covers the esophageal orifice.[95] In rostral displacement of the palatopharyngeal arch, this fold of tissue appears to be displaced forward, overlying the apices of the arytenoid cartilages. Cook[106] first described the condition in 1974; others have diagnosed the condition since that time. 304

305

7.2.3.3.2 Causes

The displacement of the palatopharyngeal arch is associated with malformation of the thyroid cartilage and the cricopharyngeal and cricothyroid muscles. Goulden, Anderson, Davies, et al.[107] have speculated that the anomaly results from developmental defects of the fourth branchial arch, which normally forms the thyroid cartilage.

7.2.3.3.3 Pathogenesis

Cartilaginous and muscular defects have been found in this condition. A deformation of the thyroid cartilage, a lack of the cricothyroid articulation, and often an absence or agenesis of the cricothyroid and cricopharyngeal muscles occur.[107,108] Abnormal pharyngeal configurations prevent normal deglutition, predisposing horses to develop aspiration pneumonia.

7.2.3.3.4 Clinical Signs

The condition may be present from birth. In most cases, abnormal respiratory noise and poor athletic performance are the presenting complaints. In severe cases, horses may exhibit dysphagia, nasal discharge of food material, persistent coughing, and belching.[106,107]

7.2.3.3.5 Diagnosis

The diagnosis is based on the clinical signs and history confirmed with endoscopic examination. The rostrally displaced palatopharyngeal arch obscures the normal view of the apices of the arytenoid cartilages. Postmortem examination or surgical exploration of the larynx confirms the presence of developmental defects.

7.2.3.3.6

Treatment

Rostral displacement of the palatopharyngeal arch is an endoscopic sign representing a major deformation of the laryngeal structures. Resection of the arch by conventional surgery or laser surgery has not enabled successful athletic performance.[109] Severely affected horses are euthanized.

7.2.3.4

Epiglottic Entrapment

7.2.3.4.1

Definition

The aryepiglottic fold is the mucous membrane that extends from the lateral aspect of the arytenoid cartilages to the ventrolateral aspect of the epiglottis, where it blends with the subepiglottic mucosa and the glossoepiglottic fold. In epiglottic entrapment, this membrane envelops a portion of or all of the epiglottis.[95,110]

7.2.3.4.2

Causes

The cause of epiglottic entrapment is not understood completely. In most cases, the epiglottic cartilage and associated soft tissues appear normal. In occasional cases, congenital epiglottic hypoplasia or inflammation of the upper respiratory tract structures appears to contribute to entrapment.

7.2.3.4.3

Epidemiology

Epiglottic entrapment occurs predominantly in Standardbred and Thoroughbred racehorses, with males and females equally affected.[111]

7.2.3.4.4

Pathogenesis

Billowing of the entrapping membranes during respiration decreases the cross-sectional area of the pharynx and effectively obstructs the airflow, particularly during expiration.

7.2.3.4.5

Clinical Signs

Most horses show exercise intolerance and respiratory stertor. Horses occasionally may cough during exercise or while eating. Because entrapment may be an intermittent finding, one should confirm the persistence of the entrapment during exercise by endoscopic examination during exercise or immediately following completion of exercise.

7.2.3.4.6

Diagnosis

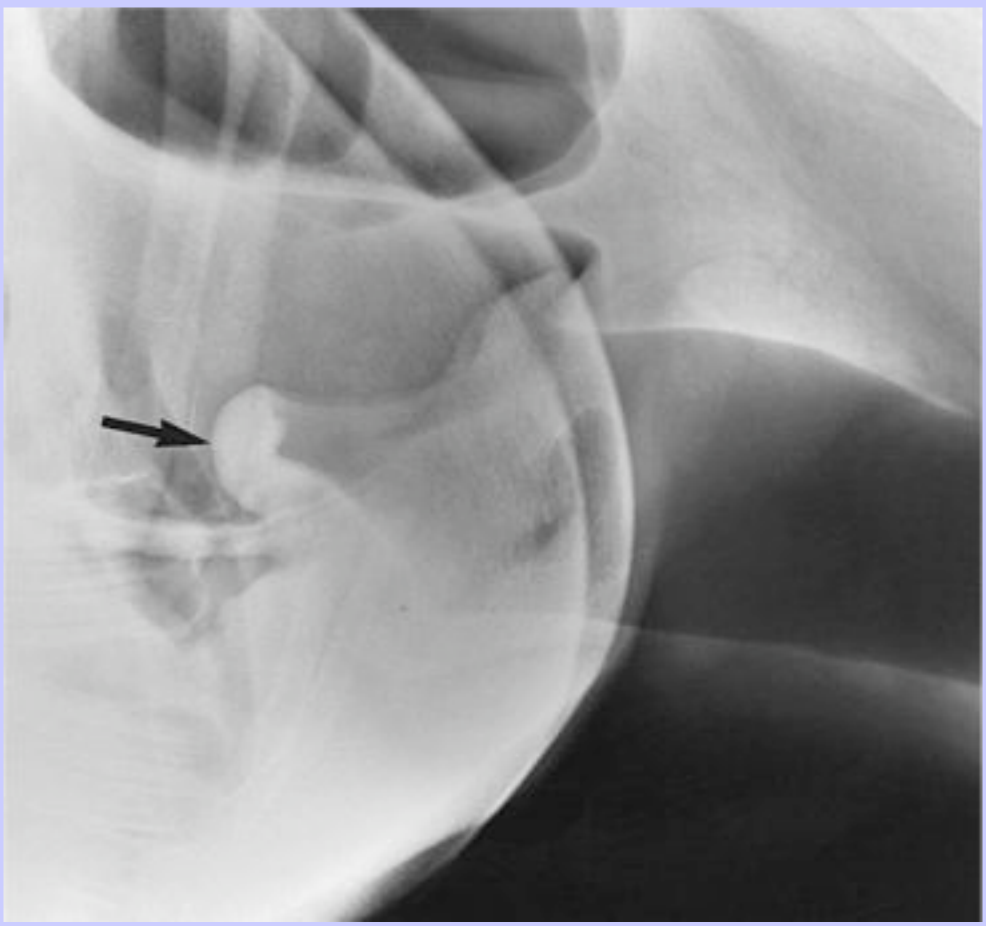
Diagnosis is based on endoscopic examination. The membrane obscures the normal serrated margin of the epiglottis and its dorsal vasculature. In contrast to dorsal displacement of the soft palate, one still can appreciate the shape of the epiglottis. Ulceration of the free margin of the fold and erosion of the entrapped

epiglottis may be apparent.[95] Epiglottic length may be determined radiographically.[98] One also may visualize the entrapment on lateral radiographs (Figure 7-12).

7.2.3.4.7
Treatment

Entrapment requires surgical correction. A number of different approaches have been advocated. One may accomplish resection or division of the tissue (1) under general anesthesia via pharyngotomy, laryngotomy, or by an oral approach; (2) transendoscopically, using a contact Nd:YAG laser; or (3) transnasally, using a hooked bistoury.[111,112] Augmentation of the hypoplastic epiglottis by Teflon submucosal injections has been advocated to increase the bulk of the epiglottis and thus offset some of the structural defects that may predispose to entrapment.[103] One performs this procedure through a ventral laryngotomy.

Figure 7-12 Seven-year-old male Standardbred with an entrapped epiglottis. Lateral radiograph of the larynx demonstrates a rounded distal end to the epiglottis *(arrow)*. The epiglottis also appears shortened. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



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7.2.3.5 Epiglottitis

7.2.3.5.1 Definition

Epiglottis is an acute inflammatory disorder affecting the epiglottis.

7.2.3.5.2 Causes

The cause of epiglottitis is unknown. Racehorses in active training are affected most commonly. Suggested causes include respiratory tract infection, allergens, palate displacement, epiglottic entrapment, inhaled foreign material, poor quality hay, and the stress of race training.[113]

7.2.3.5.3 Clinical Signs

Primary clinical signs include exercise intolerance, respiratory noise, and coughing. Dysphagia and respiratory distress are less common signs.

7.2.3.5.4 Diagnosis

Diagnosis is by endoscopic examination. Signs include epiglottic thickening and ulceration, edema and discoloration of the epiglottic mucosa and aryepiglottic folds, and dorsal elevation of the epiglottic axis. Ulceration may expose the cartilage at the tip of the epiglottis or may be evident as granulation tissue on the underside of the epiglottis. Dorsal displacement of the soft palate is a frequent accompanying finding and may obscure visualization of the epiglottis. In such cases, lateral pharyngeal radiographic or transoral endoscopic evaluation of the epiglottis is of diagnostic value. Epiglottitis frequently is misdiagnosed as epiglottic entrapment on endoscopy.

7.2.3.5.5 Treatment

Treatment is conservative, consisting of stall rest, systemic antiinflammatory drugs, and topical treatment with antiinflammatory drugs and antibiotics. Most horses respond satisfactorily to treatment, but some occasionally require surgical resection of fibrotic or abscessed subepiglottic tissues. Long-term complications include epiglottic deformity, intermittent or persistent palate displacement, and epiglottic entrapment.

7.2.3.6 Pharyngeal Cysts

7.2.3.6.1 Definition

Pharyngeal cysts are fluid-filled structures that have been found in the subepiglottic tissues, in the dorsal nasopharynx, and within the soft palate.[114,115] Depending on their location, the cysts may represent embryonic remnants of the thyroglossal and craniopharyngeal ducts. The cysts usually are considered congenital. Acquired cysts are associated with secretory cells and may occur following obstruction or

inflammation of the mucous glands.[95,115] Pseudostratified cuboidal or columnar epithelium or squamous epithelium usually line the cysts, which often contain thick, yellow mucus.

7.2.3.6.2 Clinical Signs

A foal affected with a large pharyngeal cyst may exhibit signs of respiratory obstruction or dysphagia in the immediate postnatal period. Cough, nasal discharge, nasal milk reflux, and signs of pneumonia may be evidence of accompanying aspiration pneumonia. More typically, the affected horse is asymptomatic until rigorous training begins and signs of abnormal respiratory noise and exercise intolerance become evident. Horses may cough occasionally as well. Dorsal displacement of the soft palate may coexist.

7.2.3.6.3 Diagnosis

Confirmation of diagnosis is by visualization of smooth-walled structures 1 to 5 cm in diameter. Subepiglottic cysts may distort the appearance of the epiglottis, causing it to assume a more upright position in the pharynx. Endoscopic differentiation between subepiglottic and soft palatal cysts may be difficult. Lateral radiographs of the pharynx and larynx or transoral endoscopic examination of the epiglottis may be useful in distinguishing cysts from conformational defects of the epiglottis.

7.2.3.6.4 Treatment

Treatment is surgical resection of the cyst via a ventral midline laryngotomy. Some cysts may be accessible via Nd:YAG laser techniques, eliminating the need for general anesthesia. Broad-spectrum antimicrobials are indicated because of the potential for development of aspiration pneumonia, and the clinician should initiate therapy immediately. A course of 7 to 10 days may be indicated postoperatively, depending on the involvement of the lower respiratory tract structures.

7.2.3.7 Arytenoid Chondritis

7.2.3.7.1 Definition

Arytenoid chondritis is an abnormal enlargement of the arytenoid cartilages associated with chronic inflammation.[116]

7.2.3.7.2 Epidemiology

The condition is primarily a disease of horses performing at high speeds. Although the racing Thoroughbred or Standardbred primarily is affected, the condition has been described in other breeds.[117]

7.2.3.7.3 Pathogenesis

The cause of arytenoid chondritis is unknown, but trauma, inflammation, and infection have been cited as potential causes. In most cases the onset of clinical signs is insidious and progressive, but occasionally, arytenoid chondritis may follow an acute episode of laryngeal infection. Histopathologic findings in this condition are consistent with a chronic recurrent inflammatory process. Involved cartilage is greatly

thickened and laminated with fibrous connective tissue. Intralumenal granulomata and sinus tracts are observable in some cases. Airway obstruction is caused by physical enlargement of the affected arytenoid cartilage and axial movement of the cartilage as it enlarges. The condition is typically unilateral, though often secondary contact damage ("kissing lesion") occurs to the contralateral cartilage. In most cases the condition is progressive and debilitating, and thus the clinician should give a guarded to poor prognosis.[115]

7.2.3.7.4 Clinical Signs

Clinical signs include exercise intolerance and inspiratory stridor during exercise. In some horses one easily can elicit a cough with tracheal compression. Palpation of the larynx suggests that the cartilages are less resilient than normal.[118] In severe cases of chondritis, dyspnea at rest may be evident.

7.2.3.7.5 Diagnosis

Diagnosis is based on endoscopic examination. Mildly affected horses retain arytenoid mobility yet have 306
ulcerations of the body of the affected cartilage or granulomata that project into the laryngeal lumen. In 307
more advanced cases, the affected arytenoid cartilage is immobile and deviated axially. Such cases may mimic laryngeal hemiplegia, but most have additional findings of ulceration, granuloma, deformity of the corniculate process, or a kissing lesion on the contralateral cartilage.[117] Other abnormalities, such as aryepiglottic entrapment or dorsal displacement of the soft palate, may be evident. Radiographs may reveal excessive mineralization of laryngeal cartilages.

7.2.3.7.6 Treatment

Treatment includes medical and surgical approaches. Rest, nonsteroidal antiinflammatory therapy, antimicrobials, and throat sprays or nebulization may be helpful in some cases.[117] In horses that retain arytenoid mobility yet have an intralumenal granuloma, transendoscopic laser debulking is indicated. More advanced cases require surgical resection of the affected cartilage. Several techniques have been advocated. Arytenoidectomy involves removal of the corniculate and arytenoid cartilages, including the muscular process. Partial arytenoidectomy (with ventriculectomy and cordectomy) involves resection of the laryngeal saccule, vocal fold, and arytenoid cartilage, excluding the muscular process and the rostral strip of the corniculate.[119] Subtotal arytenoidectomy involves removal of the arytenoid cartilage except for the muscular process.[120,121]

7.2.3.8 Laryngeal Hemiplegia

7.2.3.8.1 Definition

Laryngeal hemiplegia is a failure of abduction of a structurally normal arytenoid cartilage because of decreased or absent motor activity in the cricoarytenoideus dorsalis muscle, the primary arytenoid cartilage abductor. The fundamental defect is neuropathy of recurrent laryngeal nerve that innervates all of the intrinsic muscles of the larynx with the exception of the cricothyroideus. Paralysis of the cricoarytenoideus dorsalis prevents phasic abduction of the arytenoid cartilages during inspiration or maintained abduction of the cartilages during exercise. Denervation of the arytenoideus transversus, cricoarytenoideus lateralis, ventricularis, and vocalis also prevents adduction of the arytenoids and slackening of the vocal folds.[94]

7.2.3.8.2

Causes

In the majority of cases no cause for the laryngeal paralysis is found; thus it has been termed *idiopathic laryngeal hemiplegia* (ILH). Most of the clinically detectable cases of ILH involve the left recurrent laryngeal nerve. ILH is a manifestation of a generalized distal axonopathy affecting all long nerves in large-statured horses. The left recurrent laryngeal nerve is the longest nerve in the horse and typically the only one in which axonopathy leads to a clinically evident deficit. Other theories advanced to explain the recurrent laryngeal neuropathy include (1) mechanical compression or stretch of the left recurrent laryngeal nerve as it courses over the aortic arch, (2) bacterial- or viral-induced neuropathies, and (3) vitamin deficiencies.[122] In occasional cases, laryngeal hemiplegia has been known to occur following perivascular or perineural injections, organophosphate intoxication,[123,124] lead poisoning, plant poisoning, guttural pouch mycosis, neoplasia, traumatic accidents to the neck, and paralaryngeal abscessation.[123,125]

7.2.3.8.3

Epidemiology

ILH generally is considered to be a disorder of larger-breed horses and rarely is reported in Arabians and ponies. Clinical signs usually occur after 3 years of age at a time when work begins. However, left laryngeal asymmetry has been observed endoscopically in foals.[125] Males are reported to be affected more commonly than females, although the physical size difference may be an important consideration in this regard because ILH occurs more frequently in larger horses (>160 cm in height) with long necks and narrow chests.[122] ILH has been reported more frequently in certain family lines of horses, thus causing some to propose it to be a heritable disorder.[126,127] However, the exact mode of transmission remains uncertain.

7.2.3.8.4

Pathogenesis

In ILH, distal degeneration of the nerve fibers in the left recurrent laryngeal nerve and subsequent atrophy of the intrinsic laryngeal musculature occur.[128,129] Similar, although less severe changes also occur in the right recurrent laryngeal nerve of hemiplegic horses. Mild involvement of other peripheral long nerve fibers—for example, of the distal hindlimb—also occurs. The finding of bilateral recurrent neuropathy, along with changes in other peripheral nerves, is inconsistent with the hypothesis that neuropathy is a sequela of compression or stretching of the nerve as it courses along the aortic arch. The findings, however, are not inconsistent with the development of a distal axonopathy following an energy-dependent disorder, an antioxidant disorder, or a filamentous neuropathy.[130]

7.2.3.8.5

Pathophysiology

Inadequate abduction of the arytenoids provides an inspiratory resistance to air flow. In experimentally induced hemiplegia, a significant increase in inspiratory or expiratory resistance at rest is not evident. However, during moderate exercise intensities, a significant increase in inspiratory resistance and a subsequent inspiratory airflow limitation develops.[131] The effect of this limitation on gas exchange has been evaluated. During eupneic breathing, no detectable alterations in gas exchange occur. However, during exercise, the physiologic hypercapnia and hypoxemia normally found worsens.[132,133] The ensuing hypoxemia may contribute to the exercise intolerance. The inspiratory resistance increases the work of breathing and *may* predispose to the development of respiratory muscle fatigue.

7.2.3.8.6

Clinical Signs

Most horses with unilateral laryngeal hemiplegia have a history of exercise intolerance and the production of an inspiratory noise described as a whistle or a roar. In contrast, horses with bilateral laryngeal paralysis may show respiratory distress and require emergency tracheostomies. In cases of bilateral paralysis secondary to toxins (e.g., organophosphate overdose), clinical signs may not be apparent for several weeks following the toxic insult.[134] 307 308

7.2.3.8.7

Diagnosis

Physical examination and palpation of the larynx may reveal atrophy of the cricoarytenoideus dorsalis muscle. The slap test has been used to evaluate the adductor function of the intrinsic laryngeal muscles. In normal horses, when one slaps the saddle area of the horse, contralateral arytenoid cartilage adduction occurs. This adduction is detectable endoscopically or by palpation of the larynx during the procedure. The reflex is absent in horses with idiopathic recurrent laryngeal neuropathy.[135] However, the demonstration of a normal laryngeal reflex when the horse is at rest does not indicate that the horse is completely free of laryngeal hemiplegia.[126] Ultimately, the clinician usually makes the diagnosis by an endoscopic examination wherein one observes asymmetric positioning or range of motion of the arytenoid cartilages and relaxation of the vocal folds on the affected side. Box 7-3 provides a proposed grading scheme and may aid in the decision for surgical intervention. For example, horses with grade IV can be expected to benefit from surgery, whereas horses with grades I and II usually are not compromised during exercise and thus are not good surgical candidates. Horses with grade III are considered suspect, some of these horses demonstrating dynamic collapse of the airway and thus requiring surgical intervention. Ideally, one should perform endoscopic examination of the horse during treadmill exercise to ascertain the importance of slight asymmetry at rest in the production of exercise intolerance. A recent study documented progression of laryngeal hemiplegia, as judged by clinical signs and endoscopic examination, in 15% of horses.[136] The mean age of onset of progression was 7 years.

7.2.3.8.7.1

BOX 7-3 GRADING SCHEME FOR LARYNGEAL ANATOMY

Grade I: Synchronous full abduction and adduction of left and right arytenoid cartilages.

Grade II: Asynchronous movement such as hesitation, flutters, adductor weakness of the left arytenoid during inspiration or expiration or both, but full abduction induced by swallowing or nasal occlusion.

Grade III: Asynchronous movement of the left arytenoid during inspiration or expiration or both, but full abduction not induced and maintained by swallowing or nasal occlusion.

Grade IV: Significant asymmetry of the larynx at rest and lack of substantial movement of the left arytenoid.

From Ducharme NG, Hackett RP: The value of surgical treatment of laryngeal hemiplegia in horses, *Compend Cont Educ Pract Vet* 13:472, 1991.

7.2.3.8.8 Treatment

Surgical correction of the airflow obstruction is necessary. Laryngoplasty, a prosthesis that maintains the affected arytenoid in abduction, is implemented most commonly. Complications associated with the surgery include failure to maintain abduction of the arytenoid cartilage, dysphagia leading to coughing while eating or rarely aspiration pneumonia, chronic infection of the prosthesis leading to a suture sinus, ossification of cartilage, esophageal obstruction, intralaryngeal granulomatous polyps, right-sided laryngospasm during exercise, laryngeal edema, and chondritis.[137] Ventriculectomy, surgical ablation of the lateral ventricles in an effort to induce adhesions between the vocal cords and laryngeal walls, also has been performed, singly or with the laryngoplasty. Prosthetic laryngoplasty prevents the increase in inspiratory resistance and flow limitation during moderate exercise.[132] However, ventriculectomy alone fails to alleviate the increased inspiratory impedance during exercise.[133] Surgical procedures that attempt to restore innervation to the cricoartyenoideus dorsalis muscle minimize the risk of serious postoperative complications. Described techniques include transplantation of a nerve-muscle pedicle into the cricoartenoidus dorsalis muscle, implantation of the cut end of the second cervical nerve into the left cricoarytenoideus muscle and anastomosis of the first cervical nerve to the abductor branch of the left recurrent laryngeal nerve.[137–139] A modest improvement was notable in the experimental models but a report of nerve-muscle pedicle graft in racehorses revealed results comparable to those of prosthetic laryngoplasty.[140] Reinnervation of the cricoarytenoideus muscle following nerve-muscle pedicle grafting takes 6 to 12 months depending on the amount of muscle atrophy and thus is used primarily in young, unraced horses or in horses in which a rapid return to athletic performance is not necessary.

In horses with bilateral laryngeal paralysis, tracheostomy may be required until resolution or lessening of paralysis occurs. Affected horses should not be stressed or subjected to undue movements. The clinician should institute broad-spectrum antimicrobial and antiinflammatory therapy.

7.2.3.9 *Streptococcus equi* Infections (Strangles)

7.2.3.9.1 Causes

Streptococcus equi subspecies *equi,* a gram-positive β-hemolytic bacterium of the Lancefield Group C, is 308
the causative agent of strangles, a highly contagious disease of equidae. The disease was recognized in the 309
thirteenth century and occurs worldwide.[141] Recent multilocus enzyme electrophoresis studies have confirmed a close genetic relationship of *S. equi equi* and *S. zooepidemicus,* indicating that the former is a clone derived from the more genetically diverse *S. zooepidemicus*.[142] This finding has led to the recommendation that *S. equi equi* be reclassified as a biovar of *S. zooepidemicus*.[143] Although isolates of these two organisms show greater than 92% DNA homology, immunity is species specific: immunization with *S. zooepidimicus* does not protect against challenge by *S. equi equi*.

S. equi equi (hereafter referred to as *S. equi*) is not a normal inhabitant of the equine upper respiratory tract and does not require prior viral infections for successful colonization and infection.[144] Based on morphologic features of the bacterial colony, three strains of the organism occur that differ in virulence. The typical and highly virulent encapsulated *S. equi* strains produce golden, honey-colored mucoid colonies on blood agar. Atypical *S. equi* colonies exhibit a matte appearance within 24 hours of incubation.

Nonencapsulated colonies are glossy, dry, and small.[145] Differences in the hyaluronic acid content of the capsule are responsible for the morphologically distinctive features of *S. equi* colonies.

Laboratory diagnosis of Lancefield group C streptococci *(S. equi, S. zooepidemicus, S. equisimilis)* traditionally is based on fermentation patterns of lactose, sorbitol, and trehalose. Typical *S. equi* isolates fail to ferment any of these sugars, whereas atypical *S. equi* isolates may ferment lactose or trehalose but not sorbitol.[146] In some laboratories, polymerase chain reaction (PCR) assays aid in confirming *S. equi* isolates.[147]

7.2.3.9.2 Epidemiology

The infection occurs primarily in horses 1 to 5 years old but is not restricted to this age group. Foals up to 3 months of age born from immune mares are known to be resistant to the development of strangles.[148] In susceptible equine populations, morbidity is nearly 100%, whereas mortality is low (up to 10%) if appropriate therapy is instituted.[149] Once having been infected, approximately 75% of the horses are immune to the organism for 4 years.[150] In another study, 83% of young horses affected with clinical strangles as foals were resistant to co-mingling exposure 6 months later.[151] Immunity is not lifelong: epidemiologic studies report attack rates in horses greater than 3 years of age of 18%, 29%, and 35%.[149,152,153]

The organism is transmitted (1) via direct contact with nasal secretions or lymph node discharges from infected horses or (2) via exposure to fomites such as contaminated equipment, pails, halters, leads, brushes, clothing, horse vans, or stalls. Recent additions to a stable are most often the cause of a strangles outbreak because a recovering horse may shed the organism for several weeks. In closed herds, epidemics have been attributed to exposure of horses to asymptomatic chronic carriers of the organism. Such horses harbor the organism in the guttural pouches for as long as 39 months in the absence of clinical signs. Other sites of long-term carriage of the organism include the paranasal sinuses.[154]

The organism also can survive for long periods in the environment depending on the temperature and the substrate. Jorm[155] found that *S. equi* survived for 63 days on wood at temperatures of 2° C and for 48 days on glass or wood at temperatures of 20° C. A 1:200 dilution of phenol and disinfectants such as povidone-iodine, chlorhexidine, and glutaraldehyde kill the organism within 90 minutes.

Although the organism traditionally is thought to infect only equidae, a fatal pneumonia attributed to *S. equi* infection in a dromedary camel was reported recently.[156] In the literature on human beings, descriptions of two cases of *S. equi* bacteremia exist, but infections in human beings are considered rare.[157]

7.2.3.9.3 Pathogenesis

After infective droplets are inhaled or ingested, the organism adheres to the epithelial cells of the buccal and nasal mucosa of the horse. Within hours of adhesion, the organism has translocated below the mucosa and gained access to the local lymphatics and lymph nodes where replication occurs extracellulary.[141] Spread of the organism to sites other than the upper respiratory tract lymphoid tissue may occur by hematogenous or lymphatic pathways.

At least three virulence factors of *S. equi* contribute to its pathogenicity and include the hyaluronic acid capsule, the M-like protein, and a leukocidal toxin.[158] The hyaluronic acid capsule possesses a strongly negative charge and apparently repels phagocytic cells. Nonencapsulated strains of *S. equi* are able to colonize the surface of the upper respiratory tract and stimulate production of serum antibody but are unable to induce detectable pathologic changes in the retropharyngeal and submandibular lymph nodes.[159] The second virulence factor, the M-like protein (SeM), is a 58-kd cell wall antigen. This protein functions by limiting deposition of complement components (C3b) on the bacterial surface and preventing activation of the alternative complement pathway. SeM also binds fibrinogen and impairs neutrophil killing of the organism.[142] The M-like antigen also appears to be important for adherence of the organism to the oropharyngeal epithelium. Another recently identified M-like protein, SzPSe, exhibits antigenic cross reactivity with the M-like proteins of *S. zooepidemicus*. Its contribution to the virulence of *S. equi* is currently unknown.[142]

Additional factors that may contribute to the pathogenicity of *S. equi* include extracellular proteins that are mitogenic for peripheral blood mononuclear cells.[160] These mitogenic factors have been hypothesized to 309
induce release of interleukin-1 (IL-1) and tumor necrosis factor α from antigen-presenting cells 310
(macrophages, monocytes) and to contribute to the development of fever, malaise, neutrophilia, and hyperfibrinogenemia.[160]

7.2.3.9.4 Clinical Signs

The incubation period ranges from 2 to 6 days. In typical cases, horses are febrile, exhibit malaise, and develop a serous nasal discharge that becomes mucopurulent. Submandibular and retropharyngeal lymph nodes are initially firm but become fluctuant before rupturing 7 to 10 days after the onset of signs. Lymphadenopathy may be asymmetrical and may become so severe that dysphagia and respiratory distress ensue. Swelling of the throat-latch area or of Viborg's triangle may be apparent. The affected horse may stand with its neck stretched out and be reluctant to swallow. A soft moist cough may be heard. The average course of the syndrome is 23 days.[144]

In atypical *S. equi* infections, a mild inflammation of the upper respiratory tract occurs and is characterized by a slight nasal discharge, cough, and fever. Abscessation of the lymph nodes occurs only in a small number of the cases.[145,146,161]

7.2.3.9.5 Clinical Pathology

A marked neutrophilic leukocytosis, hyperfibrinogenemia, and an anemia of chronic infection are found in typical strangles cases.

7.2.3.9.6 Diagnosis

Diagnosis usually is based on clinical signs and the isolation (culture) or detection (PCR) of *S. equi* from a lymph node, a nasopharyngeal swab, or lavage fluid from the guttural pouches. Blood cultures may become positive for *S. equi* on days 6 to 12 following infection.[162]

Identification of chronic asymptomatic carriers can be difficult. In one recent study, endoscopic abnormalities in the guttural pouches were evident in most asymptomatic carriers.[163] However, the absence of visible pathologic signs in one asymptomatic carrier emphasizes the need to obtain samples for microbiologic studies. When PCR and culture results from asymptomatic carriers were compared, many more samples were found to be positive using PCR than with culture alone.[164]

7.2.3.9.7 Treatment

Treatment is a function of the stage of the disease. Sweeney, Benson, Whitlock, et al.[165] suggested a treatment protocol that is outlined next. Penicillin is the drug of choice, although *S. equi* is sensitive to oxytetracycline and the potentiated sulfonamides.[141,154]

The treatment plan is as follows:

1. For horses exposed to the organism, penicillin therapy is indicated to prevent seeding of the pharyngeal lymph nodes. Antimicrobial therapy should continue for as long as the horse remains exposed to the organism. Once the therapy stops, a risk exists that the horse may develop strangles.

2. For horses exhibiting signs of infection in the absence of lymph node abscessation, penicillin G therapy can arrest the progression of the disease. One should isolate the horses during their treatment protocol.

3. For horses exhibiting evidence of lymph node abscessation, administration of penicillin slows the progression of lymph node abscessation and generally is contraindicated. Hot-packing the area(s) promotes abscess formation. Once achieved, the clinician should lance the abscess and flush it with a 2% tamed iodine solution. One should treat horses in isolation.

4. For horses that are systemically ill or that develop complications such as dysphagia and respiratory distress require supportive care in addition to high levels of intravenous penicillin. This therapy may entail intravenous fluid therapy, a tracheostomy, nonsteroidal antiinflammatory drugs, and feeding via the nasogastric tube.

7.2.3.9.8 Sequelae

Complications most frequently result from metastasis of the organism to other organ systems with the formation of purulent foci.[165] These complications include the following:

1. Internal abscessation of the mesentery or of parenchymatous organs. The causes of internal abscessation are not known, although inadequate antimicrobial therapy during respiratory catarrh and lymphoid abscessation may be contributory.[166] The horse may have a history of intermittent colic, periodic pyrexia, anorexia, depression, and weight loss. The clinician should direct diagnostic techniques, including rectal palpation, thoracic radiography, thoracocentesis, abdominocentesis, ultrasonography, computed tomography, and nuclear scintigraphy,[167] at determining the location of the abscess. Cases of internal abscessation can be difficult to differentiate from neoplastic causes of weight loss and colic because both processes may induce similar abnormalities in the peritoneal fluid (leukocytosis and hyperproteinemia). Differentiation between the two processes may not be possible

unless exfoliated neoplastic cells are identified.[168] Hematologic and serum biochemical abnormalities in cases of internal abscessation include (1) a neutrophilic and monocytic leukocytosis, (2) hyperfibrinogenemia, (3) hyperglobulinemia and hypoalbuminemia, and (4) hypocalcemia.[166,168] However, these changes are not unique to abscesses and may not be found in horses with intraabdominal neoplasms.[168] Thus the diagnosis of internal abscessation represents a medical challenge. Once one confirms the diagnosis, long-term penicillin therapy is indicated and may be required for several months. Clinical signs, rectal palpation, repeated abdominocentesis, and multiple complete blood counts (CBCs) are useful to monitor the progress of therapy.

2. Purpura hemorrhagica. This aseptic vasculitis occurs following reexposure to *S. equi* by natural
infection or following vaccination. In horses with purpura hemorrhagica, IgA titers to the SeM and to 310
nonspecific proteins in the *S. equi* culture supernatant (SC-P) are significantly greater than those 311
titers found in horses with uncomplicated strangles.[169] Furthermore, the isolation of immune complexes consisting of IgA and M-like proteins in the sera of horses with purpura hemorrhagica has led to the suggestion that this isotype is involved in the development of purpura.[169,170] The immunologic basis for the increase in serum IgA levels is not known: possible explanations include uncontrolled expansion of B cell populations that produce IgA against antigens of *S. equi* or failure of IgA removal mechanisms because of hepatic dysfunction. In the horse, purpura hemorrhagica has been likened to Henoch-Schönlein purpura, an immune complex–mediated disease of human beings.[170]

   The clinical signs of purpura vary and range from a mild transient reaction to a severe and fatal form. Horses with purpura develop pitting edema of the limbs, head, and trunk and petechiation and ecchymoses of the mucous membranes. Horses also may have colic following edema and hemorrhage of the bowel. The vasculitis may cause skin sloughing and infarcts of skeletal muscle. Death may ensue as a result of pneumonia, cardiac arrhythmias, renal failure, or gastrointestinal disorders.

   Confirmation of diagnosis is by skin biopsy characterized as a leukocytoclastic vasculitis, isolation of the organism, or demonstration of elevated IgA titers to *S. equi*. Treatment entails administration of intravenous penicillin (20,000 IU/kg 4 times daily), dexamethasone (0.1 mg/kg intravenously or intramuscularly), intravenous fluids, and antiinflammatory drugs. Clinical recovery occurs as the source of the antigen is removed and as the immune response is suppressed. Coincidental with recovery is the production of IgG to *S. equi* antigens.[169]

3. Guttural pouch empyema and chondroid formation (see the previous discussion).

4. Septicemia and the development of infectious arthritis, pneumonia, and encephalitis. These conditions warrant a poor prognosis.[171]

5. Retropharyngeal abscessation. Upper respiratory tract obstruction may cause the horse to develop respiratory distress and dysphagia. Endoscopy of the nasopharynx demonstrates collapse of the nasopharynx, deviation of the larynx, or drainage of purulent material into the nasopharynx when external pressure is applied to the parotid region.[172] Radiographs demonstrate a soft tissue density in the retropharyngeal area, thickening of the roof of the pharynx, reduction in the diameter of the pharyngeal airway, and distortion or compression of the guttural pouches, pharynx, and trachea.[173]

The retropharyngeal abscess may rupture into the pharynx and cause a secondary pneumonia or may rupture dorsally into the guttural pouches.[165,172,173]

6. Laryngeal hemiplegia. The condition occurs when abscessed lymph nodes encroach on the recurrent laryngeal nerve.[174]
7. Tracheal compression following abscessation of the cranial mediastinal lymph nodes. Tracheal compression has been reported. The horse may show respiratory distress or exhibit stertorous breathing. Laryngeal hemiplegia may be an additional complication if the abscess compressing the trachea involves the recurrent laryngeal nerves.[174]
8. Endocarditis or myocarditis following abscess formation.
9. Agalactia in periparturient mares.
10. Suppurative necrotic bronchopneumonia, which may result in death.
11. Abscess formation in the central nervous system.[175]
12. Myopathies. Two types of muscle disorders associated with *S. equi* infection have been described recently. One fatal form is characterized by a vasculitis and infarction of skeletal muscle and pulmonary and gastrointestinal tissues. Horses typically have signs of colic and usually are euthanized because of unrelenting pain. Postmortem examination reveals muscle infarctions that are compatible with purpura hemorrhagica. The second type of muscle disorder affects Quarter Horses that have malaise, significant muscle atrophy, and chronic active rhabdomyolysis. Immunocytochemical stains of fast-twitch fibers are positive for equine IgG. Horses responded to treatment with penicillin and dexamethasone with the muscle mass normalizing within 2 months.[176]

The clinician should follow these control measures to reduce transmission of *S. equi* during an outbreak[177]:

1. Prevent spread of infection to horses on other premises and to new arrivals immediately by stopping all movement of horses on and off the premises.
2. Identify symptomatic and asymptomatic carriers by sampling nasopharygneal or guttural pouch regions at weekly intervals and testing for *S. equi* by culture and PCR.
3. Isolate infectious horses from those screened negative for *S. equi*. Cordon off the isolation area and have infectious horses looked after by a dedicated staff wearing protective clothing and footgear. Disinfect stalls, aisles, and equipment.
4. Once clinical signs have disappeared, perform at least three consecutive swabs or lavages for *S. equi* culture, coupled with endoscopic examination of the guttural pouches to confirm that horses are noninfectious.

7.2.3.9.9 Prevention

Vaccines currently available include Strepguard with Havlogen (*S. equi* extract); Strepvax II (aluminum hydroxide adjuvanted *S. equi* extract), and Pinnacle I.N. (an attenuated live culture of *S. equi*). None of the vaccines guarantees prevention of strangles in vaccinated horses. Parenteral (killed) vaccines induce strong 311 312

serum bactericidal activity, but these antibodies are not necessarily protective because mucosal immunity plays a significant role in the resistance to infection.[178] In contrast, mucosal immunization induces a combination of systemic and local responses associated with the production of a greater variety of immunoglobulin isotypes and specificities that closely mimic those induced by natural disease. Although intranasal vaccines are expected to produce fewer adverse reactions than parenteral vaccines, lethargy, inappetence, fever, lymphadenopathy, lymph node abscessation, purpura hemorrhagica, and intramuscular abscesses have occurred after vaccination. One should not administer intranasal vaccines concurrently with other parenteral injections because of the risk of abscess formation at the site of the intramuscular injection. One should wash one's hands thoroughly following vaccination. Vaccination of infected horses during an outbreak on a farm may be associated with the development of purpura hemorrhagica. Because of the lack of scientific studies, whether the clinician should implement vaccination in the face of an outbreak is uncertain. During vaccination, effective immune response takes 7 to 10 days to develop.

7.2.4 VIRAL INFECTIONS

Viruses of known pathogenicity to the horse that produce or are associated with respiratory tract disease include equine influenza virus, EHV1, EHV2, EHV4, equine arteritis virus (EAV), and equine rhinovirus A and B.

7.2.4.1 Equine Influenza

7.2.4.1.1 Causes

The influenza viruses are enveloped myxoviruses containing single-stranded RNA. Based on the internal nucleoproteins and matrix antigens, influenza viruses have been classified as three types: A, B, and C. Types B and C infect only human beings, but type A infects many different species, including human beings, horses, swine, and avian species.[178] Natural cross-species infections have occurred between human beings and swine, and experimental infection of human beings with equine influenza virus (subtype 2) is possible, although natural infections do not occur.[179] Type A is classified further into subtypes based on the surface antigens, the hemagglutinin (H) and neuraminidase (N). Two subtypes are recognized as infectious for the horse: subtypes H7N7 and H3N8. Subtype H7N7 was first isolated in Prague in 1956 and has been called equine-1 influenza (A/equine/Prague/56). Subtype 1 is thought to have disappeared from horses worldwide because an isolate has not been confirmed since 1980. Subtype H3N8 was first isolated in Miami in 1963 (prototype) and was designated as equine-2 influenza (A/equine/Miami/63). Several variants of subtype 2 (antigenic drift) have been identified and include A/equine/Fontainebleau/79, A/equine/Kentucky/81, A/equine/Saskatoon/90, and A/equine/Newmarket 2(N2/93).[178,180,181] Compared with the human influenza virus, the equine influenza virus is stable antigenically, not experiencing major antigenic shifts. This stability has been postulated to result from the shorter life span of horses relative to human beings and the lower mutation pressure placed on the equine influenza viruses because of low specific antibody titers.[179]

7.2.4.1.2 Epidemiology

Influenza most commonly affects 2- and 3-year-old horses and may be the most common cause of respiratory illness in racehorses, with some tracks experiencing influenza outbreaks 2 to 3 times within a racing season.[180–182] This phenomenon may reflect poor ventilation systems (closed shedrows), lack of

adequate immunization protocols, and rapid transmission of the virus from exercise ponies to racehorses.[180] The reservoir of equine influenza virus between epizootics remains unknown. Some speculate that the virus is maintained in the horse population itself and that asymptomatic carriers shed virus when stressed. Alternatively, the virus may be harbored in other species such as birds, which are asymptomatic but shed the virus in their feces.[179]

7.2.4.1.3

Pathogenesis

The incubation period is 1 to 3 days, with the virus infecting the upper respiratory tract and the lungs to a lesser extent. Healthy respiratory tract epithelium is ciliated and contains mucus that forms a protective layer over the cell surface. The mucus normally acts as a mechanical barrier between air and tissue and contains antibody and protein substances that deter the attachment of viral particles to the epithelium.[183] Neuraminidase activity of the viral particles alters the efficiency of the mucociliary apparatus, allowing the virions to attach via hemagglutinin antigens to sugar groups (sialic acid) on the surface of the epithelial cells. The epithelial cell internalizes the bound virus and surrounds it with an endosome. However, in the mildly acidic conditions of the mature endosome, the hemagglutinin antigen protein becomes activated and promotes fusion of the viral and cellular membranes, leading to release of the nucleocapsid into the cytoplasm.[184] Cell necrosis and desquamation follow viral replication within the respiratory tract epithelium. Exposure of irritant receptors causes hypersecretion of submucosal serous glands and further damage to the mucociliary apparatus. Inflammation leads to massive lymphocyte infiltration and edema. Recovery of the normal epithelial architecture can require more than 6 weeks.[185] Humoral and cellular immunity are important in providing protection against viral disease: IgA blocks viral penetration but is not bactericidal; certain IgG isotypes, IgGa and IgGb, opsonize the virus particles and enhance phagocytosis.[186]

7.2.4.1.4

Clinical Signs

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Signs of influenza appear 3 to 5 days following exposure to the virus. Horses exhibit a sudden onset of fever 313
(103° to 105° F), which may be biphasic; a serous nasal discharge; anorexia; depression; and a dry, deep cough. Some horses exhibit myalgia, myositis, and limb edema and are thus reluctant to move.[179] A mild form of azoturia (myoglobinuria) occurs in some cases. One may find submandibular lymphadenopathy, as well as endoscopic evidence of pharyngitis and tracheitis. A mild bronchitis with minimal changes in lung sounds may occur. From experimental studies, inoculation with A/equine-1 produces a milder (subclinical) syndrome unless the animal is stressed before infection, whereas A/equine-2 usually generates the typical clinical signs. The course of the infection usually lasts 2 to 10 days in uncomplicated cases. Exercise exacerbates the clinical signs.[187] Secondary bacterial infections may occur, and donkeys and mules appear to have greater susceptibility to secondary infections than horses.[188] Horses remain infectious for 3 to 6 days after the last signs of illness.

In young foals, influenza is severe, producing signs of viral pneumonia, which may lead to death within 48 hours.

7.2.4.1.5

Clinical Pathology

Horses initially may exhibit a lymphopenia followed by a monocytosis.

7.2.4.1.6

Diagnosis

The clinician may diagnosis equine influenza by two methods:

1. Detection of virus. Based on studies of influenza outbreaks, viral culture and isolation alone from nasopharyngeal swabs appears to be the least sensitive method for diagnosing influenza,[180] probably because the chances of viral isolation are greater when samples are collected within the first 24 hours.[182] However, a stallside assay (Directigen Flu-A test, Becton Dickinson, Franklin Lakes, New Jersey), allows rapid detection of influenza antigen and an immediate diagnosis.[185] Detection of viral particles in materials obtained from nasopharyngeal swabs via PCR permits diagnosis of influenza infections within 48 hours of sample submission.
2. Serologic testing. Several diagnostic methods for detecting influenza virus antibodies in the horse are available and include complement fixation, hemagglutination inhibition, single radial hemolysis, viral neutralization, and enzyme immunoassay.[189] Acute and convalescent serum samples are needed to establish a definitive diagnosis, and although viral neutralizing antibodies may be detectable within a week of infection, the horse may take up to 28 days to exhibit a rise in hemagglutination titers.[179] Serologic testing has been considered to be a sensitive method for diagnosing influenza outbreaks, although in one epidemic, approximately 24% of horses with clinical signs of influenza failed to seroconvert.[180]

7.2.4.1.7

Treatment

Treatment is primarily symptomatic, ensuring that the horse continues to maintain adequate hydration and is not subjected to undue stresses. Nonsteroidal antiinflammatory drugs may be indicated to reduce the fever, eliminate the myalgia, and improve the appetite. The risk is that the owner will return the horse to strenuous exercise before the horse has rested a sufficient time. Horses that suffer severe infections may be unfit for competition for 50 to 100 days after infection. During the infection, frequent examinations of the respiratory tract are indicated to detect the development of secondary complications such as pneumonia, pleuropneumonia, and myocarditis.

7.2.4.1.8

Prevention

Regular vaccination significantly reduces the population at risk. In fact, the suggestion has been made that at least 70% of the equine population (horses, ponies, donkeys) should be vaccinated to prevent epidemics of influenza.[190] Killed and modified live vaccines are commercially available. The inactivated vaccines contain both subtypes of influenza virus and some of their strain variants (e.g., A/equine-2/KY 81). Equine subtypes 1 and 2 do not immunologically cross-react: exposure to one subtype does not protect against exposure to the other. The modified live vaccine (Flu Avert, Heska, Fort Collins, Colorado) contains only A2 strains and is administered intranasally.

In one study the use of a killed vaccine reduced neither the rate of respiratory tract disease nor the severity of clinical scores compared with nonvaccinates.[191] This vaccine failure may be a function of the number of boosters horses received before infection and of the strain of A2 contained within the vaccine.

Little data is currently available concerning the performance of the modified live vaccine during influenza outbreaks. From experimental trials, the vaccine appears to have phenotypic stability, is efficacious against heterologous virus challenge, and can be administered safely to strenuously exercised horses.[192,193] The modified live vaccine is not recommended for pregnant mares.

Vaccination of young athletic horses is recommended more frequently (at 4- to 6-month intervals) than for horses that have received regular boosters for years. Vaccinating foals before 6 months of age may be ineffective in inducing an antibody response to influenza (killed vaccine) because of interference by maternally derived antibodies.[194,195] Some undesirable side effects of vaccination occur, including fever, depression, pain, swelling at the vaccine site, and muscular stiffness, but these usually resolve within 1 or 2 days.[182] Administering two intranasal vaccines simultaneously is not recommended.

7.2.4.1.9 Sequelae

Secondary bacterial pneumonia and pleuropneumonia are potential complications that may follow viral respiratory diseases in horses that have not been rested adequately before being returned to training or that have undergone other potentially stressful events such as long trailer rides. Myocarditis, pericarditis, and 313
cardiac arrhythmias are other possible sequelae to influenza infections. 314

7.2.4.2 Equine Herpesvirus Types 1 and 4

7.2.4.2.1 Causes

Currently, five equine herpesviruses are recognized as causing disease in horses. Three are classified as α-herpesviruses (EHV1, EHV3, and EHV4), and two are grouped with the γ-herpesviruses (EHV2 and EHV5). EHV4, formerly called EHV1 subtype 2, serologically cross-reacts with EHV1 but is genetically distinct from that virus.[196] EHV1 and EHV4 share many common clinical features. EHV1, in addition to causing respiratory disease, also causes abortions, myeloencephalitis, and neonatal deaths. EHV4 is associated predominantly with respiratory disease in young horses but *sporadically* causes abortion, neonatal deaths, and neurologic disease.[197–200]

7.2.4.2.2 Epidemiology

EHV1 and EHV4 may persist within a herd because of recrudescence of latent infections during periods of stress or immunosuppression. Dexamethasone or cyclophosphamide administration is a reliable reactivation stimulus for viral shedding in experimental studies.[201] For some years the site of viral persistent was unknown. However, in a study of 40 abattoir horses, EHV1 and EHV4 were detectable by PCR methods in the bronchial lymph nodes of 88% of the horses examined. Of the trigeminal ganglia examined in nine of the horses, one was found to contain only EHV1, four had detectable EHV4 in the ganglia, and three had evidence of EHV1 and EHV4.[202] EHV2 also was isolated from all of the horses examined, leading some researchers to speculate that EHV2 activates the promoter gene of EHV1.[203] In addition to the clinical trials, experimental studies of EHV1 and EHV4 also have provided data supporting the fact that lymphoid and neural tissues are sites of viral latency.[201,203]

Realizing that virus can establish latency, one easily can understand why respiratory infections by EHV1 or EHV4 can develop in foals, weanings, and yearlings in closed herds. Immunity to EHV1 or EHV4 is short-lived (3 to 5 months) so that most horses are reinfected during their breeding or racing careers. Reexposure usually results in mild or inapparent infections, except in the case of broodmares in which reinfection may lead to abortion in the last trimester. Immunity following abortion is generally of longer duration, and in the field, repeat abortions rarely occur in the same mares. No clear relationship exists between gestational age or the level of virus-neutralizing antibody and the incidence of virus-induced abortion.[204] Perinatal disease characterized by weakness and respiratory distress is usually evident within 18 to 24 hours of birth, with foals dying within 24 to 72 hours.[205]

7.2.4.2.3 Pathogenesis

Infection occurs via inhalation of the virus or contact with infected tissues. The virus is delicate and does not survive in the environment; thus close contact appears to be important for transmission.[205] Kydd, Smith, Hannant, et al.[206] have suggested that EHV1 enters the upper respiratory tract and, in the absence of mucosal antibody, immediately infects the epithelium lining of the nasopharynx and tonsils. Subsequent events depend on whether EHV1 becomes a productive or limited infection. Productive infection results in the intercellular spread of EHV1 through susceptible cells (including leukocytes) in the stroma until the vascular or lymphatic endothelium becomes infected. Thereafter infection spreads via leukocyte adhesion to infected endothelium, leading to the development of cell-associated viremia. As a result of viremia and of inhalation of viral particles, the virus disseminates to the lower respiratory tract. In a limited infection, infected cells are phagocytized and viral antigens are processed and presented to lymphocytes in mucosal lymphoid nodules or lymph nodes. The studies of Burrell[89] and Sutton, Viel, Carman, et al.[207] have demonstrated lower respiratory tract involvement in which hyperemia and mucous accumulations develop 2 to 12 weeks after the initial infection. EHV1 also has a tropism for lymphocytes and is reported to cause immunosuppression.

The pathogenesis of EHV4 has not been investigated completely and may be similar to that of EHV1.

7.2.4.2.4 Clinical Signs

Clinical signs of rhinopneumonitis appear 1 to 3 days following infection and are indistinguishable from influenza. Horses are febrile and exhibit a cough and a serous nasal discharge, which may become mucopurulent with secondary bacterial infections. Horses develop rhinitis, pharyngitis, and tracheitis detectable endoscopically. A severe disseminated EHV1 infection that caused fever, depression, respiratory distress, and eventual death has been described in yearlings and 2-year-old horses.[208,209]

7.2.4.2.5 Clinical Pathology

An epidemiologic survey by Mason, Watkins, McNie, et al.[210] during an outbreak of EHV1 infection on a racetrack in Hong Kong documents a monocytosis in infected horses (>500 cells/μl) during the first 5 days of clinically apparent infections. In experimental infections of EHV1, a decrease in neutrophils and monocytes occurs on day 3 after infection, but the absolute values of both cell types remain within normal limits.[207]

7.2.4.2.6 Diagnosis

One may attempt viral isolation from citrated blood samples. Virus has been recovered from mononuclear cells for up to 2 weeks after infection or from nasopharyngeal swabs. PCR on clinical materials is rapid and enables distinction between EHV1 and EHV4.[211] Serologic diagnosis is made by demonstrating a fourfold increase in titers. Virus neutralization, immunoassay, complement fixation, and a radial immunodiffusion enzyme assay can detect antibodies against EHV1.[212] Postmortem examination of foals who died from 314
EHV1 or EHV4 infection revealed interstitial pneumonia, pleural and peritoneal effusions, hypoplasia of 315
the thymus and spleen, focal necrosis of the liver, and viral inclusion bodies within the hepatic parenchyma. Similar pathologic findings are observed in aborted fetuses.

7.2.4.2.7 Treatment

Treatment is supportive, minimizing stresses and ensuring adequate rest. Limited trials using antiviral drugs experimentally or clinically have been described. Acyclovir, is an acyclic nucleoside analog that inhibits viral DNA polymerases. Acyclovir is activated by virus-induced thymidine kinase before being converted to acyclovir triphosphate by cellular kinases. During outbreaks of EHV1 involving neurologic disease or neonatal deaths, acyclovir has been administered at doses ranging from 8 to 16 mg/kg orally 3 times daily to 20 mg/kg orally 3 times daily.[213,214] The efficacy of acyclovir in treating respiratory disease has not been demonstrated. One potential drawback of acyclovir use is the development of viral resistance in strains lacking thymidine kinase. Another antiviral drug, (s)-1-[(3-hydroxy-2-phosphonyl methoxy)-propyl]cytosine, or HPMPC, has been shown to be efficacious when given before EHV1 challenge in naïve foals but has not been used clinically in North America.[215]

Because evidence of immunosuppression during herpetic infections exists, the clinician should give horses broad-spectrum antimicrobials for 7 to 10 days and order rest for 4 to 6 weeks before the horse commences light exercise.

7.2.4.2.8 Prevention

Currently available vaccines are produced from EHV1 and EHV4, and although the vaccine does not prevent infection, it reduces the severity of clinical signs.

7.2.4.3 Equine Herpesvirus 2

EHV2 is a slowly replicating virus, often referred to as equine cytomegalovirus. Opinions as to the role of EHV2 in producing equine respiratory diseases have been equivocal because the virus can be recovered from various sites from ill and apparently healthy horses.[87,216] Foals that are infected experimentally with EHV2 develop pharyngitis and lymphoid hyperplasia, thus implicating this as a causative agent of chronic lymphoid hyperplasia in young horses.[87] The virus also has been isolated from young horses in race training with pharyngeal lymphoid hyperplasia.[89] EHV2 has been isolated from 2- to 3-month-old foals during outbreaks of pneumonia in Hungary, Japan, Australia, New Zealand, and in the United States.[216–220] In some of these outbreaks, EHV2 was the sole infectious agent isolated from the respiratory tract, whereas in other outbreaks, bacterial agents—*Streptococcus zooepidemicus* and *Rhodococcus equi*—complicated the pneumonitis.[219] This

has led some to speculate that if the foals are stressed or infected with a large dose of virus during a period in which maternal antibodies are waning, then the virus may invade the cells of the immune system and cause further immunosuppression. Blakeslee, Olsen, McAllister, et al.[87] reported a dose-related immunosuppression following in vitro infection of lymphocytes with EHV2, and this effect has been documented in vivo using a rabbit model.

EHV2 also has been isolated from ocular, nasal, and tracheobronchial swabs of horses with keratoconjunctivitis, implicating it in the production of this clinical sydrome.[221]

The suggestion has been made that most foals are exposed to EHV2 at 2 to 3 months of age and shed the virus for 2 to 6 months until a humoral antibody response develops and eventually is associated with low viral recovery.[216] Nearly 100% of older horses are seropositive for EHV2.[222]

After natural exposure, EHV2 is recoverable from nasal and nasopharyngeal swabs, from the kidney, bone marrow, spleen, mammary gland, salivary gland, vagina, tracheal mucus, neural tissue, and from the cornea and conjunctiva.[222,223] Leukocytes and lymph nodes draining the respiratory tract are major reservoirs of EHV2 DNA.[222]

Diagnosis of EHV2 depends on isolation of the virus, serologic evidence of an active viral infection, and the presence of clinical signs. Treatment is symptomatic. Efficacy of antiviral agents has not been examined in EHV2 infections.

7.2.4.4 Equine Viral Arteritis

7.2.4.4.1 Causes

Equine arteritis virus, an enveloped single-stranded RNA virus, is a member of the order Nidovirales and family Arteriviridae. EAV is genetically similar to coronaviruses (also a member of Arteriviridae) but has a dissimilar viral structure and complement-fixing antigen.[224] Only one strain has been identified, the Bucyrus strain, but isolates vary in virulence.[225]

7.2.4.4.2 Epidemiology

Before the 1984 epizootic of equine viral arteritis (EVA) in Kentucky, little attention was paid to this virus.[226] Most infections were assumed to be transmitted by inhalation until the important role of venereal transmission was demonstrated in those outbreaks. The virus is maintained in the equine population by long-term and short-term carriers in stallions. The duration of the carrier status ranges from several weeks to years. Testosterone is essential for maintenance of persistence. Long-term infections do not occur in colts exposed to the virus before the onset of peripubertal development or in mares.[226–228] During the carrier state, the virus is present solely in the reproductive tract, principally in the ampulla of the vas deferens. Primary exposure to the virus is presumed to be via the venereal route. Susceptible mares bred to carrier stallions almost always become infected with EAV. Viral shedding into the respiratory tract allows secondary horizontal transmission of the virus to occur.

A significant difference in seropositivity to EAV exists among the horse breeds. Approximately 84% of Standardardbreds and 93% of Austrian Warmblood stallions have circulating antibodies to EAV, whereas less than 5% of Thoroughbred and less than 1% of Quarter Horses are seropositive.[229] 315 316

7.2.4.4.3

Pathogenesis

Following intranasal challenge (aerosolization), the virus invades the respiratory tract epithelium and the alveolar macrophages. By 72 hours after infection, replicating virus are detectable in the bronchopulmonary lymph nodes, endothelium, and circulating macrophages. Dissemination of the virus by hematogenous routes allows infection of mesenteric lymph nodes; spleen; liver; kidneys; nasopharyngeal, pleural, and peritoneal fluid; and urine.[226] By 6 to 8 days after infection, the virus has localized within the endothelium and medial myocytes of blood vessels, where it causes a necrotizing arteritis, a panvasculitis.[224] The vascular lesion may be caused by a direct cytopathic effect of the virus on the endothelium and medial myocytes or from the effects of anoxia or thrombosis induced by cell damage.

With venereal transmission (by natural cover or by artificial insemination), the virus can be isolated from swabs of the rectal and vaginal mucosa during the febrile periods.

7.2.4.4.4

Clinical Signs

Clinical signs vary, ranging from severe disease to subclinical infections. The variation may be a function of host factors, such as age and immune status, and virus factors, including the virulence, the amount of infective virus, and the route of infection.[230] The incubation period ranges from 3 to 14 days (6 to 8 days if transmitted venereally). In the acute disease, horses are febrile (105° F) for 1 to 5 days, anorectic, and depressed and may cough. Other clinical signs include a serous nasal discharge, congestion of the nasal mucosa, intermandibular lymphadenopathy, conjunctivitis, lacrimation, and less frequently, corneal opacification. Edema of the sheath, scrotum, ventral midline, limbs, and eyelids occurs because of vasculitis. Other signs may include respiratory distress, stiffness, soreness, diarrhea, icterus, skin rash on the neck, and papular eruptions on the inside of the upper lip. Most adult horses recover uneventfully.

Abortions occur from the tenth to the thirty-fourth day following exposure (during the third to tenth months of gestation), but the mechanism is not known. Abortion can occur with or without preceding clinical evidence of infection.[231] EVA has not been associated with teratologic abnormalities in fetuses or foals.

Neonates may die suddenly or develop severe respiratory distress followed by death.[226,232]

7.2.4.4.5

Clinical Pathology

Infection causes leukopenia, lymphopenia, and thrombocytopenia.

7.2.4.4.6

Diagnosis

EVA is a reportable disease. Although virus may persist in the buffy coat for up to 36 days after infection, viral isolation can be difficult.[230] The clinician should submit citrated blood samples and should attempt isolation of virions from nasopharyngeal and conjunctival swabs, vaginal swabs, and semen from an

infected stallion.[233] PCR enhances viral detection and specificity. One also should attempt serologic diagnosis. Virus neutralization, complement fixation, immunodiffusion, and immunofluorescence are techniques used to demonstrate changes in antibody titers. A fourfold or greater rise in titer or a change in status from seronegative to seropositive may indicate a recent infection. Postmortem examinations of aborted fetuses do not show signs of arteritis as seen in adults. Fetuses do have evidence of edema, excessive pleural fluid, and petechiation on the mucosal surfaces of the respiratory and gastrointestinal tracts, but focal necrosis of the liver or intranuclear inclusions are not features of the disease.[225]

7.2.4.4.7 Treatment

Treatment is primarily symptomatic, keeping the horse hydrated and providing analgesics (nonsteroidal antiinflammatory drugs) as needed. Fever in stallions can lead to sperm damage and temporary infertility, so one should administer nonsteroidal antiinflammatory drugs. The clinician should isolate horses for 3 to 4 weeks to minimize the chances of transmission.

7.2.4.4.8 Prevention

Vaccination of seronegative stallions with a commercially available modified live vaccine is credited with preventing the establishment of carrier states in the stallion or with further increases in the carrier state during the 1984 epizootic in Kentucky. Stallions were bred only to mares that were vaccinated or seropositive from previous natural exposure to EVA. Following vaccination, clinical immunity was found to develop rapidly and to last for 1 to 3 years.[226] Primary vaccination does not prevent reinfection and limited shedding of virus. Clinicians should vaccinate mares 3 to 4 weeks before breeding to an EAV carrier stallion and should isolate such mares for another 3 weeks after being bred to a carrier stallion for the first time.[231] Vaccination is not recommended for pregnant mares or in foals less than 6 weeks of age. Passively derived antibodies to EAV decrease to undetectable amounts by 8 months postpartum for foals born to seropositive mares. Vaccination should be effective at this time if necessary.[234]

7.2.4.5 Equine Rhinoviruses

Among those viruses that formerly were called equine rhinoviruses—classified as members of the family Picornaviridae and genus *Rhinovirus*—two serotypes were identified. Serotype 1 recently has been renamed equine rhinitis A virus (ERAV) and has been reclassified as a member of the genus *Aphthovirus*. The reclassification is based on (1) homology of the nucleotide sequence of the ERAV genome with that of foot-and-mouth disease virus, (2) the physicochemical properties of ERAV, and (3) the production of viremia and 316
persistent shedding following ERAV infection.[235] Serotype 2 has been renamed equine rhinitis B virus 317
(ERBV) and has been reclassified as the sole member of the genus *Erbovirus*. A third serotype designated P313/75 also has been identified and currently is assigned to the family Picornaviridae. However, based on sequence similarity and serologic data, investigators have proposed that P313/75 be classified as a distinct serotype of *Erbovirus* and tentatively have named it ERBV2.[236]

Infection of horses with ERAV causes acute fever, nasal discharge, coughing, anorexia, pharyngitis, laryngitis, and submandibular lymphadenitis.[237,238] Infection produces moderate increases in the neutrophil-to-lymphocyte ratios (from 50:49 in healthy horses to 57:43 in infected horses) and in plasma fibrinogen.[238] The importance of ERAV as a cause of respiratory disease has not been recognized previously because clinical

signs may be mild and because viral isolation is difficult.[239] The incubation period is 3 to 8 days, with shedding of viral particles from pharyngeal secretions, urine, and feces a feature of the infection. Young horses are infected more commonly and in one study, 73% of 2-year olds showed serologic evidence of ERAV infection.[238]

Equine rhinovirus A has a broad host range that includes rabbits, guinea pigs, monkeys, and human beings. Experimental and epidemiologic evidence of ERAV infection of human beings exists. High antibody titers were found in the sera of 3 of 12 stable workers, whereas no ERAV antibody was found in the sera of 159 non–stable workers.[240] One can attempt diagnosis of ERAV infection by serologic testing (fourfold changes between acute and convalescent titers) or PCR identification of the virus in nasopharyngeal swabs.[239]

Equine rhinovirus B is also an equine respiratory tract pathogen. In a survey of horses with acute respiratory disease, researchers isolated ERBV from 30% of the horses sampled. Of the 28 horses from which the virus was recovered, a serologic diagnosis was made in only 6 horses. Researchers attributed the low diagnosis rate to the difficulty of initially collecting samples during the acute phase: antibody levels rise rapidly from day 6 after infection to peak between days 14 and 18.[241] Thus in contrast to ERAV, viral isolation may be the more successful diagnostic method.

The clinical significance of ERBV2 (P313/75) is unknown. ERBV2 was isolated from a horse with a history of 6 days of intermittent fever that began 4 days after an umbilical hernia operation and castration.[242] The clinical importance requires further investigation.

No vaccine is currently available for ERAV or ERBV.

7.2.4.6 Tracheal Disorders

7.2.4.6.1 Definition

Inflammations, obstructions, compressions, lacerations, and containment of foreign bodies are the predominant disorders of the trachea in the horse.[243–246] Tracheitis often accompanies viral upper respiratory tract diseases (see previous discussion) and manifests as areas of hyperemia and epithelial desquamation. Lacerations usually result from trauma, whereas compressions may result from extraluminal masses such as streptococcal abscesses or neoplasms such as lymphosarcomata or lipomata. Intraluminal obstructions may follow fibrotic stricture formation following trauma, tracheostomy, or pressure necrosis from inflated cuffs of endotracheal tubes or may be caused by granulomatous reactions within the trachea.[243] Foreign bodies, such as twigs, probably are inhaled during deglutition. Tracheal collapse may represent a congenital defect in miniature horses and ponies.[247,248]

7.2.4.6.2 Clinical Signs

Clinical signs vary and range from stertorous breathing and dyspnea to chronic and persistent coughing and bilateral nasal discharge with a foul odor. Mild manipulation of the trachea also may induce paroxysmal bouts of coughing.

7.2.4.6.3 Diagnosis

The diagnosis is made on physical examination or endoscopic evaluation of the trachea. A history of a tracheal surgery or traumatic episode should alert the diagnostician to the possibility of an intraluminal obstruction or stricture. The clinician may visualize foreign bodies on examination but may need radiographs to delineate the extent of their involvement. One also should perform radiographic examination of the thorax to determine if a concomitant pneumonia, pneumomediastinum, or pneumothorax exists.

7.2.4.6.4 Treatment

One directs treatment at the primary cause. One does not treat primary inflammations following viral infections unless a persistent fever and a secondary bacterial tracheitis and pneumonia develop. The clinician may extract foreign bodies but may require tracheotomy to retrieve them if the endoscope is not long enough. In the adult horse a 2-m endoscope is necessary to reach the level of the carina located at the fifth to sixth intercostal space. Extraluminal masses require careful drainage or excision, with the potential for spread of infection into the mediastinum. The clinician should isolate horses with *Streptococcus equi* infections from other equids. Rupture of the trachea may require temporary tracheotomy distal to the rupture to ensure patency of airflow. A 2-week course of broad-spectrum antimicrobial therapy may be indicated, especially if the mucociliary clearance mechanism is compromised. Complications such as subcutaneous emphysema, cellulitis, and pneumomediastinum usually resolve over 2 weeks. Horses with tracheal defects have a poor to guarded prognosis for return to athletic potential.

7.3 The Normal Lung

7.3.1 ANATOMY

The lungs of the horse differ from those of other domestic species in that they lack deep interlobar fissures and 317

distinct lung lobes. Superficially, however, the left lung consists of a cranial and caudal lobe, whereas the right 318 lung contains a cranial, an intermediate, and a caudal lobe.[249] The intrathoracic trachea bifurcates into the right and left main stem bronchi at the level of the fifth or sixth intercostal space and enters the hilum of each lung. At its division from the trachea, the right bronchus assumes a straighter, more horizontal position relative to the left bronchus, a configuration that may predispose the horse to develop right-sided pulmonary disorders. Each bronchus divides into lobar, segmental, and subsegmental bronchi with the eventual formation of bronchioles. In the distal part of the bronchial tree the terminal bronchioles lead into poorly developed respiratory bronchioles or open directly into alveolar ducts.[249,250] The tracheobronchial lining consists of tall columnar, pseudostratified epithelium interspersed with serous and goblet cells. The goblet cells and the underlying submucosal mucous glands function to produce mucus consisting of an outer gel and an inner sol layer.[251] Mucus serves to prevent epithelial dehydration, contains protective factors that guard against infectious agents, and is an integral part of the mucociliary apparatus. The rapid beating of the cilia moves mucus and any particulate matter to the pharynx to be swallowed. Approximately 90% of material deposited on the mucinous layer is cleared within 1 hour.

Ciliated cells decrease in frequency with successive divisions of the airways so that at the level of the bronchioles the epithelium is composed predominantly of low ciliated and taller nonciliated Clara cells.[252] Glands are also absent at this level. The bronchiolar epithelium becomes contiguous with that of the alveoli, which are characterized by two distinct cell types. Type I pneumocytes cover most of the alveolar surface with

thin cytoplasmic extensions 0.2 to 0.5 μm thick.[251] Type II cells are cuboidal and contain the characteristic lamellar cytoplasmic inclusions thought to constitute surfactant components. Type II cells also are considered to be stem cells, replacing the type I cells when damaged.

Lymphocytes are scattered throughout the pulmonary epithelium, are associated with the bronchioles and bronchi, and occur in discrete nodules or patches.[253] These cells, along with alveolar macrophages, provide an integral component of the pulmonary immune surveillance system. Macrophages are derived from blood monocytes via the interstitium and are cleared continuously from the alveoli. They are the predominant cell type recovered from the bronchoalveolar lavage in normal horses (see Table 7-1). Pulmonary interstitial macrophages are able rapidly to ingest particles introduced within the pulmonary circulation.[254] The ability of these macrophages to localize antigenic particles within the pulmonary vasculature may predispose the equine lung to development of acute pulmonary inflammation (e.g., during endotoxemia).

Additional cells within the lung parenchyma include the subepithelial and free mast cell. These cells bear IgE on their cell surface and release biogenic amines in response to specific antigen stimulation. They appear to be important in the pathogenesis of several equine pulmonary disorders, including anaphlaxis and lungworm infections.

Tracheobronchial secretions consist of substances secreted from mucous and serous glands, as well as serum transudates. The principal component of the secretions is water, with approximately equal amounts of protein, carbohydrate, lipid, and inorganic material. Contained within the protein fraction of the tracheobronchial secretions are albumin, IgA, IgG (the predominant immunoglobulin in the lower lung), lysozyme, lactoferrin, haptoglobulin, transferrin, and complement components.[251]

7.3.2 VASCULAR SUPPLY

The lung has two sources of blood flow. The major source of blood is the pulmonary circulation, a low-pressure, low-resistance system that serves primarily to deliver blood to the alveoli for participation in gas exchange but also provides nutrients to the alveolar constituents. The distribution of the pulmonary arterial flow to the various lung regions depends largely on mechanical forces: gravity and pulmonary arterial, pulmonary venous, and alveolar pressures.[255] In the standing horse, a vertical perfusion gradient of the lung has been demonstrated,[256] the most dorsal part of the equine lung being less well perfused than the ventral dependent regions. The distribution of pulmonary blood flow also is influenced by vasoactive compounds such as catecholamines, histamine, and eicosanoids and by changes in alveolar oxygen and carbon dioxide. Alterations in pulmonary vascular resistance may help to match ventilation with perfusion and optimize gas exchange.

The bronchial circulation, the second source of blood flow to the lungs, provides nutrient support to the lymphatics and vascular and airway components. Bronchial circulation provides arterial blood to the pleural surface and anastomotic connections with the alveolar capillary bed derived from the pulmonary circulation.[250] The magnitude of the anastomotic flow depends on the relative pressure in the bronchial and pulmonary microvasculature and on the alveolar pressure. Thus in the dorsal part of the lung, where pulmonary arterial flow is poor, blood flow from the bronchial circulation may be favored.[257] The degree of anastomotic bronchial blood flow through the alveolar capillaries also increases with systemic arterial hypoxemia and alveolar hypoxia.[255] The bronchial circulation undergoes hypertrophy (angiogenesis) in response to inflammatory pulmonary diseases such as chronic bronchitis, bronchiolitis, and bronchiectasis. 318

7.3.3 INNERVATION

The autonomic nervous system supplies innervation to the pulmonary structures. This nervous system influences (1) airway smooth muscle tone, (2) secretion of mucus from the submucosal glands, (3) transport of fluid across airway epithelium, (4) permeability and blood flow in the bronchial circulation, and (5) release of mediators from mast cells and other inflammatory cells.[258] In the horse the autonomic nervous system can be classified functionally into three categories: (1) parasympathetic, (2) sympathetic, and (3) nonadrenergic noncholinergic (NANC) pathways.[259] The vagus nerve is an integral component of the parasympathetic and NANC systems. The vagus nerve not only contains the efferent fibers of these pathways, which help to alter airway resistance, lung volume, and compliance, but also contains the afferent fibers the central input of which regulates the pattern of breathing. Stimulation of the parasympathetic fibers within the vagus nerve (i.e., the efferent limb) releases acetylcholine from the postganglionic fibers and combines with the muscarinic receptors of the airway smooth muscle to cause bronchoconstriction. Yet because atropinization (antagonism of muscarinic receptors) does not normally change airway resistance, the parasympathetic system appears to exert little influence on resting airway tone in the *healthy* horse.[260] However, vagal efferents are important in reflex bronchoconstriction during pulmonary disease.

As described previously, the vagus nerve also contains afferent nerve fibers that transmit information detected by three types of pulmonary mechanoreceptors: (1) the slowly adapting receptors, (2) the rapidly adapting receptors, and (3) the nonmyelinated C fibers. The receptors relay information to the central respiratory neurons located within the ventral medulla and pons. Changes in breathing depth and frequency occur in response to mechanical deformations or chemical stimulation of these receptors. Thus these receptors mediate the tachypneic breathing pattern that occurs in response to inhalation of irritant substances. Stimulation of these receptors also influences airway smooth muscle tone,[261] inducing reflex bronchoconstriction in response to inhaled irritants.

In the lung, α- and β-adrenergic receptors mediate sympathomimetic effects. Postsynaptic fibers course from the sympathetic ganglion to airway smooth muscle where released norepinephrine interacts with α- and β-receptors. Postganglionic sympathetic fibers also innervate parasympathetic ganglia. The released norepinephrine inhibits cholinergic neurotransmission.[259] However, numerous β_2-adrenergic receptors are distributed throughout the pulmonary parenchyma that lack innervation by the sympathetic fibers. Circulating catecholamines are probably important in activating these receptors and in causing subsequent bronchodilation. It is of interest that β_2-adrenergic stimulation does not alter airway diameter in the healthy horse[262] but does increase airway caliber in horses with recurrent airway disease. β-agonists also promote ion transport and water secretion across human airway epithelium (in vitro) and may cause similar effects in vivo in the horse. This effect would benefit mucociliary clearance by increasing the sol component of the mucus. In addition, β-agonists stimulate surfactant secretion by type II pneumocytes, inhibit antigen-induced mast cell degranulation, and modulate cholinergic neural transmission.[258] Such effects remain to be demonstrated in the horse, however.

α-Adrenergic receptors also exist in the equine lung, but their stimulation induces bronchoconstriction only in heavy and not in normal ponies.[263] This finding suggests that, in health, these receptors are probably unimportant in the regulation of bronchomotor tone.

An additional autonomic pathway, the NANC inhibitory system, also has been demonstrated in the equine lung, and its fibers course within the vagus nerve.[259] Although the mediators involved in neural transmission have not been identified definitively, some speculate that vasoactive intestinal peptide or peptide histidine isoleucine or

both may be the neurotransmitters.[264] Stimulation of this pathway causes a 50% reduction in smooth muscle tone and thus exerts a vasodilating effect.[265] Interestingly, horses with recurrent airway disease appear to lack NANC innervation in the bronchioles, and this dysfunction may contribute to the airway hyperactivity observed.[259]

7.3.4 LYMPH DRAINAGE

Lymph drainage from the lung is accomplished by (1) the deep lymphatics, which begin at the level of the alveolar ducts and run with the conducting airway and arteries toward the hilar lymph nodes, and (2) the superficial lymphatics, which drain the visceral pleura through a plexus converging on the hilum.[251]

7.3.5 PULMONARY PHYSIOLOGY

The major function of the lung is gas exchange: diffusion of oxygen and elimination of carbon dioxide. During eupnea, an adult horse breathes at a frequency of about 12 breaths per minute and a tidal volume near 6.5 L. This represents a total minute ventilation of 77 L/min, or more than 100,000 L/day. During this same period, the resting horse consumes approximately 2.1 L/min of oxygen (3000 L/day) and produces approximately 1.7 L/min of carbon dioxide (2400 L/day).[266] Thus the lung provides an important means by which normal arterial oxygen and carbon dioxide tensions and arterial pH are maintained. In the absence of lung disease, pulmonary arterial 319
oxygenation (Pao_2) depends on the inspired fraction of oxygen (normally 0.21) and the effective level of alveolar 320
ventilation. This is approximated by a version of the alveolar gas equation:

$$PAo_2 = PIo_2\ (PAco_2)/R$$

where PAo_2 is the alveolar oxygen tension, PIo_2 is the inspired oxygen tension, $PAco_2$ is the alveolar carbon dioxide tension, and R is the respiratory exchange quotient (the ratio of CO_2 production to O_2 consumption). Because an admixture of venous blood with the pulmonary arterial circulation occurs, alveolar oxygen tension exceeds arterial oxygen tension by approximately 10 mm Hg.

In the absence of alterations in inspired oxygen tensions, arterial hypoxemia (Pao_2 <85 mm Hg) may result from basically four processes, including (1) hypoventilation, (2) diffusion impairment, (3) V/Q ($\dot{V}$ / $\dot{Q}$) inequality, and (4) shunts.[267]

Alveolar and thus arterial CO_2 levels depend on the rate of CO_2 elimination relative to its production, given by the following equation:

$$PAco_2 = \dot{V}CO_2/\dot{V}A$$

where $\dot{V}$ CO_2 is the rate of CO_2 production and $\dot{V}$ is alveolar ventilation. *Hypoventilation* is defined as inadequate CO_2 elimination relative to production, resulting in an elevated $Paco_2$ (hypercapnia, hypercarbia). Hypoventilation can result from a variety of abnormalities. A convenient diagnostic approach follows the control of breathing from (1) the respiratory center in the medulla, (2) the efferent nerves (phrenic), (3) the bellows (diaphragm and chest wall muscles), (4) the pleural space, and (5) the airways. Some situations in which hypoventilation may develop include (1) drug administration (barbiturates, diazepam, xylazine); (2) diseases of

the brainstem following infection (encephalitis), trauma, hemorrhage, or neoplasia; (3) diseases of the respiratory muscles, including botulism, trauma, or fatigue; (4) pleuritis and space-occupying lesions; and (5) choke and upper airway obstruction.

In patients suffering from hypoventilation, arterial oxygenation improves with oxygen administration (by increasing PIo_2), but the most efficacious mode of correcting the hypercapnia and consequent hypoxemia is by mechanical ventilation. Diffusion impairment occurs when time is inadequate for equilibration of alveolar oxygen tensions with pulmonary capillary oxygen tensions. Under normal resting conditions, equilibration of oxygen tensions occurs within 0.25 second, approximately one third of the contact time of the blood in the pulmonary circulation.[267] Some evidence suggests that in the exercising horse, the arterial hypoxemia that normally develops at high exercise intensities in part is caused by the decrease in time available for oxygen diffusion.[268,269] During rest, diffusion impairment is unlikely to occur. Arterial hypoxemia caused by diffusion impairment can be corrected by administration of supplemental oxygen.

V/ $\dot{Q}$ inequalities are the primary cause of arterial hypoxemia and occur when alveolar ventilation and pulmonary blood flow are mismatched despite the existence of several reflexes that normally tend to prevent this problem. For example, a fall in the $\dot{V}/\dot{Q}$ ratio causes alveolar hypoxia, which induces pulmonary vasoconstriction and decreases perfusion to that lung region. (But this reflex also increases pulmonary vascular resistance and thus the work of the right side of the heart.) A second reflex is hypocapnic bronchoconstriction. When the $\dot{V}/\dot{Q}$ ratio increases, regional ventilation of those lung units is reduced by smooth muscle constriction. $\dot{V}/\dot{Q}$ inequalities interfere with O_2 and CO_2 transfer so that hypoxemia and hypercapnia may ensue. Usually the consequent hypercapnia increases chemoreceptor drive and thus increases alveolar ventilation, which restores normocapnia. However, because of the shape of the oxyhemoglobin dissociation curve, the increase in oxygen tension in the normal alveoli caused by the hyperventilation does not increase the oxygen content of the blood coming significantly from these units. Several factors can exacerbate the hypoxemia of $\dot{V}/\dot{Q}$ inequalities, including hypoventilation (sedation) and decrements in cardiac output (mechanical ventilation with positive end-expiratory pressure). $\dot{V}/\dot{Q}$ inequalities respond to oxygen therapy, although this may lead to increases in arterial carbon dioxide tension by (1) a reduction of the chemoreceptor (hypoxic) drive, (2) an increase in $\dot{V}/\dot{Q}$ inequalities, and (3) a shift in the carboxyhemoglobin dissociation curve to the right, decreasing its affinity for carbon dioxide.[270] Mechanical ventilation may be indicated in severe $\dot{V}/\dot{Q}$ inequalities if hypercapnia is progressive.

Passage of blood through abnormal cardiovascular communications (atrial septal defects, ventricular septal defects, patent ductus arteriosus) or through pulmonary capillaries within the walls of atelectatic or fluid-filled alveoli causes shunts and consequent hypoxemia. Such defects may be considered as one extreme of $\dot{V}/\dot{Q}$ inequality ($\dot{V}/\dot{Q} = 0$) and are resistant to correction by oxygen therapy.[270] In shunts caused by pulmonary disease, mechanical ventilation and positive end-expiratory pressure increase end-expiratory lung volume and thus the alveolar surface area available for gas exchange. The incremental increase in end-expiratory lung volume also may help to redistribute the excessive extravascular lung water from the alveoli to the interstitium.

7.3.6 METABOLIC FUNCTIONS

The entire cardiac output passes through the pulmonary circulation, thus providing an ideal means by which 320
hormones and drugs may be metabolized to inactive compounds. Indeed, relative to the hepatic blood flow 321
(approximately 25% of the cardiac output), the contribution of the lungs to the total body clearance of drugs or xenobiotics may be significant. The lung contains hydrolytic enzymes that cleave peptides such as bradykinin and angiotensin I, thus serving to inactivate (bradykinin) or to bioactivate (angiotensin) compounds. The enzymatic activity responsible is concentrated within the caveolae of the pulmonary capillary endothelium or in pouchings in the plasma membranes of these cells.[271] Other pulmonary enzymes exist that are capable of cleaving phosphate groups from nucleotides (adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate), of oxidizing steroid hormones (testosterone), of inactivating prostaglandins E and F, and of metabolizing biogenic amines such as 5-hydroxytryptamine, norepinephrine, and tyramine.[255,271,272]

7.3.7 DEFENSE AGAINST INFECTION

The sterile environment of the lung results from several mechanisms, including the mucociliary apparatus, which clears particulate debris from the lung at a rate of approximately 17 mm/min,[273] and vagally mediated reflexes, which serve to initiate coughing and concomitant bronchoconstriction. However, the predominant line of defense against noxious agents and bacteria is the alveolar macrophages of the distal airways and alveoli. Recent investigations have shown depression in alveolar macrophage function following exercise or long van rides.[274,275] Viral infections also depress macrophage function. Helping to maintain immunosurveillance and the sterile pulmonary environment are polymorphonuclear cells that respond to chemotactic stimuli and lymphocytes.

7.4 Disorders of the Lower Respiratory Tract

Disorders of the lower respiratory tract are common in horses of all ages. Presenting complaints vary with these disorders and may include exercise intolerance, cough, nasal discharge, fever, dyspnea, increased respiratory rate or effort, or generalized depression and inappetance. Careful clinical examination including auscultation while rebreathing often confirms the anatomic site of the problem. Ancillary diagnostic testing such as bronchoalveolar lavage, transtracheal wash, thoracic ultrasound, or thoracic radiography often are indicated to confirm a suspected diagnosis.

7.4.1 BACTERIAL PNEUMONIA

7.4.1.1 Causes

Normally the lungs contain only small numbers of bacteria (colony-forming units) that are considered to be transient contaminants in the process of being removed by clearance mechanisms.[276] When pulmonary defense mechanisms are overwhelmed, aspirated bacteria from the oropharynx may proliferate and cause pneumonia. The most common gram-positive bacteria involved are *Streptococcus zooepidemicus* (β-hemolytic), *Staphylococcus aureus,* and *Streptococcus pneumoniae* (α-hemolytic). The most frequent gram-negative isolates are *Pasteurella* and *Actinobacillus* spp., *Escherichia coli, Klebsiella pneumoniae,* and *Bordetella bronchiseptica.*[26,27,277–280] *Pseudomonas* is rarely a cause of equine pneumonia, and its isolation from tracheobronchial aspirates suggests environmental contamination of equipment (endoscope). *Nocardia* organisms also have been isolated from horses with pulmonary infections, but these are rare and appear to require significant derangements of the defense mechanisms.[281] The anaerobic bacteria most commonly isolated are *Bacteroides fragilis, Peptostreptococcus anaerobius,* and *Fusobacterium* spp. Polymicrobic infections are not uncommon in cases of equine pneumonia and may represent a synergy between aerobic/facultatively anaerobic and anaerobic bacteria. Mechanisms of synergy may involve protection from phagocytosis and intracellular killing, production of essential growth factors, and lowering of local oxygen concentrations.[278]

7.4.1.2 Pathogenesis

Bacterial pneumonia develops following viral infections, athletic events (races), trailor rides, and general anesthesia and also occurs in horses that are overcrowded, maintained on poor nutrition, or are exposed to inclement weather. Laryngeal and pharyngeal dysfunction also may predipose the horse to develop pneumonia by aspiration of oropharyngeal bacteria. Such predisposition occurs in horses (1) with primary neuropathies of the ninth and tenth cranial nerves (equine protozoal myeloencephalitis, botulism, *Streptococcus equi* infections, guttural pouch mycoses), (2) with primary myopathies of the pharyngeal, laryngeal, or esophageal musculature (vitamin E and selenium deficiency, megaesophagus), (3) following laryngeal surgery, or (4) with esophageal obstructions.

The pathogenic mechanisms described subsequently apply to the development of lung abscesses and pleuropneumonia.

Viral-induced modifications of respiratory tract defenses include (1) enhanced susceptibility to bacterial attachment and colonization following damage to the epithelial cells, (2) diminished mucociliary clearance and physical translocation of bacterial particles deposited on the bronchial ciliated epithelium, and (3) decreased surfactant levels and collapse of the airways because of viral destruction of alveolar type II cells.[282]
The ensuing anaerobic environment predisposes to macrophage dysfunction. In addition, the alveolar exudate 321
that accompanies viral pneumonitis may provide a nutrient medium for multiplication of aspirated bacteria. 322

Exercise, with aspiration of track debris and oropharyngeal secretions and with the development of exercise-induced pulmonary hemorrhage has been suggested as creating an ideal environment for bacterial growth and pneumonia.[283] An exercise-associated increase in bacterial contaminants of the lower respiratory tract has been demonstrated experimentally. Strenuously exercised horses exhibit a ten- and a 100-fold increase,

respectively, in the number of aerobic and anaerobic bacteria isolated from transtracheal aspirates relative to preexercise samples.[284] The bacterial contamination, along with exercise-associated increases in bronchoalveolar cortisol concentrations, decreased pulmonary alveolar macrophage viability, and impaired phagocytic function, enable bacteria to proliferate and cause pneumonia.[274,275,283,285,286]

Transportation remains one of the most common causes of pneumonia and pleuropneumonia in the horse because physically restraining the head of the horse and preventing postural drainage enhances bacterial colonization of the lower respiratory tract.[283,287] Inflammation and increased numbers of bacteria are found in the transtracheal aspirates of horses within 6 to 12 hours of confinement with the head elevated.[288] Another hypothesis is that dehydration, associated with reduced fluid consumption before or during transportation, reduces mucociliary clearance and contributes to bacterial proliferation. Neither the prophylactic administration of antibiotics nor the intermittent release from the confined head posture reliably reduces tracheal bacterial numbers or prevents the accumulation of purulent tracheobronchial secretions in horses confined with their heads elevated.[288,289]

Horses undergoing general anesthesia may develop pneumonia when endotracheal intubation introduces oropharyngeal contaminants to the lower respiratory tract. Anesthesia may compromise mucociliary clearance, and compression atelectasis and vascular congestion may cause regional ischemia and necrosis of lung regions, providing local conditions suitable for bacterial multiplication.[283]

7.4.1.3 Clinical Signs

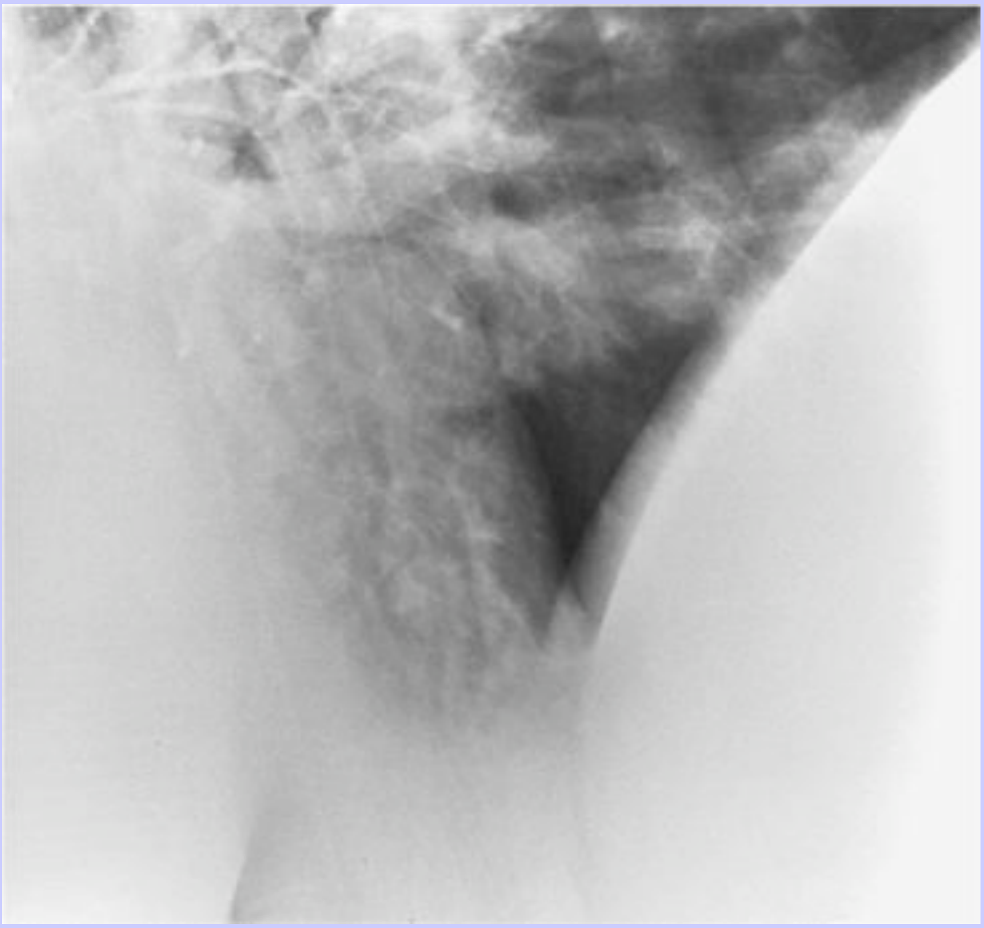
In early cases, clinical signs may not be obvious, being limited to a gurgling sound of exudates in the trachea, fever, or depression. As the pneumonia progresses, horses may exhibit intermittent fever, tachypnea or respiratory distress, nasal discharge, coughing, inappetence, exercise intolerance, and weight loss. Nasal discharge is usually mucopurulent but may be hemorrhagic in some cases.[278] Auscultation of the thorax reveals increased harsh breath sounds dorsally, crackles, and wheezes and dullness of respiratory sounds ventrally. Manipulation of the trachea or larynx may induce a cough. Halitosis and a foul-smelling nasal discharge suggest an anaerobic infection. Submandibular lymphadenopathy may be apparent in aspiration pneumonia associated with strangles or with viral infections.

7.4.1.4 Diagnosis

Clinical signs and history aid in diagnosis. Clinical pathologic data supportive of a bacterial pneumonia include a leukocytosis and an absolute neutrophilia, with or without a left shift. Neutropenia also may be evident if gram-negative organisms are involved. A hyperfibrinogenemia (>500 mg/dl), hyperglobulinemia, hypoalbuminemia, and anemia of chronic inflammation are compatible with the diagnosis of chronic bacterial pneumonia. Endoscopic evaluation of the upper respiratory tract may demonstrate a defect in the laryngeal pharyngeal function if this is the inciting cause. One may observe a mucopurulent exudate with or without traces of blood by endoscopy in the lower respiratory tract. Thoracic radiographs demonstrate a radiopacity in the anteroventral thorax and a loss of clarity in the lung fields caudal to the heart. Air bronchograms occasionally are found in the adult horse with bacterial pneumonia (Figure 7-13). Ultrasound may demonstrate consolidation of ventral lung lobes and extension of the pneumonia to the pleural surfaces. Tracheobronchial aspirates yield degenerative neutrophils, damaged epithelial cells, and microorganisms. The presence of 322
squamous epithelial cells supports a diagnosis of aspiration pneumonia, provided that the tracheal catheter was 323

not misplaced in the pharynx during sampling. The clinician should perform aerobic and anaerobic cultures on the tracheal aspirates.

Figure 7-13 Two-year-old Thoroughbred with pneumonia. A patchy area of pulmonary consolidation is notable in the ventral dependent portion of the lung silhouetting the heart and diaphragm. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



7.4.1.5

Treatment

The clinician should direct treatment at the causative organism, but in the absence of culture sensitivity results, one should administer broad-spectrum antimicrobials. Appropriate therapy might include intravenous aqueous sodium or potassium penicillin and an aminoglycoside or third-generation cephalosporin (Table 7-2). The aminoglycosides are efficacious against most gram-negative aerobes, but lack efficacy against anaerobes. However, metronidazole is effective against most anaerobes, including the penicillin-resistant *Bacteroides fragilis* and routinely is included in the treatment protocol. Depending on the culture sensitivity results, one also may administer potentiated sulfonamides. The clinician should use aminoglycosides judiciously in

animals that have renal compromise or are dehydrated. (See the section on treatment for parapneumonic effusions.)

TABLE 7-2 Antimicrobials Commonly Used to Treat Respiratory Conditions

ANTIMICROBIAL	DOSAGE*
Amikacin	16–18 mg/kg, IV, s.i.d.
Ampicillin sodium	11–22 mg/kg, IV or IM, t.i.d. or q.i.d.
Ceftiofur	2.2 mg/kg, IV or IM, b.i.d.
Chloramphenicol	50 mg/kg, PO, q.i.d.
Enrofloxacin	7.5 mg/kg, PO or IV, s.i.d.
Gentamicin	6.6 mg/kg, IV or IM, s.i.d.
Metronidazole	15–25 mg/kg, PO, t.i.d. or q.i.d.
Oxytetracycline	5.0 mg/kg, IV, b.i.d.
Potassium Penicillin G	22,000 IU/kg, IV, q.i.d.
Procaine Penicillin G	22,000 IU/kg, IM, b.i.d.
Rifampin	5–10 mg/kg, PO, b.i.d.
Sodium Penicillin G	22,000 IU/kg, IV, q.i.d.
Trimethoprim-sulfonamide	15–20 mg/kg, PO or IV, b.i.d.

* b.i.d., Twice daily; IM, intramuscularly; IV, intravenously; PO, orally; q.i.d., 4 times daily; s.i.d., once daily; t.i.d., 3 times daily.

With gram-negative infections and the potential for endotoxemia, small doses of flunixin meglumine (0.25 mg/kg 3 times daily) may be given to inhibit arachidonic acid metabolism. Other treatment modalities that have been advocated include nebulization of mucolytics, the use of bronchodilators and expectorants, and prophylactic measures against laminitis. The goal of supportive care should be to minimize stress and ensure adequate ventilation and hydration. Ideally, horses should be bedded on paper or on other materials free of dusts or molds and should be fed forages of excellent quality. One should also direct attention to correcting the primary cause of the pneumonia. Depending on the chronicity of the pneumonia, one should note clinical improvement in 48 to 72 hours. The prognosis can be excellent if the pneumonia is treated aggressively, but the clinician should forewarn the owner of potential complications (see the following discussion). The clinician should administer treatment for 2 to 6 weeks depending on the extent of the pneumonia and the underlying inciting cause. (See the section on treatment for parapneumonic effusions.)

Preventive measures that help deter the development of pneumonia include (1) adequate immunization protocols with vaccination against equine influenza virus, EHV1, and EHV2, every 4 to 6 months (in the performance horse); (2) the minimization of stresses such as long van rides in which the head is restrained constantly; and (3) the use of management or husbandry methods that minimize dust or noxious gas accumulations within the stall, prevent exposure to inclement weather, and provide adequate nutrition for the horse.

7.4.2 LUNG ABSCESSES

7.4.2.1 Causes

In foals less than 6 months of age, *Rhodococcus equi* and *Streptococcus zooepidemicus* are the most common bacterial isolates recovered.[290] In adult horses, *Streptococcus* and *Actinobacillus* species are the most gram-positive and gram-negative organisms implicated in the development of pulmonary abscesses.[290–292] This discussion predominantly addresses lung abscesses in the adult horse.

7.4.2.2 Pathophysiology

Lung abscesses may develop as a consequence of inhaling bacterial organisms resident to the oropharynx or contaminating the environment *(Rhodococcus equi)*. The abscess may develop as a consequence of a focal pneumonia or may be a component of pleuropneumonia complex. Racehorses may be at an increased risk for developing lung abscesses when bacteria proliferate in the blood in the airways and alveoli following episodes of EIPH. The regional location of lung abscesses in these cases supports this hypothesis.[292]

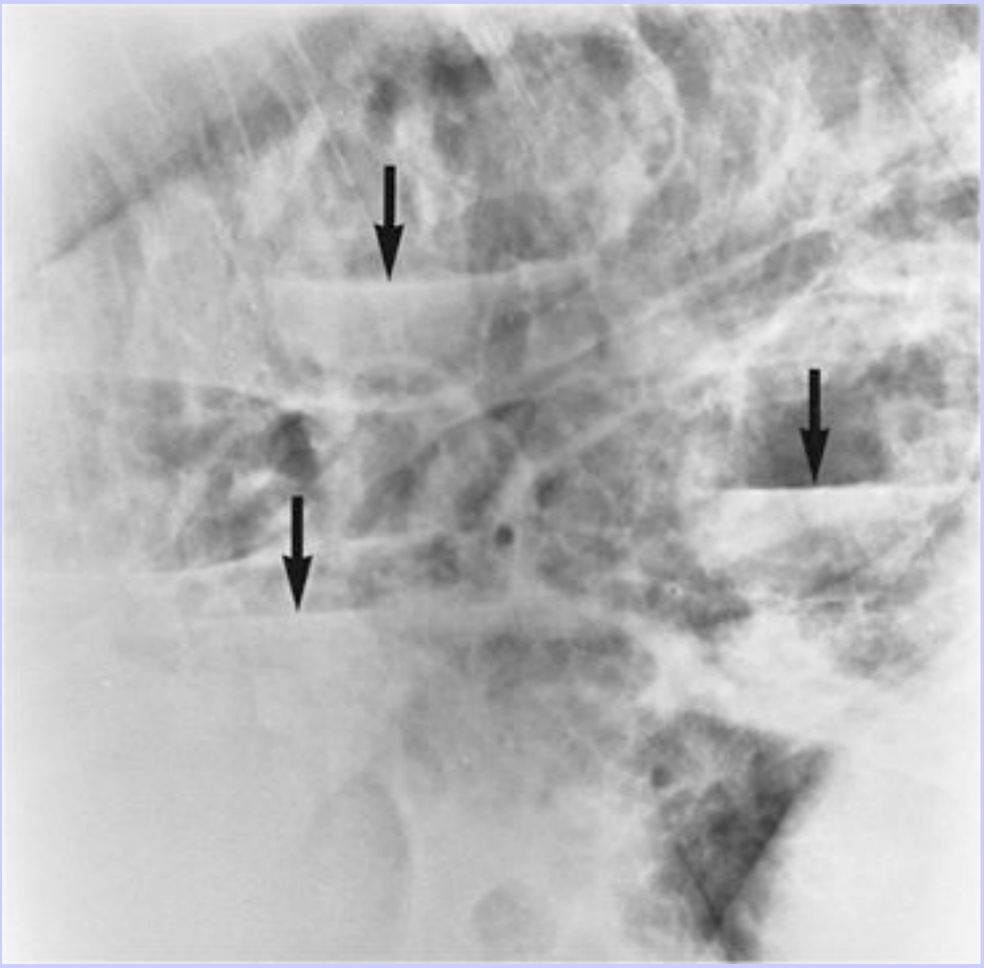
7.4.2.3 Clinical Signs

Clinical signs may vary, but most horses have periods of pyrexia, inappetance, lethargy, mild tachycardia, and respiratory-related signs ranging from tachypnea to distress.[291,292] Other signs that may be evident include cough, purulent nasal discharge, thoracic pain, epistaxis, and halitosis.

7.4.2.4 Diagnosis

The adult horse may have a history of transport, strenuous exercise, surgery, prior pneumonia, or 323
administration of medications. In most cases, evidence of an infection is found on the CBC and serum 324
chemistry panel: a mature neutrophila, hyperfibrinogenemia, hyperglobulinemia, and anemia. Radiography and ultrasound are useful imaging modalities, and one may use them concurrently to detect lung abscesses (Figures 7-14 and 7-15). Pulmonary abscesses appear on ultrasonography as encapsulated cavitated areas filled with fluid or echogenic (white) material. Cardiac structures or air-filled lung may overlie an abscess, obscuring its detection. In one study, two thirds of the abscesses were located in the caudodorsal lung field, whereas the remaining abscesses were located in the caudoventral region.[292]

Figure 7-14 Nine-year-old saddle horse. Multiple cavitating pulmonary abscesses are present within the lungs. Arrows indicate air-fluid interfaces within the abscesses. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



Transtracheal aspirates or percutaneous aspirates of lung abscesses adhered to the body wall may help the clinician decide the choice of antimicrobials. One should culture isolates under aerobic and anaerobic conditions.

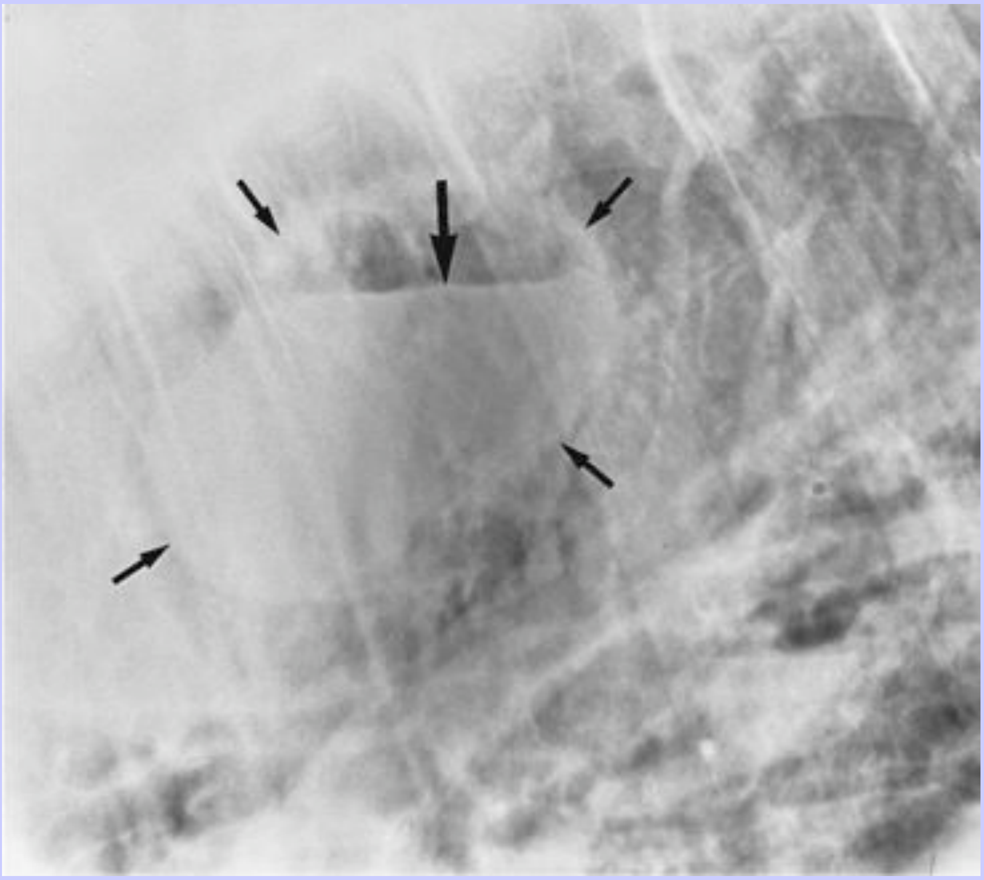
7.4.2.5

Treatment

Long-term antimicrobial therapy (8 to 10 weeks) along with prolonged periods of rest (5 to 6 months) before resuming strenuous exercise is recommended. (See the section on treatment for parapneumonic effusions for specific antimicrobial recommendations.) In human beings receiving antimicrobial treatment for pulmonary absceses, an 80% reduction in the size of abscess cavities less than 3 cm in diameter usually occurs within 1

month of appropriate therapy.[293] Furthermore, 70% of cavitary lesions in human cases of lung abscessation resolve completely by 3 months.[294] For lung abscesses unresponsive to therapy, the clinician should consider drainage via a thoracotomy or thoracoscopy.[295]

Figure 7-15 A large air-capped pulmonary abscess. Small arrows indicate an abscess; large arrow indicates the air-fluid interface. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



Prognosis for resumption of athleticism is good if treatment is initiated early. In one study, 23 of 25 Standardbreds and 13 of 20 Thoroughbreds raced after the diagnosis and treatment of a lung abscess with no significant effect on race performance.[292]

7.4.3 PARAPNEUMONIC EFFUSIONS AND SEPTIC PLEURITIS

7.4.3.1 Causes

Between two thirds and three quarters of cases of septic pleuritis arise as an extension of pneumonia or pulmonary abscessation.[296–298] Septic pleuritis also may occur in cases of thoracic trauma, esophageal

rupture, or penetration of the esophagus or stomach by a foreign body.[283,299–301] The aerobic or facultatively anaerobic organisms most often isolated from horses with pleuropneumonia are bacterial species that normally reside in the oropharyngeal cavities: *Streptococcus* spp., *Pasteurella* and *Actinobacillus* spp., *Escherichia coli,* and *Enterobacter* spp. Anaerobic organisms frequently isolated include *Bacteroides* spp., *Peptostreptococcus* spp., *Fusobacterium* spp., and *Clostridium* spp.[301–303]

7.4.3.2 Epidemiology

Risk factors for the development of pleuropneumonia are the same as those associated with pneumonia (see 324
the previous discussion) and include long-distance transport, strenuous exercise, viral respiratory tract disease, 325
surgery, dysphagia, general anesthesia, and systemic illness (enteritis). These conditions may enhance aspiration of oropharyngeal organisms or may impair clearance of such organisms.[283,297,304]

7.4.3.3 Pathogenesis

The causative factors of equine pleuropneumonia are those that suppress the pulmonary defense mechanisms and allow bacterial contamination of the lower respiratory tract to progress to pneumonia or abscess formation. The subsequent extension of the infectious process into the pleural space causes pleuritis. The distribution of the pulmonary lesions—cranioventral with the right cranial and middle lung lobes more severely afflicted—is consistent with inhalation or aspiration of bacteria rather than infection from a hematogenous spread. Fluid accumulates within the pleural cavity as the parenchymal inflammation increases the permeability of the capillaries in the overlying visceral pleura, causing an outpouring of protein and cells. Bacteria also may invade the pleural fluid.

7.4.3.4 Clinical Signs

Depending on the chronicity of the disease, the clinical signs may vary and may be confused with signs of colic or rhabdomyolysis. In the acute stages, horses are febrile and lethargic, have a slight nasal discharge, and exhibit a guarded cough, shallow breathing pattern, and painful, stilted gait.[303] The right hemithorax is affected more often than the left, presumably because of the more direct route of the right main stem bronchus.[283] Thoracic auscultation may be abnormal as evidenced by pleural friction rubs and ventral dullness. In severe acute cases the horse may exhibit nostril flaring; tachycardia; jugular pulsations; toxic mucous membranes; a guarded soft, moist cough; and a serosanguinous fetid nasal discharge. In chronic cases of pleuropneumonia (duration greater than 2 weeks), horses may have bouts of intermittent fever and exhibit weight loss and substernal and limb edema.

7.4.3.5 Diagnosis

Diagnosis is based on historical information, clinical examination, imaging results, and microbiologic and cytologic analysis of tracheal and pleural fluid aspirates.

On auscultation of the thorax, one may hear vesicular sounds only dorsally, with an absence of lung sounds ventrally. One may hear bronchial or tracheal sounds if lung consolidation exists. Cardiac sounds radiate over a wider area of the lung field than normal, a finding distinct from clinical cases of pericarditis. On percussion of the chest, the clinician may elicit a painful response (pleurodynia) and detect an area of dullness or decreased resonance ventrally.

Laboratory assessment may demonstrate normal or toxemic leukogram and chemistry findings in the acute cases, whereas in chronic cases, one may find anemia, neutrophilia, hyperfibrinogenemia, and hyperproteinemia.

Ultrasound is the diagnostic technique of choice in cases of pleuropneumonia or pleural effusion. Using a 3.5- to 5-MHz transducer (sector scanner or linear probe), one can detect free or loculated fluid, pleural thickening, pulmonary and mediastinal abscesses, pulmonary consolidation, inundation of airways with fluid, fibrinous adhesions, and concurrent pericarditis. Pleural fluid may displace the lungs axially and dorsally. The fluid may appear anechoic or hypoechoic depending on the relative cellularity.[303] Free gas echoes within the pleural fluid may reflect (1) the presence of anaerobic organisms,[33] (2) the presence of air introduced during a thoracocentesis, or (3) the presence of air introduced by a bronchopleural fistula.

Ultrasonography enables accurate placement of the catheter during thoracocentesis and ensures productive yields during placement of a chest drain (Figures 7-16 to 7-18). Ultrasound examination fails to demonstrate deep parenchymal lesions if the overlying lung is normally aerated.

Thoracic radiography also remains a useful technique for evaluating horses with pleuropneumonia, permitting detection of a pneumothorax or an abscess located deep in aerated lung tissue. Pneumothorax may develop following transtracheal aspiration, thoracocentesis, thoracic drainage, or pleuroscopy. Pneumothorax also may develop as a sequela to gas-producing organisms in the pleural cavity or to air leaks from bronchopleural 325
fistulae.[283] Although radiographs may detect pulmonary abscesses, they may fail to detect lesions obscured by 326
the cardiac silhouette.

Figure 7-16 Ultrasound of pleural effusion. Anechoic *(black)* area represents pleural effusion *(PE)*. The echogenic *(white)* tortuous fibrin strand is attached to the parietal pleura of the diaphragm. *V,* Ventral; *D,* dorsal; *arrow,* diaphragm. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)

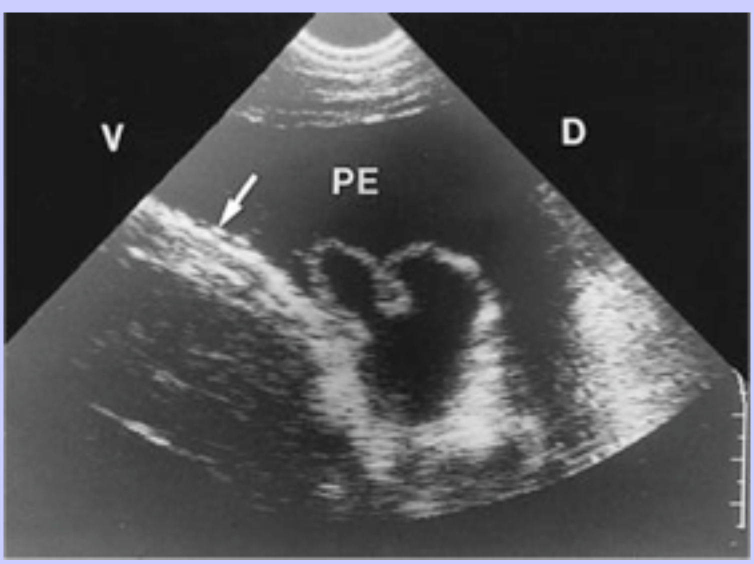
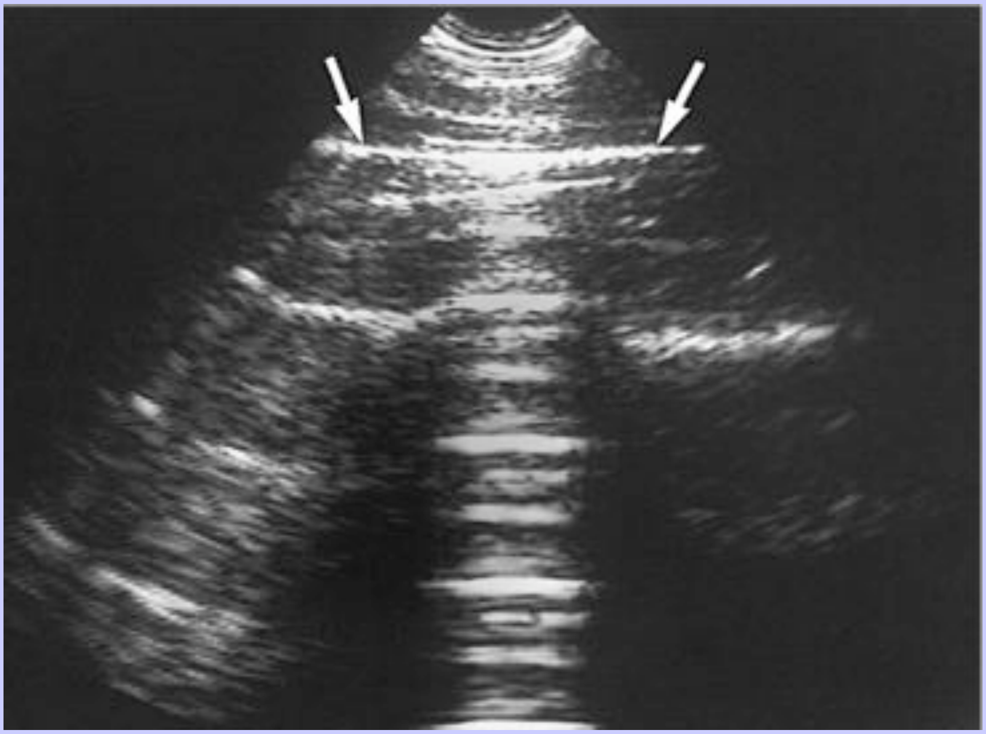
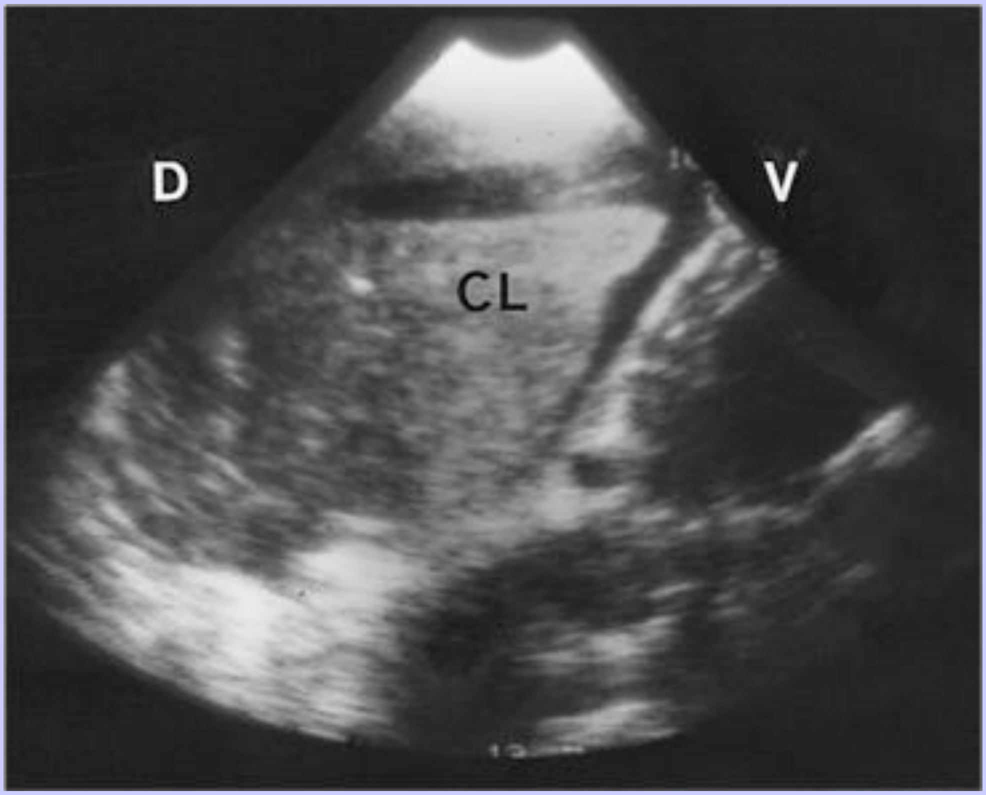


Figure 7-17 Normal thoracic ultrasound demonstrates a highly echogenic interface *(arrows)* between the chest wall and normal lung. A reverberation artifact is notable deep to the chest wall–lung interface. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



Thoracocentesis of both sides of the chest is indicated if one notes fluid bilaterally. Not only is thoracocentesis diagnostic and prognostic, it may be a therapeutic life-saving procedure in horses with severe respiratory distress.[305] In healthy horses the fenestrated caudal mediastinium allows communication of the fluid between the two sides of the chest. In horses with pleuropneumonia, fibrin may close mediastinal fenestrations, allowing for differences to develop in the pleural fluid of the two hemithoraces. The clinician should submit fluid for cytologic examination and for aerobic and anaerobic culture. Pleural fluid in healthy horses contains fewer than 10,000 cells per μl, approximately 60% of which are neutrophils, and has a total protein of less than 2.5 g/dl. In cases of pleuropneumonia, pleural fluid white blood cell counts and protein are elevated and glucose levels may be low (<40 mg/dl). The fluid may have a foul odor if anaerobes are contributing to the infection, but the absence of an odor should not preclude the possibility of an anaerobic component.

Figure 7-18 Ultrasound of ventrally consolidated lung *(CL)* surrounded by a small amount of pleural effusion. *V,* Ventral; *D,* dorsal. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



The clinician always should obtain a tracheobronchial aspirate to recover the inciting bacterial agents and to examine tracheobronchial secretions cytologically (Gram stain, cell types). One should submit aspirates for aerobic and anaerobic culture and a Gram staining to provide guidance on antimicrobial selection. During the course of the disease, one may obtain multiple tracheobronchial aspirates to identify new or resistant bacterial organisms.

One can use thoracoscopy, performed in standing sedated horses or in horses under general anesthesia, in acute and chronic cases of pleuropneumonia (1) for guided placement of drains into abscesses and loculated pleural effusions; (2) for assessment of the extend and progression of pleural disease; and (3) for the evaluation of therapeutic responses and the efficacy of pleural lavage/drainage.[306]

7.4.3.6

Treatment

Treatment aims at (1) removing excessive pleural fluid; (2) administering systemic antimicrobials to inhibit bacterial growth; (3) providing antiinflammatory and analgesic drugs that deter the development of secondary complications; and (4) providing supportive care.

Under ultrasound guidance, one can remove pleural fluid aseptically through the seventh or eighth intercostal spaces at a locale dorsal to the costochondral junction using a 24-32 French chest tube. One may remove as much as 30 to 50 L. If blood discoloration of the pleural fluid is caused by the underlying disease, the fluid remains blood-tinged throughout the drainage.[305]

The clinician should place indwelling chest tubes (1) if large volumes of foul-smelling fluid with microorganisms are obtained; (2) if the pleural fluid has a pH less than 7.2 or a glucose concentration less than 40 mg/dl; or (3) if the horse responds poorly to intermittent drainage.[298–305] One should avoid the rapid removal of large volumes of fluid from the chest to guard against the development of hypovolemia. To the end of the indwelling chest tube one should attach a Heimlich valve or a nonlubricated condom with its tip snipped off. Complications of chest tube placement include pneumothorax; lung laceration; hemothorax; cardiac arrhythmias; bowel, liver or heart puncture; and localized swelling.[305] 326

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Appropriate antimicrobial therapy is based on the results of the culture and sensitivity. Pending microbiologic test results, one should administer broad-spectrum intravenous antibiotics in most cases of pleuropneumonia because the infections are polymicrobic. Penicillin is one of the drugs of choice, for it is efficacious against *Streptococcus* spp., *Staphylococcus* spp., and many anaerobes. Metronidazole is added routinely to the penicillin to broaden bactericidal activity against anaerobes, especially *Bacteroides fragilis*. Aminoglycosides lack efficacy against anaerobic bacteria and have poor penetration into respiratory tract secretions but still are administered with penicillin. Third-generation cephalosporins such as ceftiofur sodium, with activity against gram-negative aerobes and facultative anaerobes, showed excellent efficacy in the treatment of spontaneous clinical and posttransport pneumonia associated with *Streptococcus zooepidemicus, Actinobacillus* spp., and *Pasteurella* spp.[307]

Depending on the clinical response of the horse, 10 to 14 days after the onset of initial therapy, the clinician usually replaces parenteral antimicrobials with oral antibiotics. Chloramphenicol, a bacteriostatic drug, has excellent broad-spectrum activity against gram-negative, gram-positive, and anaerobic bacteria and can be given orally. (One must use caution when handling the drug.) Enrofloxacin, a fluoroquinolone, has excellent antibacterial action against aerobic gram-negative bacteria, many gram-positive bacteria, and mycoplasma bacteria but has limited activity against *Streptococcus* spp. and anaerobes.[308] Concentrations of the enrofloxacin in lung tissue are similar to those in serum.[309] Erythromycin and rifampin attain good concentrations in the lung and pleural fluid and within phagocytic cells, but rifampin is expensive and erythromycin may induce a fatal colitis, precluding their use in adult horses.[310]

Antimicrobial therapy should continue for 2 to 4 months until the horse is gaining weight, hematologic and serum chemistry values have normalized, and no evidence of respiratory tract disease exists. One should implement limited exercise (hand walking) and avoidance of stress. One should reevaluate refractory cases using the techniques of ultrasonography, thoracocentesis, or transtracheal aspiration to determine if drug resistance has developed, if additional pathogens are involved, or if untoward sequelae have occurred (see the following discussion).

In refractory cases, drainage of pulmonary or mediastinal abscesses or mechanical debridement of fibrinous material (decortication) may become necessary. One should consider initially attempting drainage using a large-bore chest tube (24F) directed through the chest wall and then the capsular wall of the abscess followed by suction of the abscess contents. Lavage of the contents of the abscess may improve removal of the purulent material. One should take care to prevent spillage of the contents into the pleural cavity. For removal of

fibrinopurulent material or necrotic lung segments, a standing thoracotomy may provide necessary access.[311] After removing accessible exudates and fibrin manually, one lavages the chest cavity with a warm 1% Betadine solution, packs it with a large lap sponge, and covers it with a self-adhering dressing and pad. One must lavage the pleural cavity daily. Fistulae formation is a common sequela in these clinical cases, but this does not preclude horses from returning to racing or breeding careers.

Nonsteroidal antiinflammatory drugs (flunixin meglumin, 0.25 to 0.50 mg/kg intravenously 2 to 3 times daily) provide analgesia, increase appetite, increase the comfort of the horse, and decrease the inflammatory response.

Many horses with pleuropneumonia benefit from the administration of intravenous fluids for the first 48 to 72 hours, until the horse is comfortable enough to resume drinking and the danger of endotoxemia has lessened. Nasal insufflation of oxygen (10 to 15 L/min) may be indicated if the horse is hypoxemic or in respiratory distress. One may achieve bronchodilation using inhaled or nebulized albuterol (600 to 720 μg 3 to 4 times daily). Because gram-negative organisms are often involved in pleuropneumonia cases, one should implement prophylactic measures against laminitis.

7.4.3.7 Prognosis

A favorable response most often relates to early identification and aggressive treatment of pleuropneumonia. Survival rates[297,312] of horses treated for acute pleuropneumonia range from 49% to 98%. Prognosis deteriorates with increased duration of illness because of involvement of anaerobic bacteria and the development of complicating factors such as pleural adhesions, pulmonary necrosis, cranial mediastinal abscesses, bronchopleural fistulae, constrictive pericarditis, and laminitis.[312,384] In a recent study, 61% of Thoroughbreds treated for pleuropneumonia raced after recovery, with 56% winning at least one race.[313]

7.4.3.8 Complications

Long-term medical treatment of pleuropneumonia may induce complications such as venous phlebitis or thrombosis (from intravenous catheter placement), cellulitis or pneumothorax following thoracocentesis, diarrhea resulting from antimicrobial and antiinflammatory therapy or endotoxemia, and laminitis. With minor changes in the therapeutic approaches, many of these complications resolve and do not impede the eventual return to health of the horse. The more complicated sequelae of infectious pleuropneumonia have been 327
described in detail by Byars, Dainis, Seltzer, et al.[314] and Byars and Becht.[315] In a survey of 153 horses 328
brought to their veterinary hospital over a 4-year period with pleuropneumonia, they detailed the development of cranial thoracic masses (7.2%), bronchopleural fistulae (6.5%), pericarditis (2.6%), and laminitis (1.3%).

One should suspect cranial thoracic masses when horses exhibit tachycardia, jugular distention, forelimb extension (pointing), and caudal displacement of the heart. One can confirm the presence of empyema, loculations, or encapsulated abscesses cranial to the heart by ultrasonography. In most cases, medical therapy is effective at reducing the abscess, and thus one should elect this conservative approach initially. Additional therapeutic modalities include the administration of a diuretic (furosemide) and a chronotropic agent (digitalis) to improve cardiac performance. However, refractory cases may require ultrasound-guided drainage of the abscess performed under short-term anesthesia (xylazine-ketamine combination).

Bronchopleural fistulae develop when necrotic pulmonary tissue sloughs, providing a direct communication of the airways with the pleural cavity. One confirms diagnosis by visualization of the airways following

pleuroscopy or by the intratracheal appearance of contrast media injected into the pleural cavity. Bronchopleural fistulae may close eventually as the pulmonary tissue adheres to the chest wall or as the airways close. Thoracoscopic-guided closure of the bronchopleural fistualae may be necessary in long-standing cases.

7.4.4 PLEURAL EFFUSION

7.4.4.1 Causes

Accumulation of fluid within the pleural space most often results from imbalances in Starling's law of fluid fluxes. As described previously, pleural effusions in the horse most commonly occur with bacterial pneumonia or lung abscesses.[316] (See the section on treatment for parapneumonic effusions.) Pleural effusions also may accompany a number of thoracic neoplasms such as fibrosarcoma, gastric squamous cell carcinoma, hepatoblastoma, hemangiosarcoma, melanoma, mesothelioma, and metastatic mammary or ovarian adenocarcinoma but most commonly are associated with lymphoma.[317–326] Pleural effusion also develops in a number of other conditions, including thoracic trauma; pericarditis; peritonitis; viral, mycoplasmal, and fungal infections; congestive heart failure; liver disease; diaphragmatic herniation; hypoproteinemia; equine infectious anemia; pulmonary granulomata; and damage of the thoracic duct.[298,327–329]

7.4.4.2 Pathogenesis

Pleural fluid is really the interstitial fluid of the parietal pleura. A pressure gradient driving its formation exists because the parietal pleura is supplied by the systemic circulation and because the pressure of the pleural space is more negative than that of the subpleural interstitium.[330] Pleural liquid and protein exit the pleural space via the parietal pleural stomata. Fluid production in the pleural space increases if any of the following occur: (1) an elevation of the hydrostatic pressure gradient (congestive heart failure, portal hypertension); (2) a decrease in the colloid osmotic pressures (hypoproteinemia); (3) an increased permeability of the capillary vessels (infection, malignancy, inflammation); or (4) a decreased removal of fluid because of impaired lymphatic drainage (neoplasia) or a decrease in the pleural space (atelectasis).[331] Excessive amounts of peritoneal fluid may accumulate in the pleural cavity as the fluid moves through diaphragmatic defects or through diaphragmatic lymphatics.[330]

7.4.4.3 Diagnosis

A physical and rectal examination, CBC and chemistry panel, cardiac evaluation, thoracic and abdominal ultrasound, and abdominal centesis may be helpful in determining the cause of the pleural effusion. When one suspects infectious agents, one should perform a transtracheal aspirate. One also should obtain a Coggins test and titers against *Coccidioides immitis, Cryptococcus neoformans,* and *Mycoplasma felis,* depending on the nature of the effusion and the geographic location of the affected horse.

Cytologic and microbiologic evaluation of the pleural fluid may help to identify neoplastic cells or fungal elements. Transudates, fluid with a total protein less than 2.5 g/dl, and few cells usually are associated with congestive heart failure, liver fibrosis, hypoalbuminemia, or early neoplastic processes. Modified transudates have low nucleated cell counts (<10,000 cells/μl) and moderate to high protein levels (>2.5 g/dl) and can be found in many disorders including neoplasia. Exudates have nucleated cell counts exceeding 10,000 cells/μl

and total protein levels greater than 3.0 g/dl and are found in infections and intraabdominal diseases.[316] One can distinguish septic effusions from nonseptic effusions by biochemical analysis of pleural fluid aspirates. For example, fluid that has a pH less than 7.2, a glucose concentration less 40 mg/dl, and a lactate dehydrogenase concentration greater than 1000 IU/L has been suggested to indicate septic effusions. Chylous effusions (which are milky white to pale pink) have triglyceride concentrations exceeding that of simultaneously measured serum.[316]

7.4.4.4 Treatment

Treatment aims at the primary cause, but neoplastic conditions and pleural effusions associated with an end-stage organ failure carry a poor prognosis. Therapeutic thoracocentesis is indicated in malignant effusions when horses are experiencing breathing difficulties. Chest tube drainage and chemical pleurodesis or 328
thoracoscopy with talc poudrage may be one treatment option. The clinician should consider these procedures 329
if (1) significant improvement in the status of the horse occurs with pleural fluid evacuation and (2) reexpansion of the lung is achieved once fluid is removed. Adapting protocols from human medicine[332] (as successful management of malignant effusions in equine medicine has not been described) requires that the horse first be sedated. Then one administers a sclerosing agent in 100 to 500 ml of sterile solution through a chest tube. One clamps the tube for 1 hour before reconnecting it to continuous suction. One sclerosing agent that has been used is doxycyline (500 mg in 50 to 100 ml saline for human beings).

Poudrage is the most widely used method of instilling talc into the pleural space and usually is performed under thoracosopic guidance. Before spraying the talc over the visceral pleura, one should remove all pleural fluid: complete collapse aids in distributing the talc. In human medicine, one usually instills 5 g (8 to 12 ml) evenly over the pleural surface, places a chest tube, and applies progressive suction until the amount of fluid aspirated per day is less than 100 ml.[332]

7.4.5 FUNGAL PNEUMONIA

7.4.5.1 Causes

Primary fungal pathogens causing respiratory tract disease in the horse include *Blastomyces dermatitidis, Coccidioides immitis, Cryptococcus neoformans,* and *Histoplasma capsulatum*.

B. dermatitidis is a thermally dimorphic fungus: it exists as a mold at room temperature and as a budding, round yeastlike cell when cultured at 37° C or when replicating in the host. As a soil saprophyte, *B. dermatitidis* can be found near decomposed vegetation or rotting wood.[333] Although respiratory tract disease caused by this organism is rare in the horse, inhalation of the spores may cause a pyogranulomatous pneumonia.[334]

Coccidioides immitis, a soil saprophyte, is also dimorphic, existing as a mold on most culture media and as a nonbudding spherical form in the host tissue. Fungal pneumonia and pleuritis result from inhalation of wind-borne arthrospores. Lymphohematogenous dissemination of the organisms may lead to the development of lesions in the bones, skin, and meninges.[335]

Cryptococcus neoformans is a yeastlike fungus that reproduces by budding, forming cells 4 to 7 µm in diameter. The organism has a predilection for the respiratory tract and for the central nervous system. The

proposed route of infection is via inhalation with secondary hematogenous spread to the central nervous system. When *C. neoformans* replicates within the host or on culture media, the organism forms a large polysaccharide capsule that appears as a clear halo around the cell when organisms are stained with India ink.[336] The capsule is antiphagocytic and immunosuppressive: secretion of capsular antigens into the body fluids binds opsonizing antibody before it reaches the organism. Based on the capsular antigens, two varieties of *C. neoformans* exist: *C. neoformans* var. *neoformans* (serotypes A and D) and *C. neoformans* var. *gatti* (serotypes B and C).

Histoplasma capsulatum is a dimorphic fungus found in the soil and on decaying vegetation. Heavy concentrations of the organism accumulate in soils containing bat or bird feces. The organism is highly infectious as an airborne spore (microconidia) but is of low virulence when it converts to the yeast phase (2 to 4 μm) in the host.[336] Systemic histoplasmosis is uncommon in proportion to the equine population exposed: the horse seems particularly resistant to infection.[337] The propose route of infection is via inhalation or ingestion of the microconidia. Because the organism parasitizes mononuclear phagocytes and has an affinity for the reticuloendothelial system, it may disseminate to the liver, spleen, lymph nodes, and bone marrow.[338]

Opportunists such as *Aspergillus* spp. and *Pneumocystis carinii* cause fungal pneumonia in horses that are immunocompromised, that are neutropenic, or that have enteritis/colitis, bacterial pneumonia, or neoplasms.[339–345]

Aspergillus is a mold with septate hyphae 2 to 4 μm in diameter. The most prevalent pathogenic species is *A. fumigatus,* but *A. flavus, A. nidulans,* and *A. niger* also may cause disease.[346] These fungi are ubiquitous in the environment, growing on dead leaves, stored grain, compost piles, hay, and decaying vegetation.[336] Inhalation of *Aspergillus* spores is suspected to be common, but disease is rare unless the patient is immunocompromised. Infection is characterized by hyphal invasion of blood vessels, thrombosis, necrosis, and hemorrhagic infarction. Because *Aspergillus* occurs widely as an environmental contaminant, diagnosis may require repeated isolation or histologic demonstration.

Pneumocystis carinii formerly had been considered a protozoan organism because of its morphologic features and its susceptibility to antiprotozoal agents.[347] However, the genes encoding the small subunit ribosomal RNA (16s) and the large subunit of mitochondrial rRNA demonstrate similarities to the genes encoding for the rRNA of several different fungal species.[348] As a result, *P. carinii* recently has been reclassified as a fungus. Electron microscopy has revealed two parasite forms: trophozoite and cystic. The trophozoite is an ameboid form 2 to 5 μm in diameter with filopodia that attach to the surface of the type I epithelial cells. The trophozoite is visible with hematoxylin and eosin stains. The mature cyst is 4 to 6 μm in diameter and contains eight uninucleate intracytic bodies. The cyst stains with Gomori's methenamine silver stain and periodic acid– 329
Schiff stain. In keeping with the new taxonomic classification, some authors recommend replacing the 330
terminology of the different parasite forms by the terms *yeast cell* (trophozoite), *sporangia* (cyst), and *spores* (intracystic bodies).[348]

The infective stage or source of *P. carinii* is unknown, although several studies have reported discovery of *P. carinii* DNA in water and air samples, suggesting that these are environmental reservoirs.[349] In rodent models, *P. carinii* can be transmitted from one animal to another via the airborne route. Researchers also believe that once the organism enters the lower respiratory tract of an immunocompetent host, it remains as a lung saprophyte only to be reactivated during periods of immunosuppression.[347]

7.4.5.2
Epidemiology

Fungal pneumonia in the horse as a primary entity is uncommon. Disease usually results when debilitating conditions that favor the penetration or growth of fungi exist. Contributory factors include (1) exposure to large numbers of mycotic organisms in the environment[350]; (2) the stabling of horses within a moist environment[351]; (3) the prolonged administration of antibiotics that upset the microfloral balance or interfere with vitamin synthesis; (4) the existence of an immunosuppressive state primarily (combined immunodeficiency disorder) or secondarily because of the administration of drugs, the development of an endocrinopathy, or neoplasia.[345,346]

For the pathogenic fungi, prevalence of the disease may be determined geographically. For example, *Blastomyces dermatitidis* is endemic to the Mississippi, Missouri, and Ohio River basins; the Canadian provinces of Quebec, Ontario, and Manitoba; the Great Lakes region; and the Eastern seaboard.[334] Although *Histoplasma capsulatum* is also endemic to the Mississippi, Ohio and St. Lawrence River valleys, it also is found in the southern United States.[338,352] *Coccidiodoes immitis* is endemic to the arid and semiarid regions of North America, including the states of California, Texas, Arizona, New Mexico, Nevada, and Utah.[335,353] In contrast, *Cryptococcus neoformans* is widespread, being found in high concentrations in the soil and in avian manure *(C. neoformans* var. *neoformans)*. In Australia, an epizootiologic relationship of *C. neoformans* var. *gatti* exists with the eucalyptus tree *(Eucalyptus camaldulensis)*. Environmental dispersal of the fungus coincides with flowering of the eucalyptus tree in the spring, resulting in greater exposure and more cases of disease during that time.[354]

For the opportunistic fungus *Pneumocystis carinii,* human clinical infections develop only in immunodepressed subjects, those individuals with low $CD4^+$ counts, with certain viral infections (cytomegalovirus), or in weakened nutritional states.[347,348] To date, all reported cases of equine pneumocystosis have occurred in foals 1.5 to 4 months of age. Predisposing factors include the existence of a combined immunodeficiency disorder,[343] *Rhodococcus equi* or chronic bacterial pneumonia,[344,355] low $CD4^+$ counts,[356] and chronic debilitation or weight loss.[357,358] In two reports of equine pneumocystosis, predisposing factors could not be identified.[359,360] The occurrence of pneumocystosis in young foals may reflect an age-dependent maturation of the immune system. Cell-mediated immune responses (in vitro) of foals less than 2 months of age are reduced relative to those of adult horses, perhaps increasing the susceptibility of the foal to pneumocystosis.[361]

7.4.5.3
Clinical Signs

Horses with primary fungal pneumonia (blastomycosis, histoplasmosis, cryptococcosis, and coccidioidomycosis) may have a chronic cough, anorexia, weight loss, exercise intolerance, and nasal discharge.[362–364] Tachypnea or respiratory distress may or may not be a clinical feature of the fungal pneumonia. Pleural effusion is found more commonly with coccidioidomycosis but also has been reported in cases of cryptococcosis and blastomycosis.[334,335,364]

In addition to causing pneumonia, these fungal organisms may cause disease in other body systems. *Blastomyces dermatitidis* causes superficial abscesses around the anus, vulva, and udder.[365] In a review of 15 cases of equine coccidioidomycosis, 43% of the cases had hepatic lesions, 29% had bone or periosteal

involvement, 22% had lesions in the peritoneum, and 64% had pulmonary parenchymal disorders.[335] Other reports describe *Coccidioides immitis* lesions in the mammary gland,[366] the placenta and fetus,[367,368] and the skin.[369] *Cryptococcus neoformans* has been reported to cause granulomata in the skin, nasal cavity, paranasal sinuses, orbits, intestines, bones, meninges, placenta, and fetus.[354,370–373] *Histoplasma capsulatum* has been associated with abortions, disseminated histoplasmosis of foals, and granulomatous colitis of adult horses.[337,352,374]

Horse developing pneumonia caused by opportunistic fungi (aspergillosis, pneumocystosis) may have evidence of a debilitating or immunosuppressive problem such as colitis, peritonitis, septicemia, endotoxemia, an endocrinopathy, or chronic bacterial pneumonia. Young horses (foals) may be at an increased risk for developing opportunistic infections.

In the majority of cases of pulmonary aspergillosis, pneumonia appears to be a sequelum to mycotic invasion of the intestinal tract, the integrity of which has been compromised by severe acute enterocolitis.[339,340,342,350] Such horses typically also have received broad-spectrum antimicrobials and nonsteroidal antiinflammatory drugs and are neutropenic—factors that may also predispose them to the development of systemic aspergillosis. Nevertheless, pulmonary aspergillosis also has been reported in horses with pleuropneumonia, 330
myositis, renal failure, pituitary adenoma, and myelomonocytic leukemia.[342,345,346] 331

Horses with pulmonary aspergillosis suddenly may become febrile and tachypneic and may exhibit adventitious lung or pleural sounds and have a nasal discharge. Other horses with pulmonary aspergillosis may show only mild respiratory signs or fail to demonstrate any abnormalities of the respiratory tract.[342,374,375]

Pneumocystosis is a rare clinical entity. In the majority of cases, foals have evidence of chronic respiratory disease that has progressed to respiratory distress. Weight loss, dehydration, and inappetence also may be evident.[344,356–358,360]

7.4.5.4 Diagnosis

Tracheobronchial aspirates may reveal degenerated neutrophils, yeast cells, and bacteria. In the case of aspergillosis, the diagnosis may be difficult because one may recover fungal elements from the tracheal washings of normal horses.[24] Definitive diagnosis may require repeated isolation of the *Aspergillus* organisms or histologic demonstration of hyphal elements in the pulmonary parenchyma. Transtracheal aspirates may be of limited usefulness in diagnosing pneumocystosis because the organism rarely is isolated by this method. For horses not in respiratory distress, isolation of the organism may be possible by bronchoalveolar lavage or endobronchial brushings.[344,356–358]

In horses with fungal pneumonia, radiographs may reveal circular masses with or without fluid lines and an accentuated interstitial pattern. Radiography or ultrasonography may detect pleural effusion.

Serologic detection of an antibody response to *Coccidioides immitis, Cryptococcus neoformans,* and *Histoplasma capsulatum* has been useful in diagnosing fungal pneumonia. In cryptococcosis, serologic detection of capsular antigens (serum latex agglutination test) also has proved effective in the diagnosis.[364,370] However, the histoplasmin skin test is of little diagnostic value: in one endemic area, 73% of horses had a postive skin test.[376]

Serologic diagnosis of pulmonary aspergillosis can be difficult because titers are detectable in healthy and diseased horses.[342] Nevertheless, Moore, Reed, Kowalski, et al.[377] reported the existence of two precipitin bands against *Aspergillus* antigens in a horse confirmed to have an *Aspergillus* mediastinal granuloma. Precipitin bands were not evident in the eight control horses sampled. Recently, Guillot, Sarfati, DeBarros, et al.[378] reported the usefulness of an immunoblot analysis for the diagnosis of aspergillosis, detecting reactivity to low-molecular-mass antigens (22 to 26 kd) in the sera of diseased horses that was not evident in clinically healthy horses. The investigators suggested that these antigens were released during mycelial growth in the tissues, a phase that would not develop in clinically healthy horses. However, until this experimental assay is commercially available, one should use serologic diagnosis cautiously in suspected cases of pulmonary aspergillosis. One may confirm the diagnosis with lung biopsy and by the presence of other systemic alterations.

The clinician should investigate cases of immunosuppression by quantitation of immunoglobulin levels, by performing mitogen stimulation tests, by quantitation of $CD4^+$ and $CD8^+$ cells and by ruling out endocrinopathies and neoplasia.

7.4.5.5 Treatment

Treatment against the primary fungal pathogens requires long-term administration (10 to 12 weeks) of antifungal drugs and correction of the inciting cause of the fungal pneumonia. Two basic classes of antifungal drugs are used commonly in equine medicine. The polyene antibiotics—amphotericin B, nystatin, and natamycin—combine with ergosterol in the cytoplasmic membrane of the fungi to increase cell permeability. The second class of drugs, the azoles—miconazole, ketoconazole, itraconazole, and fluconazole—inhibit synthesis of ergosterol and cause an accumulation of aberrant sterols in the membrane. This inhibition affects nutrient utilization and causes leaky cell membranes.[336] Because fungal pneumonia is uncommon, the efficacy of various protocols has not been assessed rigorously. Selection of the antifungal drug to use also should depend on sensitivity patterns of the fungal isolates.

Amphotericin B has been used successfully to treat histoplasmosis[338] and pulmonary aspergillosis.[378] The recommended dose is 0.1 to 0.5 mg/kg administered in a 5% dextrose solution intravenously over 30 minutes, 3 times per week. The possible side effects include anorexia, anemia, arrhythmias, hepatic and renal disyfunction, and hypersensitivity reactions.[372] Neither amphotericin B nor ketaconazole were found to be curative in a series of cases of coccidioidomycosis,[335] although long-term administration of itraconazole (2.6 mg/kg orally twice daily) was eventually effective in treating coccidioidomycosis vertebral osteomyelitis in a foal.[353]

Pneumocystis carinii infections require treatment with antiprotozoal drugs: Ewing, Cowell, Tyler, et al.[358] reported success using trimethoprim sulfamethoxazole (25 mg/kg orally twice daily) in combination with procaine penicillin G in foals with pneumocystosis. Furthermore, Flaminio, Ruch, Cox, et al.[356] supplemented trimethoprim sulfamethoxazole (30 mg/kg orally twice daily) with interferon-α (100 U orally once daily) and *Propionibacterium acnes* (EqStim) in an effort to increase $CD4^+$ and $CD8^+$ counts in a foal with pneumocystosis. The pneumonia eventually resolved and lymphocyte counts returned to normal. 331

7.4.6 MYCOPLASMAL PNEUMONIA

One can isolate several species of mycoplasma from the upper and lower respiratory tracts of healthy horses and from the nasal cavities of healthy foals soon after birth.[379] *Mycoplasma felis* has been isolated from clinical and experimental cases of parapneumonic effusions[327,380,381] and foal pneumonia.[382] *M. felis* and *M. equirhinis* have been isolated, along with bacterial organisms, from the tracheobronchial aspirates of young athletic horses with inflammatory airway disease.[383] Furthermore, a recent Canadian survey documented serologic evidence of *M. felis* or *M. equirhinis* infection in 9% and 10%, respectively, of horses showing clinical signs of acute respiratory disease.[241] The pathogenesis of the inflammatory or infectious airway disease generally is believed to involve adherence of the organism to ciliated epithelium, causing loss of the cilia and subsequent death of the cell. This belief is supported by the finding of degenerative epithelial cells in the tracheobronchial aspirates of horses with mycoplasma infections.[383] Diagnosis of mycoplasmosis depends on isolation of the organism from tracheobronchial aspirates or from pleural fluid and on demonstration of seroconversion. The clinician should initiate treatment with broad-spectrum antibacterials (oxytetracycline) until antimicrobial sensitivity reports are available.

7.4.7 ACUTE RESPIRATORY DISTRESS SYNDROME

7.4.7.1 Causes

Acute respiratory distress syndrome (ARDS) is a syndrome of lung injury characterized by alveolar damage, high permeability pulmonary edema, and respiratory failure. Primary lung injury may result from aspiration (near drowning), improper administration of medications via nasogastric tubes, inhalation of smoke or noxious gases, oxygen toxicity, or pulmonary infection by viral, bacterial, mycoplasmal, or fungal agents. Secondary lung injury may be a sequelum of anaphylaxis, gram-negative sepsis/endotoxemia, trauma and embolism, and hypertransfusion.[384–389] In human medicine, strict criteria have been established to define ARDS: impaired oxygenation (ratio of Pao_2/Fio_2 <200), detection of bilateral pulmonary infiltrates in chest radiographs, and a pulmonary artery wedge pressure less than 18 mm Hg or no clinical evidence of elevated left atrial pressures.[390,391] Similar criteria have not been established in equine medicine.

7.4.7.2 Pathogenesis

Based on the human literature, the thought is that although many different insults (causing capillary permeability) may lead to ARDS, a common final pathway ultimately results in alveolar damage. This pathway may include complement and leukocyte activation with release of oxygen free radicals and inflammatory mediators (IL-1, tumor necrosis factor) and secondary destruction of surfactant.[392] The net result is a deterioration of gas exchange and pulmonary mechanics. Whether horses that survive are more predisposed to the development of interstitial lung disease is uncertain (see the following discussion).

7.4.7.3 Clinical Signs

Horses are tachypneic or in respiratory distress or both. A red-tinged or yellow frothy material, indicative of pulmonary edema, may be evident at the nares. Fever may be a component of the syndrome depending on the

primary cause. In cases of smoke inhalation, clinical signs may not become evident for several days following exposure to noxious gases.

7.4.7.4 Diagnosis

Diagnosis depends on the physical findings and history. One may detect crackles on auscultation and may auscultate fluid within the trachea. Endoscopic examination (if performed without stressing the horse) may reveal the extent of airway edema, inflammation, mucosal necrosis or sloughing, and the presence of soot (smoke inhalation). Radiographs reveal an interstitial pattern, although an alveolar pattern caused by ventral consolidation of the lung fields may follow aspiration pneumonia. Arterial blood gases reveal hypoxemia and hypocapnia, although with carbon monoxide poisoning, oxygen tensions may be normal. In such cases, carboxyhemoglobin concentrations (measured at human hospitals) exceed 10%, indicating carbon monoxide toxicity.[389]

7.4.7.5 Treatment

The clinician should direct treatment at the primary cause with the understanding that prognosis is guarded. Lipoid pneumonia resulting from aspiration of mineral oil is fatal.[385] Cases of near drowning and smoke inhalation have been treated successfully with return to athletic function in some cases.[386,387,393]

One should administer intravenous fluid cautiously. Plasma may be needed if hypoproteinemia develops. Furosemide (1 mg/kg intravenously) helps to mobilize lung extravascular water and repetitive dosing at 2- to 4-hour intervals may be required. (One should monitor electrolytes.) Humidified intranasal oxygen (10 to 15 L/min) or mechanical ventilation through a tracheotomy may be necessary. Although technically difficult to perform, adult horses in respiratory failure have been ventilated mechanically.[394] The clinician may perform tracheal suction to remove cell debris and other materials through the endoscope or through a tracheotomy. Surfactant, although expensive, may improve oxygenation and pulmonary mechanics when administered by 332
intratracheal instillation. One may use bronchodilators to treat bronchospasm. (See the section on recurrent 333
airway obstruction for dosages.) Nonsteroidal antiinflammatory drugs and corticosteroids also are indicated for treating severe permeability edema. One should administer broad-spectrum antimicrobials, including metronidazole, in many of these cases but especially those involving aspiration pneumonia, bacterial and viral pneumonia, and smoke or noxious gas inhalation lung injury.

7.4.8 RECURRENT AIRWAY OBSTRUCTION (HEAVES)

7.4.8.1 Definition

Recurrent airway obstruction is a naturally occurring respiratory disease characterized by periods of reversible airway obstruction caused by neutrophil accumulation, mucus production, and bronchospasm. The condition has been termed *chronic obstructive pulmonary disease, chronic* pulmonary disease, chronic airway reactivity, hyperactive airway disease, broken wind, and *hay sickness*. The consensus at the International Workshop of Equine Chronic Airway Disease was that the term *chronic obstructive pulmonary disease* not be used to describe this condition in horses, because the pathophysiologic and morphologic aspects of the equine disease are different from human chronic obstructive pulmonary disease.[395]

Pulmonary function testing demonstrates a decrease in lung compliance, an increase in lung resistance, an increase in the work of breathing, and the development of arterial hypoxemia, usually in the absence of hypercapnia.[396,397] During airway obstruction, affected horses are hyperresponsive to nonspecific stimuli such as histamine, methacholine, and water.[398]

7.4.8.2 Epidemiology

The condition occurs worldwide, but the highest prevalence in the United States is in northeastern and midwestern horses that are fed hay and stabled. A similar condition, found in horses in the southeastern United States maintained on pasture is termed *summer pasture associated obstructive disease*. These horses improve when stabled.[399] Recurrent airway obstruction effects middle-aged or older horses, the median age of which is 12 years.[400] No breed predilection occurs, but a familial tendency has been found: a horse born to a dam and sire with heaves is at an increased risk for developing heaves.[401]

7.4.8.3 Causes

Much debate still occurs concerning the cause of heaves. Many consider heaves to be a hypersensitivity reaction to organic dusts or molds commonly found in poorly cured hay or straw. Dust in horse stables contains well more than 50 species of molds, large numbers of forage mites, endotoxins, and inorganic components—a variable mixture of agents any one of which might induce pulmonary inflammation in a susceptible horse. The two most frequently implicated molds are *Aspergillus fumigatus* (fungus) and *Micropolyspora faeni* (a thermophilic actinomycete).[402]

7.4.8.4 Pathogenesis

The condition is characterized by periods of reversible small airway obstruction caused by smooth muscle contraction and accumulations of mucus and neutrophils. One hypothesis is that inhalation of the organic molds, dusts, and endotoxin induces an immune response, the nature of which is controversial. A T helper cell type 2 response (TH2), involving IgE-mediated degranulation of mast cells, has been proposed as the inciting cause because of the findings of (1) increased anti– *Micropolyspora faeni* and anti–*Aspergillus fumigatus* IgA and IgE concentrations in the bronchoalveolar lavage (BAL) fluid of asymptomatic and symptomatic heaves-affected horses,[403] (2) increased histamine levels in the BAL fluid of heaves-affected horses 5 hours after natural challenge,[404] (3) increased serum IgE levels against recombinant *Aspergillus* antigens in heaves-affected horses,[405] and (4) increased numbers of BAL cells that are positive for IL-4 and IL-5 messenger RNA in heaves-affected horses.[406]

A T helper cell type 1 immune reaction with secondary neutrophil influx and release of cytokines also has been implicated in the pathogenesis of heaves because (1) correlation of antigen-specific IgE levels with expression of disease is not absolute,[405,407] (2) pulmonary eosinophilia, as occurs in the TH2 disorder of human asthma, is not a feature of heaves,[408] (3) increases in BAL fluid allergen-specific IgGa and IgGb noted by Halliwell, McGorum, Irving, et al. are more compatible with a TH1 rather than with a TH2 response,[403] and (4) quantitative PCR analysis of BAL lymphocytes demonstrates an increase in TH1 (interferon-γ) but not TH2 (IL-4, IL-5) associated cytokines.[409,410]

In horses with heaves, IL-8 concentrations in the bronchoalveolar fluid are increased relative to levels found in healthy horses.[410,411] This cytokine may be derived from macrophages, neutrophils, or airway epithelial cells and is one of the primary chemoattractants for neutrophil migration into the airways. Interestingly, in murine models of obstructive airway disease, airway epithelial cell production of IL-8 is upregulated by interferon-γ and IL-17.[412] Because the mRNA levels of IL-17, a T cell–derived cytokine, also increased in airway lymphocytes isolated from heaves-affected horses (Ainsworth, unpublished data), a link between the airway TH1 responses and epithelial-derived chemokines may exist.

Neutrophil- and macrophage-derived products such as reactive oxygen species and proteases also may 333
contribute to the inflammatory process of heaves. Art, Kirschvink, Smith, et al.[413] suggested that an oxidative 334
stress caused by the release of reactive oxygen species from granulocytes and macrophages develops in the airways of heaves-affected horses. Their conclusion is based on the finding that oxidized glutathione levels and glutathione redox ratios (the ratios of oxidized glutathione to total glutathione levels) are elevated in the pulmonary epithelial lining fluid of heaves-affected horses relative to controls. They further suggested that the oxidant stress does not incite but rather exacerbates the existing inflammation. Interestingly, oxidative stress[414] has been found to activate NFκB, a transcription factor that regulates the expression of many proinflammatory cytokines and adhesion molecules including tumor necrosis factor α, IL-1β, IL-8, and intercellular adhesion molecule 1.[415] Recently, Bureau, Bonizzi, Kirschvink, et al.[416] demonstrated that levels of NFκB in the bronchial brushing cells of heaves-affected horses were increased many times, the magnitude of which correlated with the severity of the clinical disease. Furthermore, the airway epithelial cell expression of intercellular adhesion molecule 1, a protein required for migration of neutrophils from the pulmonary vasculature into the lung parenchyma, paralleled the expression of NFκB, suggesting that this protein, along with IL-8, enhances the airway neutrophilia.

Proteases released from neutrophils (and macrophages) in the airways of heaves-affected horses may contribute further to inflammatory cell influx and possible tissue damage. Researchers have found that the tracheal epithelial lining fluid of horses with heaves has increased concentrations of matrix metalloproteinases that exhibit collagenolytic, gelatinolytic, and elastinolytic activities.[417–419] The importance of these proteases, as well as other inflammatory mediators and arachidonic acid metabolites, to the initiation and development of bronchospasm, mucus secretion, and neutrophil influx needs to be determined.

7.4.8.5 Clinical Signs

Horses with heaves have a chronic spontaneous cough, a mucopurulent nasal discharge, an accentuated expiratory effort, and adventitious lung sounds. Hypertrophy of the external oblique and rectus abdominis muscles caused by continued recruitment is evident (heave line). The respiratory rate may be normal or increased (tachypnea). Exercise intolerance, weight loss, and cachexia also may be evident in severe cases. Horses are usually afebrile.

7.4.8.6 Diagnosis

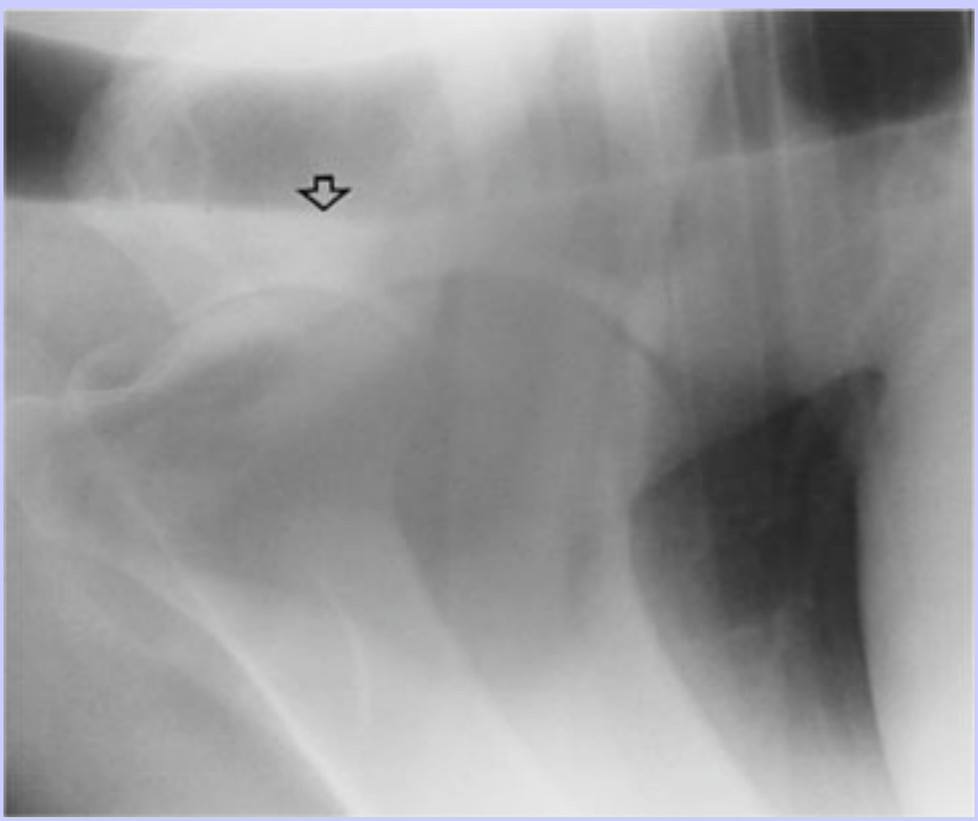
Diagnosis is based on the clinical signs and history of a seasonal disorder associated with husbandry alterations such as being fed hay, stabled, transported in a trailer, or maintained in a dusty lot. Auscultation may reveal inspiratory or expiratory wheezes, crackles, or tracheal rattles. Percussion may be normal or an expanded lung field may be detectable.

Endoscopic examination reveals excessive mucopurulent exudate within the trachea. A bronchoalveolar lavage supports a *nonseptic* inflammatory reaction with increases in mucus and the percentage of intact neutrophils. Albumin and immunoglobulin levels within the bronchoalveolar lavage may not increase.[420] Gram-positive organisms may be retrieved by tracheobronchial aspirates if a concurrent septic inflammation exists. Pollen, fungal hyphae, and Curschmann's spirals (inspissated mucus plugs) also may be visible.

Thoracic radiography may demonstrate an increase in the interstitial and bronchial pattern throughout the lung fields. However, these changes may be difficult to interpret relative to the normal aging changes that occur. One also may detect exudate within the trachea by thoracic radiography (Figure 7-19).

Some have advocated intradermal skin testing for diagnosing heaves and for detecting sensitizing allergens. Its usefulness is questionable. Theoretically, heaves-affected horses should react if sensitized to a given allergen, but (1) clinically normal horses have positive skin test reactions to the allergens and (2) heaves-affected horses lack positive reactions. Thus intradermal skin testing is not indicated for diagnosing heaves.[421–423] Furthermore, a poor correlation exists between skin test results and serum allergen testing, leading some clinicians to conclude that these assays should not be used as screening tests for allergen hypersensitivities in the horse.[423]

Figure 7-19 Tracheal fluid or exudates *(arrow)* is visible ventrally in the trachea at the level of the thoracic inlet. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



A CBC and serum chemistry panel is of limited usefulness in diagnosing heaves because no abnormalities are detectable in the leukogram or in serum biochemistries.[400]

7.4.8.7 Pathologic Signs

Postmortem examination reveals a diffuse bronchiolitis with goblet cell metaplasia, airway smooth muscle hypertrophy, excess mucus, and inflammatory cells in the small airways. Eosinophilic infiltration around the bronchioles is a variable finding, present in approximately one third of the horses. Varying degrees of alveolar overinflation and atelectases also are found, as well as evidence of fibrosis of the alveolar septa.[424] The larger airways—the bronchi and trachea—also may show variable changes, including epithelial hyperplasia with loss of ciliated cells, goblet cell hyperplasia, and inflammatory cell infiltration.[425]

7.4.8.8 Treatment

Elimination of the source of the mold or dust is the most beneficial step in the treatment regimen but also the most difficult management change to implement.[400] Horses should be fed a pelleted alfalfa or a complete pelleted feed with the transition from a hay diet to one of a complete pelleted feed made gradually over 7 to 10 days. Unless pasture exacerbates the condition, the owner should keep horses outside, blanket them in the winter, and allow access to a three-walled shelter affording protection from the wind and rain. If the horse is stabled, the stall bedding should be shredded paper, shavings, peat moss, or clay to eliminate dusts and molds. The surrounding stalls should be bedded similarly. Although such stabling changes improve lung function somewhat, airway hyperreactivity still remains.[426,427]

Medical management also has been indicated in clinical cases because stabling alone takes several weeks to quiet the pulmonary inflammation. Corticosteroids are efficacious in reducing the inflammatory reaction in the lungs, whereas nonsteroidal antiinflammatory drugs provide no benefit. The clinician initially may give prednisolone (not prednisone) at 2.2 mg/kg orally once daily in the morning for 7 to 10 days, 1.1 mg/kg once daily for 7 to 10 days, 0.50 mg/kg once daily for 7 to 10 days, and 0.50 mg/lb every other day for 7 to 10 days. Alternatively, one may give a course of dexamethasone parenterally. For the 500 kg horse, the course is 40 mg intramuscularly once daily every other day for three treatments, followed by 35 mg intramuscularly once daily every other day for three treatments, 30 mg intramuscularly once daily every other day for three treatments, etc., until the horse is weaned off of the dexamethasone. Corticosteroid use may be contraindicated in horses predisposed to laminitis or exhibiting endocrinopathies.

Inhaled steroids have been used to treat the pulmonary inflammation of heaves but may not provide a therapeutic benefit for 24 to 72 hours. Thus for horses in respiratory distress, inhaled steroids should not be the main route of glucocorticoid administration. Metered dose inhalers attached to a spacer on the Aeromask (Trudell Medical, London, Ontario, Canada) administer the drug when the horse initiates inspiration. Frequent administration of steroids to effect improvement in lung function may make this medical treatment less desirable. Some suggested corticosteroid dosages (using the Aeromask) are beclomethasone (3750 μg twice daily) or fluticasone (2 μg twice daily).

Bronchodilators also are recommended for treating recurrent airway obstruction. Three types of compounds are used to relax airway smooth muscle: the β-adrenergics, the methylxanthines, and the anticholinergics. The β_2-adrenergic compounds include clenbuterol, albuterol, salmeterol, and fenoterol. Experimental investigations with clenbuterol suggest that when given at a dose of 0.8 μg/kg once daily by mouth, the drug

may be efficacious in alleviating some of the signs of heaves. In addition to a direct smooth muscle effect, clenbuterol also may stabilize mast cells, increase mucociliary clearance, and improve airway secretions.[428] Albuterol is absorbed poorly from the gastrointestinal tract, so inhalation is the recommended method of administration. Some suggested dosages of β_2-agonists that can be administered by metered dose inhalers through the Aeromask are these: albuterol, 720 μg 3 to 4 times daily; fenoterol, 1 to 2 μg 3 to 4 times daily; and salmeterol, 210 μg once or twice daily. Administration of the β_2-agonist before corticosteroid dosing enhances the deposition of the latter in the smaller airways.[429] Long-term use of β_2-agonists has been associated with a downregulation of the β-receptors in human beings, but whether this occurs in horses is unknown.

The methylxanthines include aminophylline (theophylline), which dilates smooth muscle by increasing cyclic adenosine monophosphate levels intracellularly. Cyclic adenosine monophosphate also inhibits degranulation of mast cells and subsequent mediator release. One may give aminophylline at 4 to 6 mg/kg orally 3 times daily. However, in a recent study, aminophylline 18 mg/kg intravenously provided clinical improvement in only 50% of the cases.[430]

The third class of compounds used to effect smooth muscle dilation includes the anticholinergics, of which atropine and glycopyrrolate are representatives. Atropine provides clinical improvement when administered intravenously at 0.02 mg/kg, but the duration of effect is short-lived (2 hours) and atropine may be associated with the development of ileus, abdominal pain, tachycardia, mydriasis, and thickening of airway secretions.[430] 335
Atropine generally is used for emergency relief of airway obstruction. Glycopyrrolate has been reported to be 336
efficacious at a dose of 0.007 mg/kg, but it also may cause colic. One also can administer ipratropium bromide by inhalation at a dose of 180 to 360 mg 3 times daily with a low risk of inducing systemic side effects.[395]

Disodium cromoglycate is considered efficacious by some, acting to stabilize mast cell degranulation and inhibit the vagal efferent component of histamine response. A suggested dose from the study of McPherson and Thomson[402] was 80 mg once daily for 4 days by nebulization. Mucolytics (acetylcysteine, dembrexine) and mucokinetics (iodides, bromhexine) may provide some relief.[431] Antimicrobials are indicated if microorganisms are isolated on the tracheobronchial aspirate.

7.4.8.9 Prevention

Once the clinician has diagnosed the condition, the horse always will be susceptible to recurrences of the disease. Husbandry changes should be implemented to decrease environmental dust and organic mold exposure. During periods of hot, humid, or dusty conditions, prophylactic administration of a steroid and bronchodilator via the metered dose inhaler may be indicated. Routine immunizations against the viral respiratory pathogens and good management practices are logical suggestions.

7.4.9 INFLAMMATORY AIRWAY DISEASE

7.4.9.1 Definition

Inflammatory airway disease (IAD) is characterized by the presence of excessive amounts of mucoid or mucopurulent exudate in the nasopharynx, trachea, and bronchial bifurcation. The condition is encountered predominantly in the young performance horse.[47,89] Auscultatable pulmonary abnormalities rarely are

identified. Some horses with IAD may exhibit a cough and reduced athletic performance. The relationship between IAD in young horses and recurrent airway obstruction in mature horses is unknown: currently, the former condition is believed not necessarily to progress to the latter.[395]

7.4.9.2 Epidemiology

Depending on the study, a prevalence of 20% to 65% among racehorses in training has been noted.[89,432] The variation in prevalence may reflect the method of diagnosis: the amount of tracheal exudates detected endoscopically increases as a function of exercise intensity.[89] Thus one may miss the diagnosis of IAD if one performs endoscopic examination in the resting horse.[433]

7.4.9.3 Causes

The definitive cause of the lower airway inflammation is currently unknown and has been hypothesized to reflect the response of the lung to the presence of (1) low-grade persistent bacterial or viral infections, (2) autologous blood from exercise-induced pulmonary hemorrhage, and (3) inhaled dusts, molds, particulate matter or environmental pollutants such as H_2S, NH_3, ozone, SO_2, NO_2 and CO.

Studies in the United Kingdom and Australia suggest that bacterial agents are involved in the pathogenesis of IAD.[89,434–436] Using an inflammatory score based on the amount of mucus, total nucleated cell count, and neutrophil percentages in endoscopically obtained tracheobronchial aspirates, investigators found that as the inflammatory score increased, the percentage of positive bacterial cultures also increased.[437] The most commonly isolated organisms were *Streptococcus zooepidemicus, Streptococcus pneumoniae, Actinobacillus equuli,* and *Pasteurella* spp. Mycoplasma spp. also were isolated from some horses with IAD.[383] That the prevalence of IAD decreased with increasing age of the horse was attributed to the development of an effective immune response. In studies in the United Kingdom and in Australia (where equine influenza is not endemic), no association between the onset of IAD and seroconversion to EHV1, ERV1, ERV2, or equine influenza existed,[434,436,438] suggesting that IAD is not associated with a viral infection.

A viral cause has been proposed for IAD based on the cytologic improvements in BAL fluid constituents in horses treated with oral interferon-α. In their study of 32 Standardbred horses with a history of poor performance referable to the respiratory tract, Moore, Krakowka, Robertson, et al.[439,440] and Moore, Krakowka, McVey, et al.[441] found that relative to control horses, the BAL fluid total nucleated cell count and the percentages of BAL fluid neutrophils, lymphocytes, and monocytes increased significantly in horses with IAD. They considered the lymphocytosis and monocytosis to be consistent with a low-grade viral infection. A 5-day course of oral interferon-α (50 U per day) lowered total nucleated cell counts and converted the cell distribution to a noninflammatory profile for at least 15 days. The investigators did not describe whether the amelioration of pulmonary inflammation was associated with an improvement in athletic performance. They also did not evaluate horses endoscopically beyond 2 weeks to determine if cessation of interferon-α therapy was associated with a return of IAD.

Autologous blood, derived from pulmonary capillaries that rupture during intense exercise, also has been suggested to cause of IAD. In an experimental study, Tyler, Pascoe, Aguilera-Tejero, et al. found that instillation of blood caused a neutrophilic pulmonary inflammatory reaction in the lungs, leading them to conclude that pulmonary hemorrhage may contribute to IAD.[442]

Evidence that inhaled environmental allergens from hay or straw contribute to IAD is supported by the observations that episodes of IAD are shorter in horses bedded on shredded paper[434] and that stabling is associated with an increase in the proportion of BAL fluid neutrophils and a decrease in the percentage of lymphocytes.[91]

Hare and Viel[443] also have suggested that a type I (IgE-mediated) hypersensitivity reaction to inhaled environment allergens could be the cause of IAD. They studied young Standardbred racehorses with a history of poor performance and found evidence of peripheral blood and BAL fluid eosinophilia (12% eosinophils). Normally the percentage of eosinophils in BAL fluid is less than 2%. Because they found no evidence of pulmonary or intestinal parasitism in this group of affected horses, the investigators attributed the eosinophilic IAD to an allergic reaction.

Environmental pollutants also have been suggested as a cause of IAD. This hypothesis arises from the observation that many racetracks and training facilities are located in metropolitan areas that have significant accumulations of smog. No data exist verifying this as a cause of IAD.

7.4.9.4 Clinical Signs

Inflammatory airway disease is frequently subclinical but in some cases may be associated with poor athletic performance or a cough. With the exception of the cases described in England, pyrexia is generally not a feature of the disorder. Horses also may have evidence of EIPH.

7.4.9.5 Diagnosis

Definitive diagnosis requires endoscopic examination of the respiratory tract with the finding of exudate in the nasopharynx and in the trachea. One also may see lymphoid hyperplasia depending on the age of the horse. Other causes of poor performance—musculoskeletal and cardiovascular—must be ruled out to attribute reduced athleticism to IAD. This may require that the horse perform a standardized treadmill exercise test, enabling assessment of the upper respiratory tract (videoendoscopy), the cardiovascular system (electrocardiogram), and the musculoskeletal system (lameness exam, creatine kinase and aspartate transaminase measurement). One also should obtain a CBC and chemistry panel. With the exception of cases of IAD associated with pulmonary eosinophilia, CBC and chemistry profiles are usually within normal limits.

Further diagnostic evaluation of the respiratory system in horses with IAD should include a culture of a tracheobronchial aspirate to rule out a low-grade bacterial infection and a bronchoalveolar lavage to determine whether the cytologic profile is most representative of a bacterial, viral, or allergic cause.

Although instillation of autologous blood does decrease dynamic compliance and increase respiratory resistance[444] in the majority of investigations of IAD, pulmonary function tests have been unremarkable.[47,443] Measures of airway hyperreactivity, performed by determining dose-dependent alterations in respiratory tract resistance or dynamic lung compliance following nebulizing of methacholine or histamine, have demonstrated that some horses with IAD have hyperresponsive airways.[47,443,445]

7.4.9.6
Treatment

Because the cause of IAP remains uncertain, most treatment recommendations aim at environmental alterations to decrease exposure to dust, molds, and allergens. Horses should have pasture turnout whenever possible. Horses with bacterial infections should receive a 7- to 10-day course of an antibiotic, the selection of which is based on culture and sensitivity results from the tracheobronchial aspirate. Horses with pulmonary lymphocytosis and monocytosis may benefit from a 5-day course of orally administered interferon-α (50 U once daily). One also should implement efforts to decrease the severity or frequency of EIPH by having horses train and race with furosemide. In the absence of an infectious cause, horses also may benefit from a course of corticosteroids: prednisolone at 1 mg/lb orally once daily for 7 to 10 days, followed by 0.5 mg/lb orally once daily for 7 to 10 days, and then 0.5 mg/lb orally once every other day for 7 to 10 days.

7.4.10
LUNGWORMS

7.4.10.1
Causes

Lungworm infections in the equine species are caused by *Dictyocaulus arnfieldi*. Infections in the donkey and mule are asymptomatic but provide a source of viable eggs for clinically apparent infections in the horse and pony.

7.4.10.2
Epidemiology

Lungworm infections have been diagnosed by recovery of larvae in live animals by bronchoscopic examination[446] or at postmortem evaluation. Using this approach, the prevalence of lung worm infection is approximately 68% to 80% in donkeys, 29% in mules, and 2% to 11% in horses.[447,448]

7.4.10.3
Pathogenesis

Donkeys are asymptomatic reservoirs of the parasite, but instances of lungworm infections have occurred in horses in which no contact with donkeys could be established, suggesting horse-to-horse transmission may be possible.[448] Experimental studies provide evidence that under field conditions, *Pilobolus* fungi may facilitate 337
the spread of lungworm infections in a manner similar to that which occurs in cattle lungworm infections.[449] 338
The infective larvae ascend the coprophilous fungus as it grows on the manure and invade the sporangia. When the sporangia rupture, the infective larvae disperse with the fungal spores. After the infective larvae (0.4 mm) are ingested, they migrate through the gut wall and are carried to the lungs via the lymphatics. In hosts in which infections are patent, the larvae mature to egg-laying adults in the peripheral bronchioles. The prepatent period is approximately 2 to 3 months. Eggs are transported out of the lungs by the mucociliary apparatus, swallowed, and excreted in the feces where they become infective by 4 days.[450] First-stage larvae have survived for at least 49 days but do not survive over the winter.[451] In horses and ponies, larval development in the lungs is arrested (fifth stage), but airway inflammation still occurs.

7.4.10.4 Clinical Signs

Horses exhibit chronic coughing and an increased expiratory effort. Auscultation reveals crackles and wheezes, especially over the dorsal and caudal parts of the lung fields.[452] Signs are often indistinguishable from those associated with heaves. Donkeys do not typically exhibit clinical signs of infection.

7.4.10.5 Diagnosis

Endoscopic examination may reveal a mild lymphoid follicular hyperplasia and copious amounts of exudate in the trachea and bronchioles. Tracheobronchial aspirates or bronchoalveolar lavage may contain a predominance of eosinophils. A peripheral eosinophilia is a variable finding in these horses. Definitive diagnosis is made by identification of *D. arnfieldi* larvae in the sediment of centrifuged mucus, although this may be difficult.[451] One should examine stained and unstained cytologic preparations. In donkeys and in horses with patent infections, bronchoscopic identification of the lungworm, 16 cm in length, confirms the diagnosis. The Baermann technique is useful for diagnosing lungworms when the infections are patent.

Diagnosis most often depends on the clinical signs, the history of exposure to donkeys, and the response to anthelmintic therapy.

7.4.10.6 Treatment

Ivermectin (200 μg/kg) is effective against *D. arnfieldi* in controlled studies and field evaluations and did not cause any detrimental side effects.[453] Moxidectin (0.4 mg/kg) was found to be 99.9% effective in treating lungworm infections in donkeys.[454]

7.4.10.7 Prevention

Horses should not be pastured with donkeys or mules unless they are confirmed to be free of lungworms.

7.4.11 EXERCISE-INDUCED PULMONARY HEMORRHAGE

7.4.11.1 Causes

Strenuous exercise is associated with exudation of red blood cells from the pulmonary vasculature into the alveoli and airways of the caudal dorsal lung segments.

7.4.11.2 Epidemiology

EIPH has been detected in most breeds of horses undergoing strenuous athletic events. The prevalence of EIPH is estimated to be between 44% and 75% in the Thoroughbred, 26% in the Standardbred, 62% in the racing Quarter Horse, 50% in racing Appaloosas, 68% in steeplechasers, 67% in timber racing horses, 40% in Three-Day Event horses, 10% in pony club event horses, and 11% in polo ponies.[455–459] Indeed, EIPH probably occurs in any breed of horse that is exercised strenuously. The prevalence of EIPH increases with the

age of the horse. No clear correlation exists between EIPH and the location of stables, the condition of the track, or the track type.[460] No geographic variation exists in the prevalence of EIPH.

7.4.11.3 Pathogenesis

The mechanisms responsible for the development of EIPH are not known completely, although potential causes include (1) ventilation inhomogeneities caused by small airway disease, (2) mechanical constraints of abdominal viscera placed on the dorsocaudal lung field, and (3) stress failure of the pulmonary capillaries.

Robinson and Derksen[461] proposed that poor collateral ventilation in the horse coupled with small airway disease (and thus altered time constants for alveolar filling) caused underventilation of certain lung units. Extreme fluctuations in the alveolar pressure of these underventilated regions during exercise produced parenchymal tearing or alveolar capillary rupture. Small airway disease (IAD) has been detected in a large percentage of horses with EIPH[462]; however, its absence in the lungs of young racehorses that still exhibit evidence of EIPH suggests that small airway disease, at least, is not an inciting cause of EIPH.[463] The role of small airway disease in propagating the cycle of EIPH cannot be dismissed.

Clarke[464] speculated that visceral constraint of the diaphragm caused greater mechanical forces or stresses to develop in the dorsal thorax. Thus in the caudodorsal lung, these mechanical forces would be borne over a narrow area, leading to parenchymal tearing or rupture of capillaries during inspiration. However, evidence of widespread hemorrhage in the dorsal portions of the lung suggests that the distribution of extremely negative intrapleural pressures is more complex and not simply restricted to the region in close proximity to the caudal dorsal lung. An alternative mechanical theory has proposed that forelimb locomotory impact forces are 338
transmitted through the chest wall, setting up waves converging caudodorsally in the lung parenchyma.[465] 339
Experimental evidence supporting the mechanical theories is currently not available.

Pulmonary hypertension and secondary stress failure of the capillaries also has been suggested as the cause EIPH. Stress failure is thought to occur following development of high transmural pressures, the pressure difference between the pulmonary capillary bed and the adjacent alveoli. Pulmonary capillary pressures (which may exceed 70 mm Hg in strenuously exercised horses) disrupt capillary endothelial and alveolar epithelial tight junctions leading to hemorrhage within the interstitium and alveoli. The pulmonary hypertension is a consequence of the high cardiac output, the lack of sufficient pulmonary vascular vasodilation and the increased blood viscosity during exercise. Evidence supportive of the stress failure theory is provided by electronmicrographs prepared from lung segments taken from strenuously exercised horses. These demonstrate a breakdown of the endothelial and epithelial tight junctions and exudation of red blood cells into the alveoli.[466] Although some investigators have found no correlation between pulmonary capillary pressures in exercising horses and the development of EIPH ascertained endoscopically,[467] others have found a correlation between mean pulmonary artery pressures in exercising horses and the number of erythrocytes in postexercise bronchoalveolar lavage fluid samples.[468]

A combination of these three mechanisms likely may contribute to the development of EIPH.

7.4.11.4 Clinical Signs

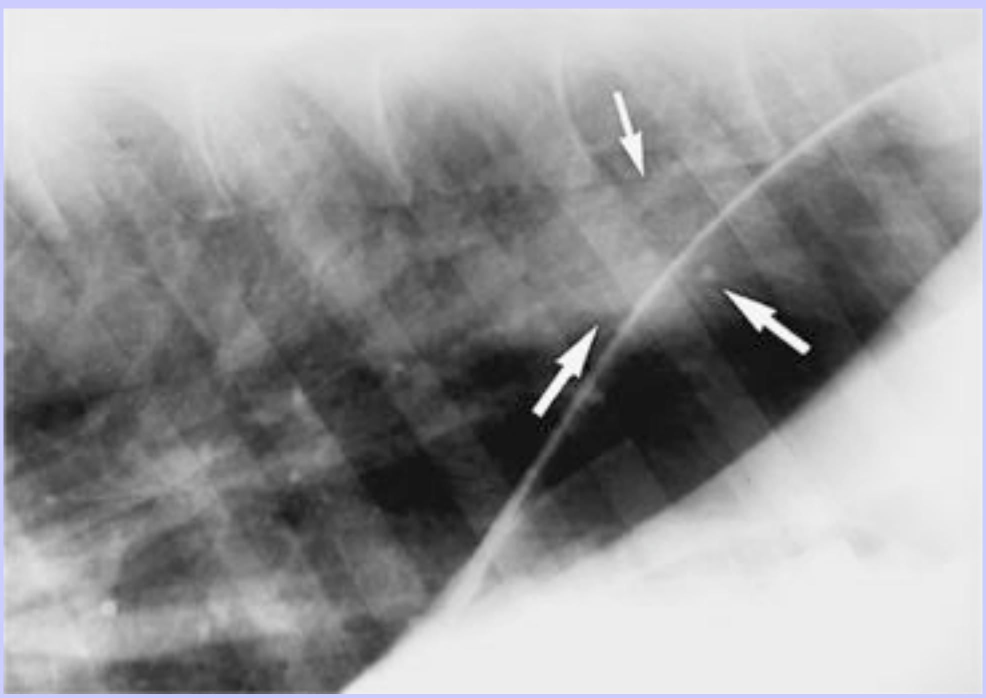
Evidence of frank blood occurs in less than 10% of the horses with EIPH. The effect of EIPH on racing performance varies. A clear association between finishing position in a race and the prevalence of EIPH in

Thoroughbreds or Standardbreds has not been demonstrated. Often horses continue to perform at levels judged to be adequate. In other cases, horses may slow or stop, and some may exhibit difficult or labored breathing, coughing, or excessive swallowing. The affect of EIPH on athletic performance may be better understood when methods to quantitate the extent of bleeding have been developed.

7.4.11.5 Diagnosis

Endoscopic examination of the upper respiratory tract and detection of frank blood within the trachea is the usual method of diagnosis. Optimal time for endoscopic examination is within 90 minutes of a race or workout. However, EIPH is not a consistent finding; it may not be apparent in a given horse examined on different occasions after the same level of exercise. Transtracheal aspirates or bronchoalveolar lavage reveals hemosiderophages, intact and degenerating neutrophils, some with intracellular bacteria, and erythrocytes. Radiographs may demonstrate increases in interstitial patterns, a radiopaque region in the caudal lung lobe (rare), and possible dorsal displacement of the major pulmonary vessels[469] (Figure 7-20).

Figure 7-20 Focal area of increased interstitial pulmonary opacity *(arrows)* in the dorsal caudal lung field represents exercise-induced pulmonary hemorrhage. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



7.4.11.6 Treatment

Rest has been recommended, but pulmonary hemorrhage likely will recur once the horse resumes training. The finding of concomitant small airway disease (IAD) in some horses suggests that one should attempt to

minimize environmental dusts and molds. Corticosteroids and bronchodilators, inhaled or administered parenterally, have not been shown to reduce EIPH but may lessen the severity of small airway disease. Cromoglycate, which is believed to stabilize mast cell membranes, has not been efficacious in the treatment of EIPH.[470]

Antimicrobials may be indicated in cases of severe hemorrhage because blood provides an excellent medium for bacterial growth to occur.

Drugs that reduce pulmonary capillary pressure have gained the most widespread use in the treatment and prevention of EIPH. Furosemide, 250 to 300 mg, is given 4 hours before a race or strenuous workout. Although furosemide admnistration has failed to prevent the development of EIPH in horses that were previously EIPH-negative,[471,472] some evidence indicates that it may reduce the severity of EIPH (red blood cell counts in bronchoalveolar lavage fluid). Nitric oxide, a potent vasodilator, also has been administered to horses in the form of nitroglycerin. However, nitric oxide reduces neither pulmonary artery pressures of strenuously exercising horses nor the severity of EIPH.[473,474] 339

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7.4.11.7 Prevention

The efficacy of treatment regimens in the prevention of EIPH under race conditions has been difficult to determine even when speed handicapping methods are used. Variables such as the administration of drugs unknown to the investigators or the inability to diagnose or reproduce EIPH within a given horse on consecutive days makes interpretation of these studies difficult. And yet controlled studies conducted on a treadmill probably do not accurately simulate race conditions, so that one also may draw false conclusions.

7.4.12 INTERSTITIAL LUNG DISEASE

7.4.12.1 Definition

Although traditionally considered to be a chronic disorder, interstitial pneumonia encompasses acute and chronic inflammatory responses that predominantly involve the alveolar walls and interstitial tissues of the lung.[475] Interstitial lung disease includes a heterogeneous group of disorders characterized by damage to the alveolar walls and loss of functional alveolar capillary units. This is a morphologic characterization of a lung disease. These authors believe it possible that cases of ARDS may progress eventually to interstitial lung disease. However, longitudinal studies supporting this hypothesis have not been conducted.

7.4.12.2 Epidemiology

Buergelt[476] has suggested that two types of equine interstitial disorders exist: one occurring in foals 6 days to 6 months of age and one developing in adult horses greater than 2 year of age. This discussion focuses on interstitial lung disorders in the adult horse.

7.4.12.3 Causes

Most of the recognized spontaneously occurring interstitial lung disorders in animals have been attributed to toxic or infectious (viral or parasitic agents) agents or to allergens.[475] Toxic lung injury in the horse has been

documented following consumption of Crofton weed, pyrrolizidine alkaloids, and *Perilla* ketones.[475–478] Interstitial pneumonia is a feature of viral infections (influenza, EHV1, EHV4, EVA), silicosis, and possibly oxygen therapy.[479,480] Other cases of interstitial pneumonia may not have a well-defined cause.[481–484]

7.4.12.4 Pathophysiology

An inciting agent initiates damage to pulmonary epithelial or endothelial cells, causing coagulative necrosis of the alveoli. Pulmonary congestion, interstitial edema, erythrocyte extravasation, and alveolar flooding characterize the acute (exudative) phase of the disease. Fibrin, protein-rich fluid, cellular debris, and inflammatory cells (neutrophils and macrophages) form hyaline membranes. During the acute phase (days 1 to 5), the horse may exhibit respiratory distress. The exudative phase is followed by a proliferative stage in which the type II pneumocytes, the pulmonary stem cells, replace damaged type I pneumocytes. Interlobar septa widen because of the proliferation of fibroblasts and inflammatory cell infiltration by neutrophils and macrophages.[475]

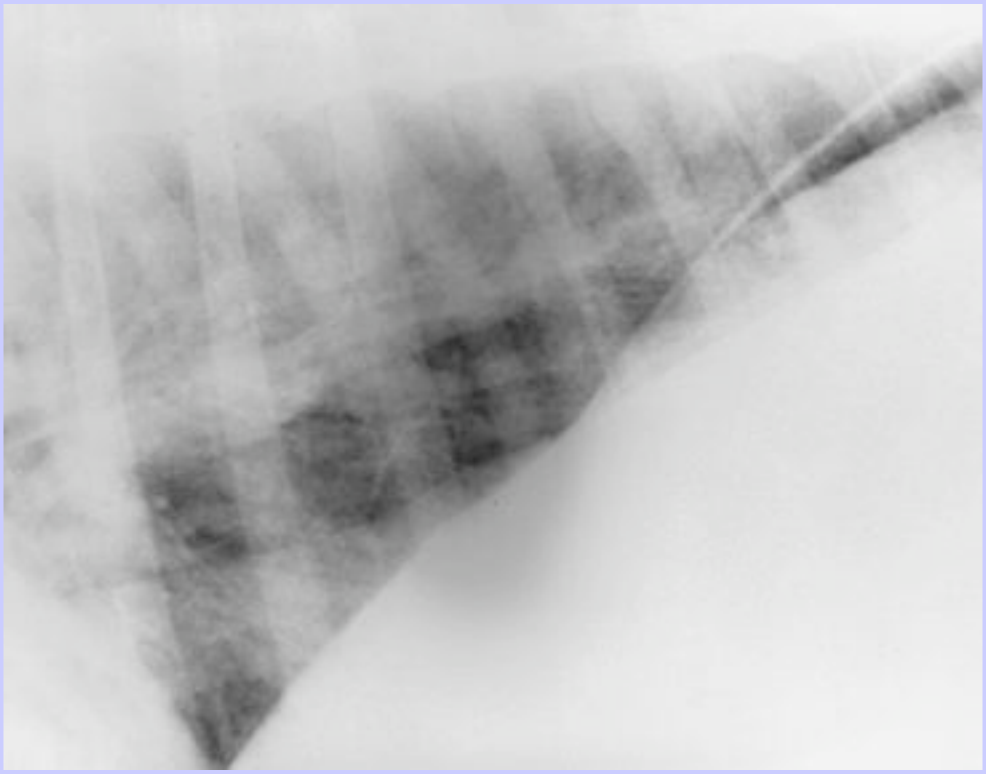
7.4.12.5 Clinical Signs

Clinical signs vary depending on the stage of the pneumonia and the causative agent. In the acute phase, horses may have respiratory distress: they are tachypneic, may be hypoxemic, and may or may not be febrile.[478,479] Horses also may have signs similar to those exhibited by heaves-affected horses with an increased respiratory rate and a significant expiratory effort, cough, and nasal discharge.[481–483] In chronic disorders, horses may exhibit exercise intolerance[482] or have a history of weight loss and anorexia. Interstitial lung disease also may be present in the absence of clinical signs.[482]

7.4.12.6 Diagnosis

The diagnosis is based on clinical signs, history, radiographic examination, isolation of a causative agent, and lung biopsy. Arterial hypoxemia may be evident on blood gas analysis. CBC and serum chemistry panels may be normal or may demonstrate leukocytosis and hyperfibrinogenemia.[485,486] Radiographs demonstrate (1) pulmonary infiltrates with discrete and diffuse nodules, suggesting neoplasia or mycotic pneumonia, or (2) an 340
increase in the interstitial pattern of the lung (Figure 7-21). Serum titer levels may indicate a high titer to 341
equine influenza virus.[479] Lung biopsy confirms the diagnosis of interstitial pneumonia. Silicosis, also a rare finding in the horse, is diagnosed by using x-ray diffraction techniques on lung tissue specimens.[480]

Figure 7-21 Overall increase in interstitial pulmonary opacity represents interstitial lung disease. (Courtesy D.S. Biller, Manhatten, Kansas, 1991.)



7.4.12.7 Pathologic Signs

The lung changes are a function of the stage and the causative agent. Postmortem examination may reveal diffuse pulmonary fibrosis and hypercellularity of alveolar septa and scarring of the interlobular septa and parts of the pleura. Severe chronic bronchitis and bronchiolitis also may be evident if concurrent airway disease exists, but this is typically not a feature of interstitial pneumonia.[482] Multifocal granulomata may be detectable.[480,483]

7.4.12.8 Treatment

The prognosis is poor for resolving the interstitial lung disorder. In one study of six adult horses presented to a veterinary clinic for chronic respiratory disease, one horse improved.[482] In another study, long-term glucocorticoid therapy improved the pulmonary disorder sufficiently to allow the horse to return to athletic performance.[485]

7.4.13 TUMORS OF THE RESPIRATORY SYSTEM

7.4.13.1 Causes

Based on postmortem surveys, the most frequently encountered equine tumors are sarcoids, squamous cell carcinomata, fibromata, melanomas, papillomata, fibrosarcomata, and lymphomata.[486–488] Neoplastic involvement of the pulmonary parenchyma or thoracic structures per se remains rare in horses, occurring at a prevalence rate of 0.15 to 0.62.[326,488]

Primary equine lung tumors include granular cell tumors (myoblastomata),[489–492] bronchial myxomata,[493] pulmonary carcinomata[486,494] pulmonary adenocarcinomata,[495,496] and pulmonary chondrosarcomata.[497,498] Primary thoracic tumors include pleural mesotheliomata,[432,499] thymomata,[326] and lymphomata.[326,500]

The incidence of primary lung tumors is much less than that of metastatic pulmonary tumors. The most common primary lung neoplasm is the granular cell tumor, with a prevalence rate of 0.15%. Most granular cell tumors involve the right lung, may be multiple or single masses, may be associated with airway obstruction, and are visible with endoscopic examination.[491,492] Based on immunohistochemical staining, granular cell tumors are thought to be composed primarily of myelinating Schwann cells with lesser numbers of scattered nonmyelinating Schwann cells.[501,502]

Metastatic lung tumors include adenocarcinoma (primary sites of origin are in the kidney, uterus, thyroid, ovary, and mammary gland),[323,324] hemangiosarcoma (primary sites of origin are in the skeletal musculature or are undetermined),[326,503–510] and lymphoma.[326,500]

Hemangiosarcoma remains a rare tumor, occurring in 2 in 1322 horses in a study by Sundbery, Burnstein, Page, et al.[487] and in 1 in 4739 cases in a survey reported by Hargis and McElwain.[506] Many of the reported cases of hemangiosarcoma involve horses less than 7 years of age.[503,507–510] Typically, horses exhibit pallor of the mucous membranes because of anemia, have evidence of pleural effusion (hemorrhagic fluid) or pulmonary hemorrhage,[508] and exhibit rapid clinical deterioration.

Although hemangiosarcoma may involve the thoracic cavity, the more common neoplasms include lymphoma and gastric squamous cell carcinoma.[326,511,512] Both tumors may be associated with the development of pleural effusion.

7.4.13.2 Clinical Signs

Horses with thoracic neoplasms may have a history of weight loss, inappetence, exercise intolerance, ventral edema, and intermittent fever. Depending on the tumor, physical examination may or may not reveal signs referable to the respiratory tract. In some cases, horses exhibited signs that were initially compatible with inflammatory airway disease, pleuropneumonia, and pulmonary epistaxis.[489,507,508] Hypertrophic pulmonary osteoarthropathy characterized by enlargement of the joints, bony swellings of the distal limbs, and generalized stiffness has been reported in cases of granular cell tumor.[513,514]

7.4.13.3
Diagnosis

Endoscopic examination may reveal the presence of masses occluding the bronchi, as in the case of primary granular cell tumors and bronchogenic carcinomata.[489,494] Pleuroscopy may reveal the extent of tumor involvement. Transtracheal aspirates, bronchoalveolar lavages, cytologic aspirates, histologic examination of biopsy specimens, and thoracocentesis may aid in the diagnosis. Radiographic or ultrasonographic evidence of multifocal interstitial densities (2 to 3 cm in diameter), tracheal elevation, or mediastinal densities also suggest neoplasms.

7.4.13.4
Treatment

The prognosis is poor for horses with neoplasms, and most patients are euthanized. In horses in which the tumor is localized and has a low metastatic potential, as exists with granular cell tumors, lung resection may be an option.[515] In cases with malignant effusion, the clinician may achieve temporary stabilization by thoracocentesis and possibly with pleurodesis (see the section on pleural effusion). 341

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7.5
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8 CHAPTER 8 DISORDERS OF THE CARDIOVASCULAR SYSTEM

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Virginia B. Reef

Identification, evaluation, and treatment of the horse with cardiovascular diseases present a significant challenge to the veterinarian. Cardiovascular lesions are common in horses. Often these lesions are minor and well tolerated; however, cardiovascular disease can become clinically significant, manifesting as exercise intolerance, arrhythmia, weakness, systemic infection, congestive heart failure, or sudden death. Because the horse is a species renowned for its physiologic murmurs and arrhythmias, the cardiovascular findings may be confusing. Moreover, the effect of a cardiac lesion on a performance animal that depends so much on circulatory function can be difficult to quantify without a thorough examination.

Equine cardiology has advanced from a study of physiologic variation and speculation to one of accurate diagnosis and focused therapy. Much of the important groundwork in clinical equine cardiology can be attributed to the studies of Detweiler and colleagues[1–4]; Hamlin, Smith, and Smetzer, and their colleagues[5–20]; and Holmes and his graduate students.[21–51] The initial information these cardiologists provided about normal function, auscultation, and electrocardiography of the equine heart was accurate, and many still commonly make clinical decisions based on the data they provided. Because of the great size of the mature horse, traditional diagnostic studies such as radiography have played a smaller role in the assessment of heart diseases. Recent laboratory developments, including Holter electrocardiography, Doppler echocardiography, and functional exercise testing, have become increasingly important in assessing cardiovascular disease. These diagnostic studies allow detection and quantitation of a variety of anatomic and physiologic disturbances of the heart and circulation.

No comprehensive studies of cardiovascular *disease prevalence* have been done. Holmes observed that 2.5% of hospitalized horses were in atrial fibrillation.[33] Else and Holmes noted myocardial fibrosis in 14.3% of horses examined at necropsy and evidence of chronic valvular disease in approximately 25% of the hearts examined.[27–29] Various cardiovascular lesions were considered to be important in 8.5% of 480 consecutive losses in a necropsy study conducted by Baker and Ellis.[52] Cardiovascular diseases have been suggested to be the third most common cause of poor performance following musculoskeletal and respiratory diseases.[53–55] Occult heart disease, cardiac arrhythmias, and vascular lesions are important reasons for unexplained sudden death.[52,56–67] Certainly, most clinicians regularly encounter manifestations of cardiovascular disease or dysfunction.

Assessment of cardiovascular disease in a horse is predicated on a competent clinical examination and on the clinician' knowledge and ability to evaluate data from various diagnostic studies. Incomplete information may impede an accurate communication of risks to the client and delay a proper course of management. A review of the currently available information regarding the diagnosis and therapy of cardiovascular diseases is the focus of this chapter. The authors assume that the reader is familiar with basic cardiovascular anatomy, physiology, and electrophysiology, and these topics have been reviewed elsewhere.[9,20,68–74] The authors briefly describe pertinent aspects of clinical cardiac anatomy and physiology relevant to causes of equine heart diseases, review important studies essential in the diagnosis of cardiovascular disease, and have designed subsequent sections to provide the student and practicing 355
veterinarian with a framework for understanding the pathophysiology, clinical identification, and management of 356
important congenital and acquired conditions of the heart and major vessels. Instrumental to each discussion are clinically relevant aspects of diagnosis and therapy. Clinical aspects of circulatory shock are described in this volume, and the reader is referred elsewhere for the management of cardiopulmonary arrest.[75,76]

8.1 Anatomic Correlates Of Cardiovascular Diseases

Many diagnostic techniques, including cardiac auscultation, cardiac catheterization, and echocardiography, are predicated on an understanding of cardiac anatomy and physiology. Furthermore, the causes of cardiovascular disease can be subdivided into anatomic and physiologic (functional) diagnoses and into etiologic considerations (Boxes 8-1 and 8-2).

The cardiovascular system is divided into two separate circulations: systemic and pulmonary. The systemic circulation has a greater venous capacity, ventricular pumping pressure, arterial pressure, and vascular resistance.[70,73] Despite these differences, the functions of these two circulations are intertwined. Systemic and pulmonary circulations are arranged in series; therefore disordered function of either side can affect the contralateral circulation. Cardiac output (CO) from the left ventricle must be equal to that of the right ventricle, because ventricular filling depends on venous return from the contralateral circulation. Isolated left ventricular (LV) failure increases pulmonary venous pressure. The resultant pulmonary hypertension imparts a pressure load on the right ventricle and is a common cause of right ventricular (RV) failure. Leftward bulging of the ventricular septum, following RV dilation, can impair filling of the left ventricle.

The heart consists of various active and passive components[73]; therefore different methods and techniques are necessary to evaluate these structures and their associated functions. For normal heart action to proceed, coordination of electric activity, muscular contraction and relaxation, and valve motion must occur. When one reviews heart anatomy, and subsequently cardiac pathology, considering the normal development of the heart and the anatomic integrity of the pericardium, myocardium, endocardium and valves, specialized impulse-forming and conduction systems, and blood vessels is useful.[74]

8.1.1 PERICARDIAL DISEASE

The pericardium limits cardiac dilation, acts as a barrier against contiguous infection, and contributes to the diastolic properties of the heart. The pericardial space is formed by the reflection of the two pericardial membranes, the parietal pericardium and the visceral pericardium (the epicardium), and normally contains such a small amount of serous fluid that echocardiography cannot detect the fluid. Pericardial effusion, tamponade, and 356
constriction can decrease ventricular filling and diastolic function greatly. Pericardial diseases, as well as mass 358
lesions impinging on the systemic veins or the heart, can lead to right-sided congestive heart failure (CHF).

8.1.1.1 BOX 8-1 CARDIAC DIAGNOSES

8.1.1.1.1 Anatomic Diagnosis

Cardiac malformation

Valvular (endocardial) disease

Myocardial disease

Pericardial disease

Cor pulmonale (pulmonary disease leading to secondary heart disease)

Disorder of the impulse-forming or conduction system

Vascular disease

8.1.1.1.2

Physiologic Diagnosis

Systemic: pulmonary shunting

- Left to right
- Right to left

Valvular insufficiency

Valvular stenosis

Myocardial (systolic) dysfunction

Diastolic dysfunction

Cardiac rhythm disturbance

Cardiac-related syncope

Heart insufficiency or failure (limited cardiac output)

Congestive heart failure

Shock

Sudden cardiac death

Cardiopulmonary arrest

8.1.1.1.3

Etiologic Diagnosis

Malformation (genetic)

Degenerative disease

Metabolic or endocrine disease

Neoplasia

Nutritional disorder

Inflammatory disease

- Infective or parasitic

- Noninfective
  - Immune-mediated
  - Idiopathic
- Ischemic injury
- Idiopathic disorder
- Iatrogenic disease
- Toxic injury
- Traumatic injury

8.1.1.2 BOX 8-2 CAUSES OF CARDIOVASCULAR DISEASE

8.1.1.2.1 Congenital Cardiac Malformation

- Simple systemic-to-pulmonary shunts (left to right)
  - Atrial septal defect
  - Ventricular septal defect
    - Paramembranous defect
    - Ventricular inlet defect
    - Subarterial defect
    - Muscular defect
  - Patent ductus arteriosus
- Patent foramen ovale (permitting right-to-left shunting)
- Valvular dysplasia
  - Pulmonic stenosis (bicuspid pulmonary valve)

Pulmonary atresia (leading to a right-to-left shunt)

Tricuspid stenosis/atresia (leading to a right-to-left shunt)

Aortic stenosis/insufficiency (bicuspid or quadricuspid valve)

Subaortic rings with stenosis

Tetralogy of Fallot

Pulmonary atresia with ventricular septal defect (pseudotruncus arteriosus)

Double-outlet right ventricle

Subaortic stenosis

Hypoplastic left-side of the heart

Other complex malformations

8.1.1.2.2 Valvular Heart Disease Causing Valve Insufficiency or Stenosis

Congenital valve malformation

Semilunar valve fenestrations causing valve insufficiency

Degenerative (fibrosis) or myxomatous disease causing valve insufficiency

Vavular prolapse

Bacterial endocarditis causing valve insufficiency with or without stenosis

Rupture of a chorda tendinea causing mitral or tricuspid valve insufficiency

Rupture of a valve leaflet causing flail leaflet and valve insufficiency

Noninfective valvulitis

Valvular regurgitation following dilation of the heart or a great vessel

Papillary muscle dysfunction causing valvular insufficiency

8.1.1.2.3 Myocardial Disease

Idiopathic dilated cardiomyopathy: ventricular dilation and myocardial contractility failure

Myocarditis

Myocardial fibrosis

- Ischemic (embolic?) myocardial fibrosis
- Parasitic *(Strongylus)* embolization

Myocardial degeneration/necrosis

- Toxic injury (e.g., monensin)
- Nutritional deficiencies (e.g., selenium deficiency)

Myocardial neoplasia

- Lymphosarcoma
- Melanoma
- Hemangioma/hemangiosarcoma
- Pulmonary carcinoma

Infiltrative myocardial disease (e.g., amyloidosis)

8.1.1.2.4

Pericardial Disease

Pericardial effusion with or without cardiac tamponade

- Infective: bacterial or viral
- Idiopathic pericardial effusion
- Constrictive pericardial disease
- Mass lesion (intrapericardial or extrapericardial) compressing the heart

8.1.1.2.5

Pulmonary Hypertension and Cor Pulmonale

Pulmonary hypertension following left-sided heart disease

Pulmonary vascular disease following left-to-right shunt

Immature pulmonary circulation

Primary bronchopulmonary or pulmonary vascular disease

Alveolar hypoxia with reactive pulmonary arterial vasoconstriction

Severe acidosis

Pulmonary thromboembolism

8.1.1.2.6

Cardiac Arrhythmias (see Box 8-9)

Atrial arrhythmias

Junctional (nodal) arrhythmias

Ventricular arrhythmias

Conduction disturbances

8.1.1.2.7

Vascular Diseases

Congenital vascular lesions

Rupture of the aorta, pulmonary artery, or systemic artery

Aneurysm of the aortic sinus of Valsalva

Aortic or aortoiliac degenerative disease

Arteritis

- Infective
- Immune-mediated

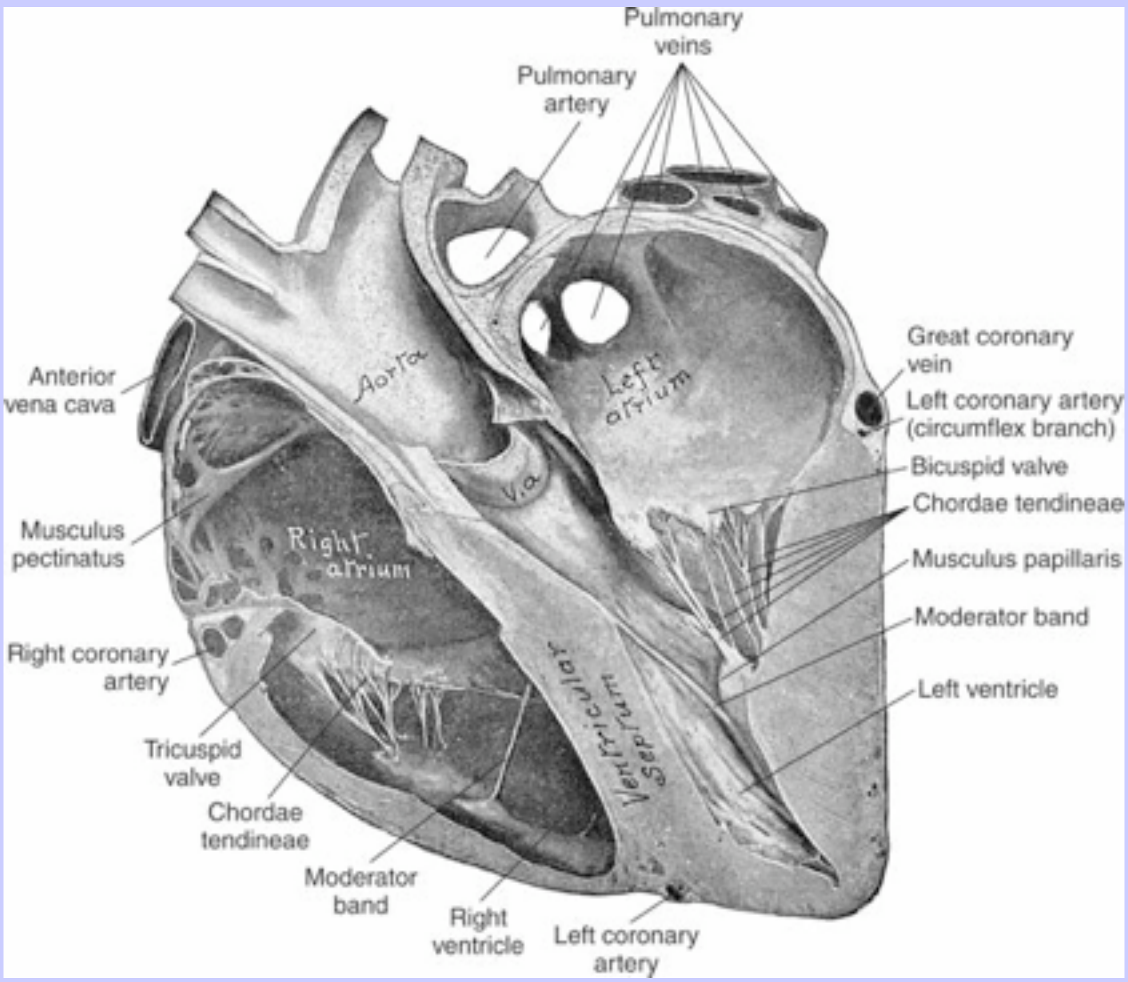
Thrombophlebitis

Pulmonary embolism

Mass lession or tumor obstructing blood flow

Pericardial effusion can develop as a primary disorder or following pleuropneumonia. Infective pericarditis can produce an effusion sufficient to cause cardiac tamponade or eventual constriction of the heart.[55,77–91] Sterile, idiopathic pericardial effusion also has been reported in horses. The volume of effusion can be substantial and can lead to cardiac decompensation.[84] Cardiac mass lesions and intrapericardial tumors have been reported sporadically.[86,89,92,93] Cranial mediastinal tumors (lymphosarcoma) or abscesses following pleuropneumonia also can compress the heart and mimic pericardial disease.[94] Clinical aspects of pericardial disease are discussed later in this chapter.

Figure 8-1 Sagittal view of the equine heart. The thicknesses of the ventricles, the position of the atria relative to the ventricles, and the relationship of the left ventricular inlet and outlet are evident. The bicuspid valve referred to in this figure is the mitral valve. The circular appearance of the left atrium and the relationship of the septal cusp of the mitral valve to the left ventricular inlets and outlets are notable. These aspects are important when examining the heart by echocardiography. *v.a.,* Segment of aortic valve. (From Sisson S, Grossman JD: *Anatomy of the domestic animals,* ed 4, Philadelphia, 1953, WB Saunders.)



8.1.2 MYOCARDIAL DISEASE

The myocardium forms the bulk of the atrial and ventricular muscular walls. The right atrium, which is larger than the left atrium, communicates with the RV inlet through the right atrioventricular or tricuspid valve. The right ventricle, appearing crescent-shaped on cross-sectional echocardiographic studies, is functionally U-shaped with the inlet at the right hemithorax and the outlet, pulmonary valve, and main pulmonary artery located on the left side of the chest. The thicker (by approximately 2.5 to 3 times) ventricular septum and attached LV wall is circular in cross section when viewed by echocardiography and is functionally V-shaped with an inlet and outlet separated by the cranioventral (septal or anterior) leaflet of the left atrioventricular, or mitral, valve (Figure 8-1). 358
The aorta originates in the LV outlet, continuous with the ventricular septum cranially and the septal mitral 359
leaflet caudally, and exits from the center of the heart and to the right of the main pulmonary artery. Ventricular or atrial dilation are recognized by echocardiography or at necropsy by distention and rounding of the affected chambers. Persistent embryologic openings in the cardiac septa are known as septal defects, with the ventricular septal defect (VSD) representing the most common cardiac anomaly in most practices (see Box 8-2).

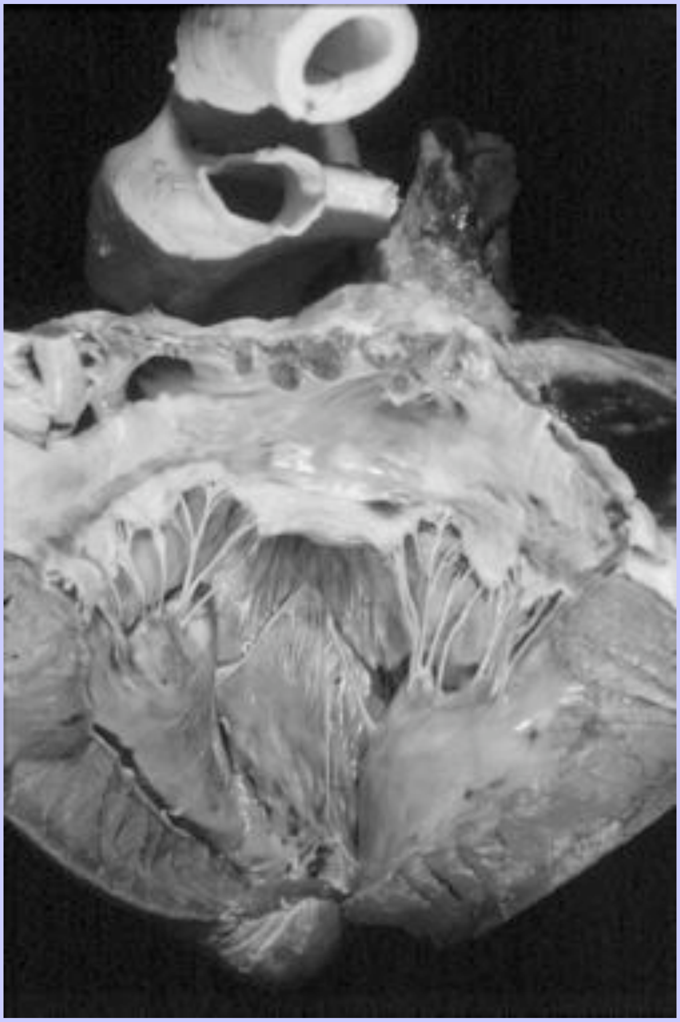
The myocardium may dilate or hypertrophy in response to exercise,[95] increased work caused by structural cardiac disease, or as a result of a noncardiac disorder. Lesions causing systolic pressure overload lead to concentric hypertrophy.[96] More common are lesions such as incompetent valves that cause ventricular volume overload with dilation and eccentric ventricular hypertrophy. Increased cardiac work also occurs in response to exercise, severe anemia, and infections. In these situations, compensatory increases in CO, sympathetic activation, and peripheral vasodilation occur to maintain oxygen delivery to the tissues.[97,98]

The overall prevalence of myocardial disease is unknown; however, multifocal areas of fibrosis commonly are found at necropsy.[28,29,99–102] Whether these areas indicate prior inflammation, toxic injury, or ischemic necrosis caused by intramural coronary disease is uncertain. Cases of multifocal or diffuse myocarditis have been observed. Myocardial inflammation and myocardial failure can lead to cardiac arrhythmias and heart failure.[3,103,104] Idiopathic dilated cardiomyopathy develops sporadically and is recognizable by echocardiography or nuclear studies as a dilated, hypokinetic left or right ventricle.[87,105] Ingestion of monensin can cause mild to severe myocardial injury.[106–111] Neoplastic infiltration is considered rare.[58,92,93,112] Myocardial contraction is dictated by electric activity of the myocardium; accordingly, cardiac arrhythmias, especially atrial fibrillation, can limit CO and cause exercise intolerance in performance animals (see the following discussion). Clinical aspects of myocardial disease are discussed later in this chapter.

8.1.3 VALVULAR AND ENDOCARDIAL DISEASES

The endocardium lines the cardiac chambers, covers the four cardiac valves, and is continuous with the endothelium of the great vessels. Normal valves govern the one-way flow of blood in the heart by preventing regurgitation of blood from higher to lower pressure zones. The atrioventricular inlet valves, the tricuspid and the mitral, are anchored by the collagenous chordae tendineae and papillary muscles and are supported by a valve "annulus" and the caudal atrial walls (see Figures 8-1 and 8-2).[74] The mitral valve consists of two major cusps and several accessory cusps.[45] The tricuspid valve is the largest valve and consists of three well-defined leaflets. Lesions of any portion of the atrioventricular valve apparatus or dilation of the ventricle can lead to valvular insufficiency (see Box 8-2). The aortic and pulmonary valves each consist of three leaflets that close during diastole to protect the ventricles from the higher arterial blood pressures. The left and right main coronary arteries originate within the aortic valve sinuses (of Valsalva).

Figure 8-2 Anatomy of the left atrioventricular (mitral) valve. Opened left atrium and left ventricle viewed from the caudal perspective. The large anterior (cranioventral or septal) leaflet in the center of the figure is notable. Chordae tendineae attach the valve to the papillary muscles. The ventricle has been cut so that the multiple cusps of the posterior (caudodorsal or mural) leaflet are visible to the left and the right of the anterior leaflet.



Valvular disorders are common.[*] Congenital valve stenosis, dysplasia, or atresia occur sporadically in foals. Degenerative diseases of the aortic, mitral, and tricuspid valves are common in mature horses,[3,103,116,145–158] and endocarditis can develop on any cardiac valve[†] (see Box 8-2). Valvular lesions of obscure cause, including nonseptic valvulitis, have been recognized sporadically. Tricuspid regurgitation, of unknown cause, often is

detectable in high performance animals, including Standardbred and National Hunt horses.[162,163] Mitral regurgitation (MR) caused by rupture of a chorda tendinea is common and has been observed in foals and mature animals.[38,122,137,164] Clinical aspects of valvular heart disease are discussed later in this chapter.

* References 5, 25, 27, 28, 38, 45, 48, 55, 80, 88, 102, 113–144.

† References 3, 81, 103, 114, 125, 129, 134, 159–161. 359

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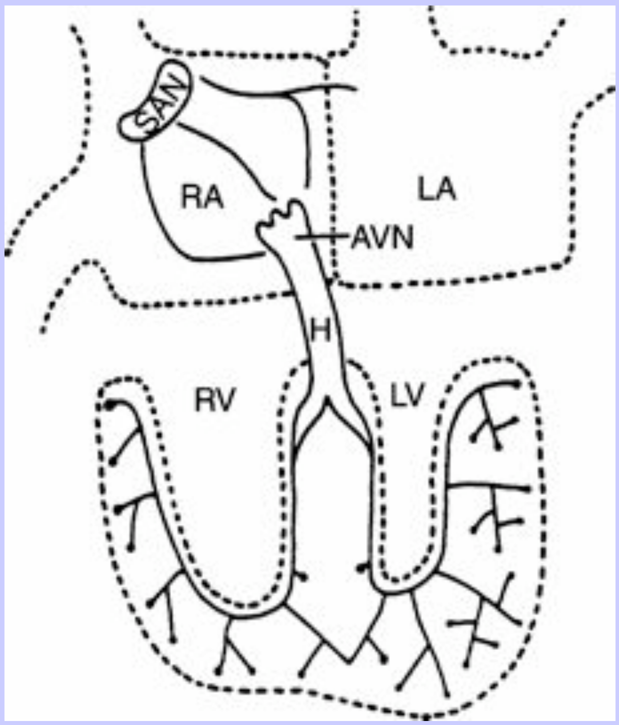
8.1.4 DISEASE OF THE IMPULSE-FORMING AND CONDUCTION SYSTEMS

The specialized cardiac tissues consist of the sinoatrial node, internodal pathways, atrioventricular node, bundle of His, bundle branches, fascicles, and Purkinje system (Figure 8-3). The sinoatrial node is a large, crescent-shaped structure located subepicardially at the junction of the right auricle and cranial vena cava. Well-documented sinus node disease, although suggested,[165] is rare; in contrast, vagally induced sinus arrhythmias are common.[9,165–167] The equine atrial muscle mass is large and predisposes the horse to develop reentrant rhythms and atrial fibrillation.[168] The atrioventricular node, situated in the ventral atrial septum, and the bundle of His, which continues on into the bundle branches, are sites for atrioventricular block, physiologic (vagal) and infrequently pathologic. Conduction is slow across the atrioventricular node.[169–171] The His-Purkinje system in the ventricular septum and ventricular myocardium can act as substrates for junctional and ventricular ectopic impulses and tachycardias. Because the horse has relatively complete penetration of Purkinje fibers to the ventricular free walls, except for a small portion of the LV free wall, the substantial equine ventricles are activated electrically in a short time (~110 ms).[7]

The autonomic nervous system extensively innervates the heart and influences cardiac rhythms.[3,103,124,172–177] Interplay between the sympathetic and parasympathetic branches normally controls heart rate and rhythm in response to changes in arterial blood pressure.[9,178] The vagus innervates supraventricular tissues extensively and probably affects proximal ventricular tissues to a minor extent. Vagal influence is generally depressive to heart rate, atrioventricular conduction, excitability, and myocardial inotropic (contractile) state. However, because vagotonia shortens the action potential and refractory period of atrial myocytes, high vagal activity may be a predisposing factor in the development of atrial fibrillation.[179] Innervation of the stimulatory sympathetic nervous system is extensive throughout the heart and has effects opposite to those of the parasympathetic system. β_1-Adrenergic receptors dominate in the equine heart,[180] but presumably other autonomic subtype receptors include α-adrenergic receptors and possibly small numbers of β_2-adrenoceptors.[181] The notable increase in heart rate that attends exercise is related to increased sympathetic efferent activity and withdrawal of parasympathetic tone.[9] Increases in heart rate to 220 to 240 beats/min are common with maximal exercise.[182–186] The exact role of dysautonomia in the genesis of cardiac arrhythmias has not been determined; however, infusion of autonomic receptor agonists and antagonists can be associated with direct or baroreceptor-induced changes in heart rate and rhythm.[187–190] Cardiac arrhythmias are discussed later in this chapter.

Figure 8-3 Schematic demonstrating the anatomy of the impulse-forming and the conduction system. Diagram shows the sinoatrial node *(SAN)*, right atrium *(RA)*, left atrium *(LA)*, atrioventricular node *(AVN)*, bundle of His *(H)*, and the bundle branches continuing into the Purkinje system in the right ventricle *(RV)* and the left ventricle *(LV)*. (Courtesy Robert L. Hamlin.)



8.1.5

VASCULAR DISEASES

The arteries and veins consist of three layers: adventitia, media, and intima. The overall structure and function of each layer varies with the vessel and location. Vascular receptors[20,70,73,181] and anatomic lesions influence vascular resistance and blood flow. α-Adrenoceptors dominate in the systemic vasculature, and blood pressure generally increases by vasoconstriction following stimulation of postsynaptic α-adrenergic receptors by norepinephrine, epinephrine, or infused α-adrenergic receptor agonists such as phenylephrine.[191,192] The presence of vasodilator β_2-adrenergic receptors is clinically relevant, insofar as infused β_2-agonists cause vasodilation in circulatory beds that contain high β-agonist adrenergic receptor density. Many vascular beds also dilate following the production of local vasodilator substances, such as nitric oxide, released during exercise, stress, or metabolic activity.[70,73] Dopaminergic receptors, when present in vascular walls, may be stimulated to cause vasodilation, provided vasoconstricting α-adrenergic activity does not dominate. Stimulation of $histamine_1$ receptors or serotonin (5-hydroxytryptamine) receptors causes arteriolar dilation, venular constriction, and increased capillary permeability.[73] Infusion of endothelin[193] or of calcium salts causes arterial vasoconstriction,

[194] whereas administration of calcium channel antagonists (e.g., verapamil and diltiazem) causes vasodilation of vascular smooth muscle. 360

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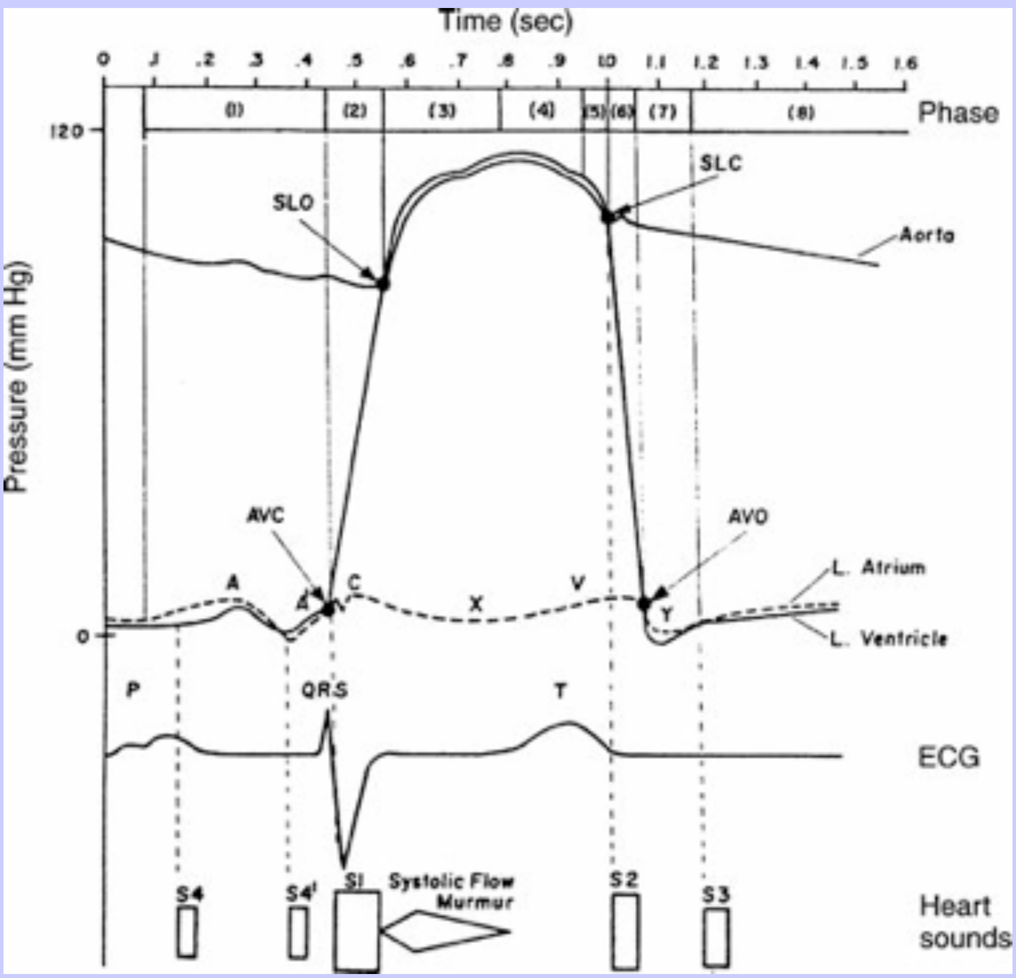
Figure 8-4 The cardiac (Wiggers') cycle of the horse. This drawing integrates the electric, pressure, mechanical, and flow events of diastole and systole and demonstrates the origins of the heart sounds. Electric activity precedes mechanical events. See the text for a full description. *AVC,* Closure of the mitral (atrioventricular) valve; *AVO,* opening of the mitral (atrioventricular) valve; *SLO,* opening of the aortic (semilunar) valve; *SLC,* closure of the aortic (semilunar) valve. (Modified from Detweiler DK, Patterson DF: The cardiovascular system. In Cattcott EJ, Smithcors JF, editors: *Equine medicine and surgery*, ed 2, Santa Barbara, Calif, 1972, American Veterinary Publications.)



Various vascular lesions have been reported (see Table 8-2). Rupture of the aorta, pulmonary artery, or middle uterine artery is devastating and typically lethal.[*] Although parasitic arteritis may predispose horses to vascular injury, the cause of most vascular lesions, including aortic-iliac thrombosis, is unknown.[197–202] Causes of vasculitis include *Strongylus vulgaris* infestation of the cranial mesenteric artery, infective thrombophlebitis of the jugular veins, equine viral arteritis, and suspected immune-mediated disease.[99,101,203] Clinical features of vascular disease are discussed later in this chapter.

* References 3, 34, 57, 103, 142, 195, 196.

8.2 Clinical Cardiovascular Physiology

The clinician must appreciate elementary aspects of normal heart function to understand changes in the pulses and auscultation and hemodynamic alterations that develop in heart disease and CHF.[204] Central to this understanding are the electric-mechanical correlates of Wiggers' cardiac cycle.[181]

8.2.1 CARDIAC CYCLE

The association between electric and mechanical events of the heart first described by Wiggers has been reviewed in standard physiology textbooks (Figure 8-4).[70,73,181] A study of this cycle gives evidence that cardiac electric activity precedes pressure and volume changes; therefore arrhythmias can have deleterious hemodynamic effects, especially during exercise, illness, or anesthesia. Understanding the cardiac cycle and considering the interrelated events during the course of an examination provides the clinician with critical information regarding cardiac rhythm and the health of the heart. Relevant aspects of this cycle are considered next.

The P wave of the electrocardiogram (ECG) results from electric activation of the atria late in ventricular diastole 361
and after the ventricles have filled passively. The ensuing atrial contraction generates the *atrial sound* (S_4), and 362
the ventricle fills to a slightly greater extent (termed the *end-diastolic volume*). The increase in atrial pressure, the atrial *a wave,* is reflected up the systemic venous system, causing a normal *jugular pulse* in the ventral cervical region. The magnitude of the atrial contribution to ventricular filling is greatest at high heart rates; therefore atrial tachyarrhythmias such as atrial fibrillation have a most serious effect during exercise.

The QRS complex heralds ventricular systole. After depolarization of the ventricular myocytes, calcium enters the cell to trigger shortening of the myofilaments and develop tension, an event that digitalis glycosides or catecholamines such as dobutamine can enhance. These changes lead to an abrupt increase in ventricular wall tension and intraventricular pressure. The atrioventricular valves close once atrial pressure is exceeded (generating the high-frequency *first heart sound,* S_1), the intraventricular pressure increases *(isovolumetric period),* and subsequently the semilunar valves open.[23,25] At this instant the ventricular walls move inward 362
(Figure 8-5) and eject blood into the great vessel (Figure 8-6). The contracting heart twists slightly during 363
systole, and the left ventricle strikes the chest wall caudal to the left olecranon, causing the *cardiac impulse* or apex beat. This early systolic movement, coincident with opening of the aortic valve, is a useful timing clue for cardiac auscultation. The delay between the onset of the QRS and the opening of the semilunar valves, termed the *preejection period,* is measurable by echocardiography and is an index of ventricular myocardial contractility such that positive inotropic drugs shorten the preejection period.[46,205–212] Blood is ejected into the pulmonary and systemic arteries with an initial velocity that generally peaks near 1 m/sec and is measurable by Doppler

echocardiography.[139,205] The aortic ejection time usually exceeds 400 ms in a horse at rest, and reductions of ejection velocity or ejection time suggest reduced LV function. A *functional systolic ejection murmur* often is audible during ejection. Such murmurs, by definition, must begin after the first sound and end before the second sound. One can palpate the *arterial pulse* during systole, but the actual timing of the pulse depends on the proximity of the palpation site to the heart. At the end of the *ejection period,* as ventricular pressures fall below those of the corresponding arteries, the semilunar valves close, producing the high-frequency *second heart sounds* (S_2) and the incisura of the arterial pressure curves.[19,23,25] The pulmonary valve may close after or before the aortic valve.[3,4,213] Asynchronous valve closure may lead to audible splitting of S_2, which is normal but can be extreme in some horses with lung disease. During the ejection period, the ventricular volume curve graphs a considerable reduction from the end-diastolic volume: this volume ejected is defined as the *stroke volume*. The ratio of the stroke volume to the end-diastolic volume is the *ejection fraction,* a commonly used index of systolic heart function. The ejection fraction commonly is estimated by echocardiography. Contraction of the ventricles causes the atrial pressures to decline, leading to the x descent of the atrial pressure curve and a brief systolic collapse of the jugular vein. Following atrial filling during ventricular systole, a positive pressure wave, the v wave, occurs in the atrial pressure curves. Severe tricuspid regurgitation (TR) accentuates this wave and may lead to pathologic systolic pulsations in the jugular furrow. Finally, a decline in ventricular pressure (isovolumetric relaxation) related to active ventricular relaxation and closure of the semilunar valves occurs.

Figure 8-5 Ventricular function and echocardiography. **A,** Derivation of the M-mode echocardiogram. The lines demonstrate typical paths of M-mode recording planes. (*1,* Ventricular/papillary muscle; *2,* chordae tendineae; *3,* anterior mitral valve *[AMV]*; *4,* aortic root and left atrium/auricle; *TW,* thoracic wall; *RVW,* right ventricular wall; *RV,* right ventricle; *S,* septum; *LV,* left ventricle; *AV,* aortic valve; *AO,* aortic outflow tract; *LVW,* left ventricular wall; *PMV,* posterior mitral valve; *LA,* left atrium) **B,** The drawing demonstrates the appearance of the M-mode echocardiogram at each level. (*PER,* Pericardium; *RS, LS,* right and left sides of the ventricular septum; *EN,* endocardium.) **C,** M-mode echocardiogram demonstrating the method of measuring left ventricular shortening fraction *(LVSF),* in which *D* is diastolic dimension and *S* is systolic dimension (LVSF = D – S/D). The prominent thickening of the walls during systole is notable. The end-systolic excursion of the left ventricular wall is visible *(arrow)*. In practice the systolic dimensions of the ventricular septum and the left ventricular wall generally are measured along the same line as demonstrated for the left ventricular lumen in systole *(S)*. (*W,* Left ventricular wall; *VS,* ventricular septum.)

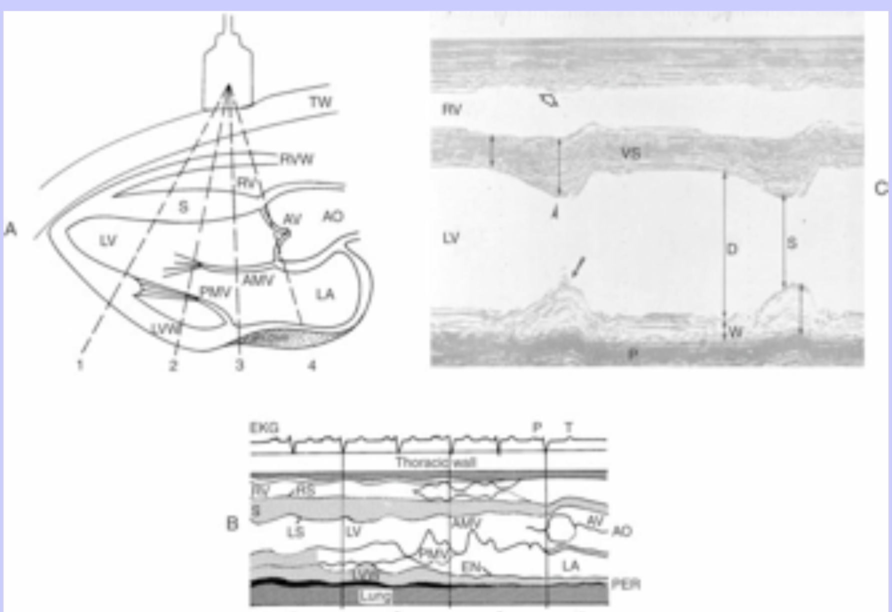
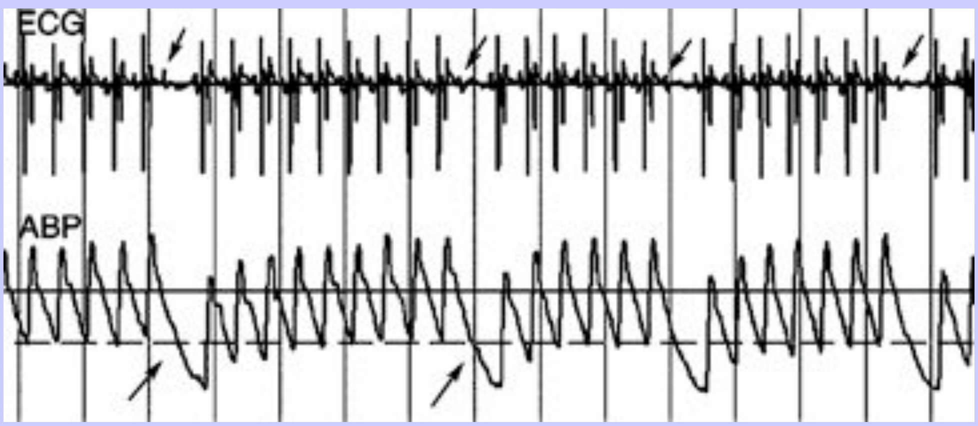


Figure 8-6 Compressed electrocardiogram *(ECG)* with simultaneous arterial blood pressure *(ABP)* recording in a horse with second-degree atrioventricular block. The progressive increase in arterial blood pressure triggers a baroreceptor reflex leading to atrioventricular conduction block *(upper arrows)* and a corresponding fall in the arterial blood pressure *(lower arrows)*. Presumably this mechanism, along with sinus arrhythmia and sinus arrest, represent vagally induced mechanisms for controlling arterial blood pressure in the standing horse.



Filling commences just as the atrioventricular valves open. Ventricular diastole can be subdivided into three general phases: rapid ventricular filling, diastasis, and atrial contraction.[70,73,181] These phases are readily observable using pulsed wave Doppler echocardiography (see Figure 8-6). Once the ventricles have relaxed and the atrial pressure exceeds the corresponding ventricular pressure, the atrioventricular valves open. At that instant, rapid filling ensues with a peak velocity of about 0.5 to 1 m/sec, but varying directly with the heart rate.[139] The ventricular pressures increase only slightly during this phase, whereas the ventricular volume curves change dramatically from the venous return. Rapid filling may be associated with a *functional protodiastolic murmur,* which is concluded by the *third heart sound* (S_3), the low-frequency vibrations caused by termination of rapid ventricular filling. The loss of atrial volume and corresponding decline in the atrial pressure (the y descent) is reflected in the jugular furrow as the vein collapses. Following rapid filling, a period of greatly reduced low-velocity filling, diastasis, ensues. This period may last for seconds during sinus bradycardia or pronounced sinus arrhythmia, and with greatly exaggerated pauses the jugular vein may begin to fill prominently. The last phase of diastole is the contribution to ventricular filling caused by the atrial contraction. A functional *presystolic murmur* has been associated with this period between S_4 and S_1.

8.2.2

ASSESSMENT OF VENTRICULAR FUNCTION

The ability of the ventricles to eject blood depends on systolic and diastolic ventricular function and on heart rate and rhythm (Box 8-3). The most commonly used measurements of overall ventricular performance and

circulatory function are invasively or noninvasively determined arterial blood pressure, rate of ventricular pressure change, CO, stroke volume, ejection fraction, LV shortening fraction, central venous pressure, pulmonary artery or wedge pressures, and arteriovenous oxygen difference.[13,24,26,47,214–227] CO, the amount of blood pumped by the left (or right) ventricle in 1 minute (liters per minute), is the product of ventricular stroke 363
volume (milliliters per beat) multiplied by the heart rate (beats per minute). Cardiac index refers to the CO divided by (indexed to) the body surface area. CO coupled with systemic vascular resistance determines the mean arterial blood pressure: an increase in either variable raises mean arterial pressure. Values for CO vary widely with the size and activity of the horse and often are influenced by drug therapy or anesthesia.[188,228–239] One also can obtain a noninvasive estimate of CO using Doppler techniques.[208–210,225,240–248] 364

8.2.2.1
BOX 8-3 DETERMINANTS OF CARDIAC FUNCTION

8.2.2.1.1
Systolic Function: Determinants of Ventricular Stroke Volume

Preload [+]—ventricular end-diastolic volume

- Determinants of diastolic function (see below)
- Plasma volume
- Venous pressure/venous return

Myocardial inotropism [+]—contractility of the myocardium

- Sympathetic activity
- Myocardial disease
- Drugs (positive or negative inotropic agents)
- Myocardial perfusion

Ventricular afterload [–]—wall tension required to eject blood

- Aortic impedance
- Vascular resistance
- Ventricular volume (tension increases with dilation)
- Ventricular wall thickness (thin walls have higher tension)

Cardiac lesions increasing workload [–]

Valvular regurgitation (common)

Valvular stenosis (rare)

Septal defects and shunts

8.2.2.1.2

Diastolic Function: Determinants of Ventricular Filling

Pleural/mediastinal factors (pressure, mass lesions)

Pericardial function (intrapericardial pressure, constriction)

Myocardial relaxation

Myocyte stiffness

Ventricular wall distensibility (chamber and myocyte compliance)

Venous pressure and venous return (must be matched with compliance)

Heart rate and ventricular filling time (shortened by tachycardia)

Myocardial perfusion (ischemia impairs relaxation)

Atrial contribution to filling (lost in atrial fibrillation)

Cardiac rhythm (arrhythmias can alter atrioventricular contraction sequencing)

Atrioventricular valve function

8.2.2.1.3

Cardiac Output

Cardiac output = Stroke volume [+] × heart rate [+]

8.2.2.1.4

Arterial Blood Pressure

Arterial blood pressure = Cardiac output [+] × vascular resistance [+]

Ventricular stroke volume depends on myocardial contractility and loading conditions (preload and afterload).[24,26] Although traditionally considered to be independent determinants of myocardial function, these variables are interconnected and influence force, velocity, and duration of ventricular contraction.[181] Ultimately, the availability of calcium to the sarcomere is modulated by the inotropic state, the initial myocardial stretch (preload), and the tension that must be generated to eject blood into the vascular system (afterload).

Catecholamines, calcium, digitalis glycosides, and phophodiesterase inhibitors increase myocardial contractility.[*] Contractility is difficult to measure in the clinical setting, but one can estimate it by observing directional changes in ejection or shortening fractions (which are also load dependent) or by Doppler echocardiographic techniques including preejection period, ejection time, aortic acceleration, and velocity time integral (see Figure 8-5). Assessment of global LV performance commonly is accomplished clinically using M-mode and two-dimensional echocardiography.[266–270] Physiologic state, altered mildly by day-to-day variation[210,271] or sedatives,[206] influences measured variables, and general anesthesia greatly affects these variables.

Ventricular fiber length or preload is a positive determinant of ventricular systolic function that depends on venous return and ventricular size and distensibility. The normal ventricle depends greatly on preload so that an increase in preload increases stroke volume. Dehydration, venous pooling, loss of atrial contribution to filling (atrial fibrillation), and recumbency reduce ventricular filling and decrease stroke volume. When the horse develops hypotension resulting from decreased ventricular filling, the clinician usually administers intravenous crystalloid to increase venous pressure and preload. One can observe increased ventricular preload in horses with heart disease. Moderate to severe valvular insufficiency and CHF increase ventricular filling pressure and preload.[38,98,272,273] The increased ventricular diastolic dimension serves as a compensatory mechanism that 364
maintains forward stroke volume in the setting of a failing ventricle or regurgitant heart valve. 365

One can estimate ventricular preload by determining ventricular end-diastolic volume or size, as measured by echocardiography or by measuring venous filling pressures.[273] The measurement of venous filling pressures (central venous pressure, pulmonary diastolic or wedge pressure) provides an accurate gauge of preload provided that ventricular compliance (distensibility) is normal and ventilation is stable. Myocardial ischemia, which impairs myocardial relaxation, and pericardial diseases, which constrict the ventricles, reduce ventricular compliance; in such cases, the venous filling pressures may not reflect changes in ventricular preload accurately.

Ventricular *afterload* represents the tension that must develop in the ventricular walls before ejection of blood into the arteries. Increases in systemic arterial resistance, aortic blood pressure, and aortic impedance usually increase afterload and resist ventricular shortening. Increased afterload decreases stroke volume, unless ventricular force of contraction increases to compensate for the load. Afterload is difficult to measure clinically, and although systemic arterial blood pressure is not identical to afterload, it often is used to estimate directional changes in afterload. LV failure, alveolar hypoxia– induced pulmonary vasoconstriction, and atelectasis are important clinical causes of increased RV afterload. Arterial vasodilators such as hydralazine decrease LV afterload.[236]

Ventricular *synergy* refers to the normal method of ventricular activation and contraction. Normal electric activation causes a burst of activation of great mechanical advantage.[20] Cardiac arrhythmias, especially ventricular rhythm disturbances, can cause dyssynergy with a resultant decrease in stroke volume. Coronary occlusions leading to ischemic myocardial necrosis also cause dyssynergy but are rare.[56,101,274]

Structural and functional competency of the cardiac valves and ventricular septa influence ventricular systolic function. Valvular insufficiency or the rare obstruction may reduce ventricular stroke volume unless adequate compensation occurs from ventricular dilation and hypertrophy or heart rate reserve. For example, many septal defects are well tolerated at rest and during moderate exercise because of eccentric hypertrophy. However, large defects can lead to substantial shunting and volume overload of the left side of the heart, causing limited LV stroke volume, arrhythmias, or CHF.

Ventricular *diastolic function* determines ventricular filling and preload.[70,73,181,227] Box 8-3 indicates factors that affect diastolic function. When diastolic function is abnormal, heart rate dependency for maintenance of CO often is greater. A common cause of diastolic dysfunction is constriction or compression of the heart caused by pericardial disease. Arrhythmias affect ventricular diastolic function. Persistent tachycardia shortens diastole, cardiac filling time, and coronary perfusion. With atrial fibrillation the atrial contribution is lost. Junctional and ventricular arrhythmias prevent normal atrioventricular sequencing. Ventricular chamber dilation or hypertrophy decreases ventricular compliance and require higher ventricular distending pressures for filling. LV diastolic dysfunction resulting from severe RV dilation or hypertrophy can be explained by bulging of the ventricular septum into the left ventricle, which impedes filling of the left side. This effect occurs clinically with constrictive pericardial disease and severe pulmonary hypertension.

Objective measures of diastolic function are complicated, and no good clinical indicators of diastolic function are currently available. However, one may assume diastolic dysfunction on recognition of one of the aforementioned conditions. Measuring transmitral and tricuspid inflow using Doppler techniques is possible, but these methods are crude and also depend on atrial pressure. Tissue Doppler imaging can measure the actual velocity of myocardial wall segments in the equine heart and may provide insight into diastolic cardiac function.

Imbalance between *myocardial oxygen demand* and delivery can reduce ventricular systolic and diastolic function and may affect cardiac rhythm. Myocardial oxygen demand is augmented by increasing myocardial inotropic state, heart rate, and ventricular wall tension (related to preload and afterload).[181] Oxygen delivery depends on coronary anatomy and vasomotion (degree of vessel constriction), diastolic arterial blood pressure, diastolic (coronary perfusion) time, and metabolic activity of the myocardium.[10,73,275–282] Normal coronary flow to the LV myocardium is highest in the ventricular septum and LV wall.[282] The immediate subendocardial layer of myocardium is probably most vulnerable to ischemic injury,[9] and altered ventricular depolarization may develop following an imbalance in myocardial oxygen delivery. Depolarization may account in part for the ST segment to T wave depression and changes in the T waves observed in hypotensive animals and in normal horses during sinus tachycardia. Coronary vasomotion is effective in augmenting coronary perfusion even at high heart rates (up to 200 beats/min in ponies); however, coronary autoregulation is not as effective if diastolic perfusing pressure decreases in the aorta.[276,277,279]

The clinician may use the arterial blood pressure × heart rate "double product" as a general estimate of myocardial oxygen demand.[181] Persistent ST segment depression or elevation, especially at normal heart rates, suggests deficient myocardial perfusion.

* References 187, 188, 191, 194, 209, 227, 232, 249–265.

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8.3 Cardiovascular Examination

8.3.1 GENERAL APPROACH

Box 8-4 summarizes a general approach to recognizing and diagnosing heart disease and assessing its severity. Undoubtedly, history and auscultation are the most important initial evaluation procedures in the cardiovascular examination of the horse. With the exception of mild abnormalities in ventricular function, normal cardiac auscultation in a horse with good exercise tolerance practically precludes significant heart disease. The initial cardiovascular physical examination should include a thorough auscultation of the heart at all valve areas, auscultation of both lung fields, palpation of the precordium, evaluation of the arterial pulses (head and limbs),

inspection of the veins, and evaluation of the mucous membranes for pallor, refill time, and cyanosis, which may develop in foals following a right-to-left cardiac shunt. One also should record an accurate resting heart rate and respiratory rate.

A point of emphasis is that one can detect most *serious* cardiac disorders initially by physical examination and a stethoscope. One easily can discover sustained or recurrent cardiac arrhythmias through cardiac auscultation and palpation of the arterial pulse and can verify diagnosis of the rhythm through electrocardiography. Pericarditis and cardiac tamponade are characterized by muffled heart sounds or pericardial friction rubs, jugular distention, and often RV failure. Significant myocardial disease usually is associated with heart failure, arrhythmias, or a cardiac murmur, especially in advanced cases in which ventricular dilation causes secondary insufficiency of the mitral or tricuspid valves. The presence of a cardiac murmur is the essential finding that leads one to suspect degenerative or infective valvular disease or a congenital heart malformation.[103,146]

Laboratory studies, electrocardiography, and echocardiography are additional tests particularly useful in recognizing the underlying basis and severity of cardiovascular disease. Mild or subtle cardiovascular disease may require a detailed examination, including exercise testing,[283–288] before one can detect abnormalities objectively.

8.3.2 HISTORY

One may suspect cardiovascular disease from the history or a serendipitous finding during a routine examination.[*] One may examine the horse with CHF for obvious generalized venous distention, jugular pulsations, or edema. Conversely, other cardiac problems such as arrhythmia or murmur can be incidental findings, detected during a routine physical, prepurchase, or insurance examination. Once one finds an abnormality, a complete cardiovascular examination is aimed at determining the lesion and the significance of disease in terms of safety, performance capabilities, and expected longevity.

The horse with clinically apparent cardiovascular disease may have subtle performance problems that are apparent only at peak performance levels. Slowing in the last quarter to three eighths of a race is a common historical complaint. In many cases, performance may deteriorate by only 2 to 3 seconds. In other horses, particularly in cases of atrial fibrillation, racing performance may decline greatly by 20 to 30 seconds or more. Horses with malignant ventricular tachycardia may stop abruptly or even fall. Horses with cardiovascular disease may have excessively high heart and respiratory rates during and after exercise and may take longer than normal time to return to a resting rate. Such horses take longer to cool out. Coughing, at rest or during exercise, and exercise-induced pulmonary hemorrhage are reported in some horses with heart disease. One always must consider cardiovascular disease along with musculoskeletal, respiratory, metabolic, and neurologic problems in the differential diagnosis of poor performance (Box 8-5).[54,55,118,292] Other performance-related problems with cardiovascular disease can include weakness, ataxia, collapse, and sudden death[52,56–67,196] (Box 8-6).

* References 32, 103, 104, 174, 224, 289–291.

8.3.3 AUSCULTATION

8.3.3.1 Clinical Method

Auscultation is an accurate method for cardiac diagnosis, but effective auscultation requires an understanding of anatomy, physiology, pathophysiology, and sound. Clinical experience regarding cardiac auscultation in the

horse is extensive,[*] and recent experience with Doppler echocardiography has refined the clinician's understanding of heart sounds and murmurs.[120,135] Experience and training are also significant factors in effective cardiac auscultation,[307,308] and one should consider auscultation an acquired skill to be honed constantly. The overall sensitivity of auscultation for identification of congenital heart disease is high, as is the sensitivity for recognition of *significant* valvular disease and *persistent* cardiac arrhythmias. Sensitivity is probably lower for primary myocardial or pericardial diseases, unless associated abnormalities such as a murmur, arrhythmia, or prominent friction rub are obvious. The specificity of auscultation in the horse—for example, the ability to distinguish a functional from a pathologic murmur or identify a specific flow 366
disturbance—has not been sufficiently studied, but most certainly depends on the clinician's knowledge and 368
experience.[307]

8.3.3.1.1 BOX 8-4 CLINICAL EXAMINATION OF THE EQUINE CARDIOVASCULAR SYSTEM

8.3.3.1.1.1 History and Physical Examination[*]

Work history and identification of possible hemodynamic dysfunction

Heart rate and rhythm

Examination of the arterial and venous pulses, refill, and pressure

Inspection of the mucous membranes

Evaluation for abnormal fluid accumulation: pulmonary, pleural, peritoneal, and subcutaneous

Auscultation of the heart and lungs

Measurement of arterial blood pressure (indirect method)

8.3.3.1.1.2 Electrocardiography

Heart rate, rhythm, and conduction sequence

P-QRS-T complexes: configuration, amplitude, duration, and axis

Postexercise and exercise electrocardiography[†]

Holter (tape-recorded) electrocardiogram[†‡]

8.3.3.1.1.3 Echocardiography

M-mode echocardiography: cardiac dimensions and systolic ventricular function, cardiac anatomy and valve motion, and estimation of cardiac output

Two-dimensional echocardiography: cardiac anatomy and size, systolic ventricular function, identification of lesions, and estimation of cardiac output

Doppler echocardiography[‡]: identification of normal and abnormal flow, estimation of intracardiac pressures, estimation of cardiac output, and ventricular function

Postexercise echocardiography: identification of regional or global wall dysfunction or valve dysfunction exacerbated by exercise

8.3.3.1.1.4

Thoracic Radiography

Evaluation of pleural space, pulmonary parenchyma, and lung vascularity

Estimation of heart size (more beneficial in foals)

8.3.3.1.1.5

Clinical Laboratory Tests

Complete blood count and fibrinogen to identify anemia or inflammation

Serum biochemical tests including electrolytes, renal function tests, and muscle enzymes: These studies may be useful for assessing arrhythmias, identifying low cardiac output (azotemia), and recognizing myocardial cell injury (cardiac isoenzymes of creatine kinase or lactic dehydrogenase, troponin concentration)

Serum protein to identify hypoalbuminemia and hyperglobulinemia

Arterial pH and blood gas analysis to evaluate pulmonary and renal function and to assess acid-base status

Urinalysis to identify renal injury from heart failure or endocarditis

Blood cultures for bacteremia and diagnosis of endocarditis

Serum/plasma assays for digoxin, quinidine, and other drugs

8.3.3.1.1.6

Radionuclide Studies[§]

Detection of abnormal blood flow or lung perfusion and assessment of ventricular function

8.3.3.1.1.7

Cardiac Catheterization and Angiocardiography[§]

Diagnosis of abnormal blood flow and identification of abnormal intracardiac and intravascular pressures

* Most important part of the cardiac evaluation.

† May be needed to identify paroxysmal atrial fibrillation.

‡ Generally done after referral.

§ Not commonly performed.

Modified from Bonagura JD: Clinical evaluation and management of heart disease, *Equine Vet Educ* 2:31-37, 1990.

8.3.3.1.2

BOX 8-5 CARDIOVASCULAR ASSOCIATION OF POOR PERFORMANCE

8.3.3.1.2.1

Arrhythmias

Atrial premature complexes

Ventricular premature complexes

Atrial fibrillation

Supraventricular tachycardia

Ventricular tachycardia

Advanced second-degree atrioventricular block

Complete third-degree atrioventricular block

8.3.3.1.2.2

Congenital, Valvular, or Myocardial Heart Diseases Associated With Murmurs

Ventricular septal defect

Mitral regurgitation

Tricuspid regurgitation

Aortic regurgitation

Cardiomyopathy with secondary atrioventricular valvular regurgitation

8.3.3.1.2.3

Occult Heart Disease

Pericardial disease

Cardiomyopathy or myocarditis

Ischemic myocardial disease (?)

8.3.3.1.2.4

Vascular Disorders

Aortic-iliac thrombosis

Jugular vein thrombophlebitis

Aortic root rupture

Pulmonary artery rupture

A prerequisite for auscultation is an appreciation of the normal heart sounds, the genesis of which has been described already (Clinical Cardiovascular Physiology). The examiner must be familiar with the causes and clinical features of murmurs and arrhythmias (Tables 8-1 to 8-3) and the areas for auscultation (Figures 8-7 and 8-8).[*] Auscultation is best carried out in a quiet area because extraneous noise makes detection of soft to moderate murmurs difficult. The clinician should palpate the arterial pulse and the precordium before commencing auscultation and should use both stethoscope chest pieces: the diaphragm (applied tightly) and the bell (applied lightly).

8.3.3.1.3 BOX 8-6 CAUSES OF SUDDEN CARDIOVASCULAR DEATH

8.3.3.1.3.1 Electric Disorders of the Heart (Arrhythmias)

Ventricular tachycardia, flutter, or fibrillation

Complete atrioventricular block

Asystole

8.3.3.1.3.2 Toxic Injury to the Heart

Acute myocardial failure

Anesthetics

Drug- or toxin-induced arrhythmia

Toxic plants

Myocardial toxins

Systemic toxin secondarily affecting the heart

8.3.3.1.3.3 Cardiac Tamponade

Bacterial pericarditis

Idiopathic pericarditis

Viral pericarditis

Trauma

8.3.3.1.3.4 Hemorrhage

Rupture of the heart (with cardiac tamponade)

Rupture of the aorta or pulmonary artery (with or without cardiac tamponade)

Arterial rupture

Middle uterine artery

Mesenteric, omental, or other large arteries

Severe pulmonary hemorrhage

Rupture of the spleen or liver

Brain hemorrhage

8.3.3.1.3.5

Embolism

Carotid air embolism

Coronary embolism or thrombosis

8.3.3.1.3.6

Electrocution

Lightning

Alternating current electrocution

8.3.3.1.3.7

Cardiac Trauma

Cardiac catheterization or needle puncture of a ventricle leading to ventricular fibrillation

Penetrating thoracic wound

The clinician should examine all auscultatory areas and can identify the locations of the cardiac valve areas using the following method:

- The left thoracic wall cardiac impulse (left apical beat), located adjacent to or under the olecranon, identifies the ventral region of the LV inlet. Mitral sounds and murmurs usually project to this location 368
as well as radiating dorsally into the left atrium. S_1 is most audible at this location. 369
- The aortic valve area is located dorsally and one or two intercostal spaces cranial to the left apical impulse. S_2 is loudest at this point, and aortic murmurs are audible well over this valve. The murmur of MR also may radiate dorsally and cranially to this area.
- Because of the central location of the aortic valve and the dextrad orientation of the ascending aorta, aortic flow murmurs and the murmur of aortic regurgitation are audible bilaterally, just medial to the triceps muscles.
- The pulmonic valve is located slightly cranioventral (generally one intercostal space) to the aortic valve; the pulmonary component of S_2 is loudest at this point. Also at this location the splitting of S_2 is most obvious.

- The main pulmonary artery is dorsal to the pulmonic valve, high on the left base. Murmurs that radiate into the pulmonary artery are most audible at this location, including some functional murmurs and the murmur of patent ductus arteriosus. 369 370

- The tricuspid valve area is located over a wide area on the right hemithorax, dorsal to the sternum and just cranial to the mitral valve. Sounds and murmurs associated with tricuspid disease usually are most audible over the right hemithorax.

Concentrating first on the individual heart sounds when assessing the heart rhythm is worthwhile, because the generation of cardiac sounds depends on the underlying electric rhythm and heart murmurs are timed relative to the heart sounds. S_4 is audible after the P wave. S_1 and S_2 encompass systole, indicating the presence of a QRS complex. A murmur detected between S_1 and S_2 is termed *systolic*. In contrast, a murmur heard after S_2 is designated as *diastolic*. The distinctive S_4 is absent in arrhythmias such as atrial fibrillation. Normal variation in the P-R interval causes gradual changes in the S_4-S_1 interval. Absence of a QRS complex, which occurs with second-degree atrioventricular block (see Figure 8-6), causes a pause in which S_1 and S_2 are absent. One also can palpate the heart sounds and precordial movements, especially over the left thoracic wall. Frequently, one can detect the cardiac movements and low-frequency vibrations corresponding to the atrial contraction (S_4), onset of ventricular contraction (S_1), and closure of the semilunar valves (S_2). With ventricular volume overloading, an accentuated apex beat and S_3 are palpable. Cardiac enlargement or displacement of the heart by an intrathoracic mass may lead to an abnormal location of the cardiac impulse. A loud cardiac murmur often is associated with a palpable vibration or *thrill*.

TABLE 8-1 Auscultation of Cardiac Arrhythmias

RHYTHM	HEART RATE PER MINUTE	HEART SOUNDS	AUSCULTATION
SINUS MECHANISMS			
Sinus rhythm	Variable	S4–1–2 (3)[*]	Rate and rhythm depend on autonomic tone.
Sinus arrest/block	<26	S4–1-2 (3)	Irregular, long pauses.
Sinus bradycardia	<26	S4–1-2 (3)	Generally regular unless escape rhythm develops.
Sinus arrhythmia	25–50	S4–1-2 (3)	Irregular, cyclical change in heart rate; often interval varies between S_4 and S_1; often associated with second-degree atrioventricular block or sinus bradycardia.
Sinus tachycardia	>50	S4–1-2–3	Typically regular, but second-degree atrioventricular block may develop if sympathetic tone decreases.
SUSTAINED ATRIAL			
TACHYARRHYTHMIAS			
Atrial tachycardia and atrial flutter	>30	S1–2 (3)	Ventricular regularity and rate depend on atrioventricular conduction sequence, sympathetic tone; consistent S_4 absent; variable-intensity S_1; may detect independent atrial sounds.
Atrial fibrillation	>30	S1–2 (3)	Ventricular response irregular; rate related to sympathetic tone; heart rates consistently above 60 beats/min suggest significant underlying heart disease or heart failure; consistent S_4 is absent.
ECTOPIC RHYTHMS			

Junctional rhythm	26–200	S1–2 (3)	Heart rate usually regular idionodal rhythms or junctional tachycardia; heart rate depends on the mechanism and sympathetic tone; inconsistent S_4; variable intensity sounds.
Ventricular rhythm	26–200	S1–2 (3)	Heart rate may be regular during monomorphic, unifocal ectopic rhythm or irregular during polymorphic or multifocal ectopic activity; the heart rate depends on the mechanism (e.g., escape rhythm versus ventricular tachycardia); variable intensity and split heart sounds may be audible.
Premature atrial and junctional beats	Varies	Early S1-S2	Intensity of S_1 may be louder or softer than normal; the sounds are not usually split; less than compensatory pause follows the premature beat.
Premature ventricular beats	Varies	Early S1-S2	Intensity of S_1 often varies and ventricular beats may be softer than normal; heart sounds may be split from asynchronous ventricular activation heart sounds; compensatory pause typically follows a premature beat.
ATRIOVENTRICULAR BLOCKS			
Incomplete (first-and second-degree)	<50	S4–1-2 (3)	Heart rate varies; cyclic arrhythmia, variable S_4-S_1 interval; some variation in heart sounds.
Complete (third-degree)	<26	S4/S1–2 (3)	Ventricular escape rhythm, usually regular; independent atrial (S_4) sounds; variable-intensity heart sounds.

* Heart sounds in parentheses may be audible.

TABLE 8-2 Auscultation of Heart Sounds and Common Cardiac Murmurs

AUSCULTATORY FINDING	TIMING	POINT OF MAXIMAL INTENSITY (VALVE AREA)
NORMAL HEART SOUNDS		
First heart sound	Onset systole	Left apex and over the mitral valve
Second heart sound	End systole	Left base and over the aortic valve
Pulmonic component	End systole	Left base and over the pulmonic valve
Third heart sound	Early diastole	Left apex and over the mitral valve
Fourth (arterial) sound	Late diastole	Ventricular inlet (left)
FUNCTIONAL MURMURS[*]		
Systolic ejection murmur	Systole	Left base (aortic/pulmonic valves)
Early (proto-)diastolic	Diastole	Ventricular inlets (left/right)[†]
Late diastolic (presystolic)		Ventricular inlets (left/right)[†]
VALVULAR REGURGITATION[‡]		
Mitral regurgitation	Systole	Left apex and over the mitral valve[§]
Tricuspid regurgitation	Systole	Right hemithorax (tricuspid valve)
Aortic regurgitation	Diastole	Left base and over the aortic valve
Pulmonary insufficiency	Diastole	Left base and over the pulmonic valve
Ventricular septal defect[||]	Systole	Right sternal border/left cardiac base (pulmonic valve)
Patent ductus arteriosus	Continuous	Dorsal left base over the pulmonary artery

Modified from Bonagura JD: Clinical evaluation and management of heart disease, Equine Vet Educ 2:31–37, 1990.

Only typical features are considered: Apex refers to the ventral part of the heart, at the point of the palpable cardiac impulse (apex beat); base refers to the craniodorsal part of the heart over the outlet valves (aortic, pulmonic) where the second heart sound is most intense.

* The exact causes of functional (flow) murmurs have not been proved; however, systolic murmurs have been recorded within the aorta and pulmonary artery using intravascular phonocardiography. The systolic ejection murmur, which begins after the first heart sound and ends before the second heart sound, is the most commonly identified murmur; the protodiastolic murmur extends from the second to the third heart sound; the presystolic murmur is short and spans the fourth and first heart sounds. Functional murmurs may be musical.

†	Ventricular inlets refer to the parts of the thorax overlying the ventricular inflow tracts and include the areas just dorsal to the mitral and tricuspid valve areas and extend ventrally to the apical regions of the ventricles.
‡	Most cases of valvular regurgitation are evident throughout systole or diastole and extend into the second heart sound (holosystolic or pansystolic) or first heart sound (holodiastolic or pandiastolic); however, late systolic murmurs, which may be related to valve prolapse and minor chordal ruptures, have been identified with mitral or tricuspid valve insufficiency, and the murmur of aortic insufficiency may not always be holodiastolic. Documented reports of valve stenosis are rare. Murmurs of atrioventricular valve insufficiency are generally audible over the affected valve, project prominently toward the respective ventricular apex, and also radiate dorsally, following the regurgitant jet into the atrium. Occasionally, murmurs of tricuspid valve disease are prominent at the extreme left cranial heart border.
§	The murmur of mitral regurgitation often radiates well into the aortic valve area and dorsally into the left atrium.
‖	Murmurs caused by defects in the right ventricular inlet septum are most audible below the tricuspid valve and above the right sternal border and generate a softer systolic ejection murmur of relative pulmonic stenosis over the left cardiac base; murmurs from defects in the right ventricular outlet septum may be loudest over the pulmonary valve; flow across large nonrestrictive defects can be soft.

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TABLE 8-3 Causes of Cardiac Murmurs

CARDIAC MURMUR	LESION IDENTIFIED BY ECHOCARDIOGRAPHY, CATHETERIZATION, OR NECROPSY
Functional murmurs*	No identifiable lesions
Congenital heart disease murmurs	Defect(s) in the atrial or ventricular septa; patent ductus arteriosus; atresia/stenosis of the tricuspid or pulmonic valve; valve stenosis; other malformations of the heart
Mitral regurgitation	High-level training; degenerative thickening of the valve; bacterial endocarditis; mitral valve prolapse into left atrium; rupture of a chorda tendinea; dilated-hypokinetic ventricle (dilated cardiomyopathy); papillary muscle lesion or dysfunction; valvulitis; malformation
Tricuspid regurgitation	High-level training†; same as mitral regurgitation; also pulmonary hypertension from severe left heart failure or chronic respiratory disease
Aortic regurgitation	Degenerative thickening of the valve; congenital fenestration of the valve; bacterial endocarditis‡; aortic prolapse into a ventricular septal defect or the left ventricle; flail aortic valve leaflet; valvulitis; malformation; ruptured aorta or aortic sinus of Valsalva
Pulmonary insufficiency	Bacterial endocarditis‡; pulmonary hypertension; flail pulmonic valve leaflet; valvulitis; malformation; rupture of the pulmonary artery
Murmur associated with vegetative endocarditis	Insufficiency of the affected valve‡

Modified from Bonagura JD: Clinical evaluation and management of heart disease, Equine Vet Educ 2:31–37, 1990.

* Functional murmur may be innocent (unknown cause) or physiologic (suspected physiologic cause). Functional murmurs are common in foals and trained athletes (athletic murmur); associated with fever and high sympathetic nervous system activity (pain, stress, sepsis); and often are heard in anemic horses. Functional murmurs depend on the physiologic state and can be altered by changing the heart rate. Such dynamic auscultation is useful in detecting functional murmurs.

† Doppler echocardiography can identify “silent” regurgitation across a right-sided cardiac valve in some horses; this is probably a normal finding of no clinical significance.

‡ Anatomic stenosis generally is caused by a large vegetation and also should be associated with a diastolic murmur of valvular insufficiency; increased flow across the valve, even in the absence of a true stenosis, may generate a murmur of “relative” valvular stenosis (e.g., with aortic regurgitation a systolic ejection murmur may occur because of an increased stroke volume).

* References 4, 16, 19, 23, 25, 27, 32, 36, 48, 72, 103, 104, 124, 162, 174, 213, 224, 291–306.

* References 4, 19, 52, 56–64, 72, 196, 289–291, 295, 296, 298.

8.3.3.2 Heart Sounds

Heart sounds should be easy to auscultate on both sides of the thorax, although some variability exists based on body type. Heart sounds are louder over the left thoracic wall. All four heart sounds generally are detectable in the standing horse, but all may not be audible at the same location (Figure 8-9).[4,23,25] The variable ventricular filling sound (S_3) is most localized and may be difficult to detect unless one places the bell lightly over the left apex. The intensity of the heart sounds should be consistent when the rhythm is regular; variation of heart sound intensity occurs with arrhythmias (irregular cardiac filling) and with volume-overloaded ventricles. Muffled heart sounds are audible with pericardial effusions or pericardial abscesses (in affected horses, the muffling may be only on one side of the thorax) but also occur in some horses with large pleural effusions and cranial mediastinal masses. Accentuation of all heart sounds, especially S_3, may be detectable with volume-loaded ventricles or with marked sympathetic activity.

S_1 varies after prolonged diastolic periods and often becomes louder (and sometimes softer). The intensity of S_1 varies whenever the rhythm is irregular, but this in itself is not diagnostic of an abnormality. Splitting of S_1, if pronounced, may indicate abnormal ventricular electric activation or ventricular premature complexes.[213] 371
Close splitting of S_1 may be more obvious with atrial fibrillation, and in the absence of an atrial sound, one 372
may misinterpret the split S_1 as a closely timed S_4-S_1 complex.

Figure 8-7 General areas for cardiac auscultation (left side). The typical flow murmur is loudest over the craniodorsal base *(upper oval)*. Murmurs of mitral regurgitation often project loudly to the left apex *(lower oval),* although dorsal radiation is also common. *Ao,* Aorta; *PA,* pulmonary artery; *AV*, aortic valve; *LV,* left ventricle; *RV,* right ventricle. (From Bonagura JD, Muir WW: The cardiovascular system. In Muir WW, Hubbell JAE, editors: *Equine anesthesia,* St Louis, 1991, Mosby.)

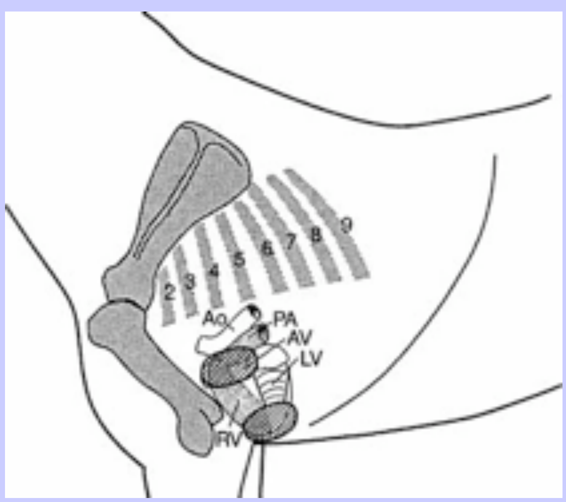
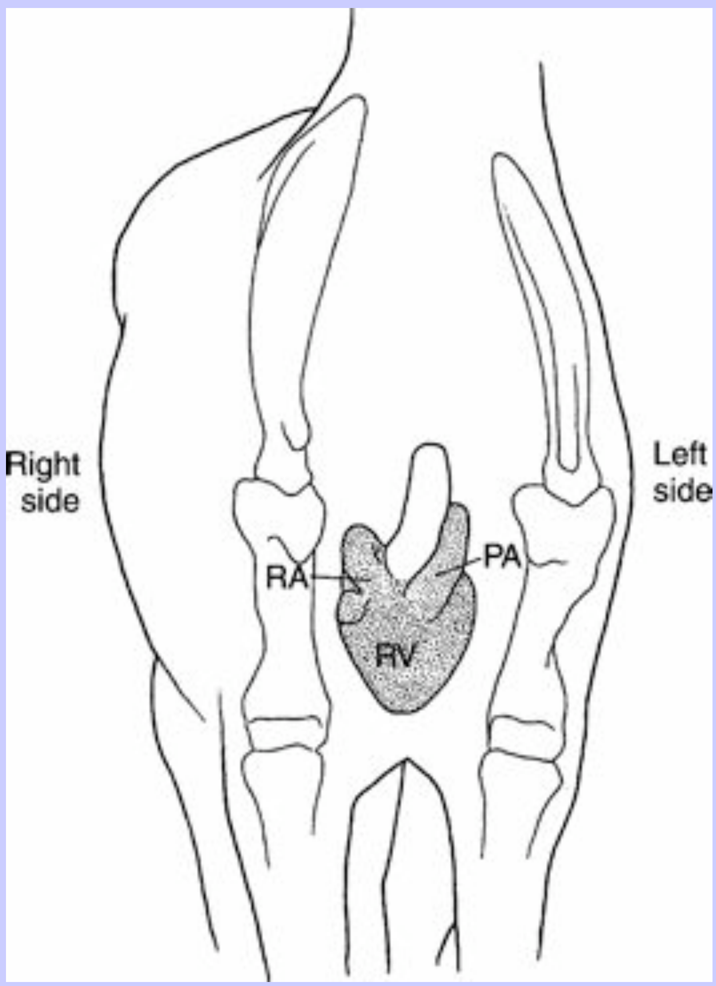


Figure 8-8 Cranial view of the heart demonstrating the U shape of the right ventricle *(RV)*. The tricuspid valve and the right ventricular inlet are located on the right side, whereas the outlet and pulmonary artery *(PA)* are on the left side. Murmurs of tricuspid regurgitation and perimembranous ventricular septal defects are typically loudest over the right hemithorax, whereas flow murmurs and those caused by subaortic or subpulmonic septal defects are loudest over the left cardiac base. *RA,* Right atrium. (From Bonagura JD, Muir WW: The cardiovascular system. In Muir WW, Hubbell JAE, editors: *Equine anesthesia,* St Louis, 1991 Mosby-Year Book.)



S_2 is loudest normally over the aortic valve area and may be split audibly over the pulmonic valve area.[3,4,213] This sound can be soft or absent following a premature beat and may be obscured by a holosystolic murmur. Audible splitting of S_2 occurs commonly in normal horses, varies with heart rate or respiration, and only infrequently is associated with pulmonary hypertension. The relative closure of the semilunar valves probably varies with the heart rate and pulmonary artery pressure,[4,213] although the pulmonic component most often is detectable after the aortic component in the healthy, resting horse. If the pulmonary component of S_2 develops a tympanic quality, becoming equal to or louder than the aortic component of S_2, the clinician should suspect pulmonary hypertension. Identification of a loud pulmonic S_2 is a useful clinical finding in cases of right-sided heart failure because it often indicates that a lesion on the *left* side of the heart has led to pulmonary hypertension and right-sided CHF.

Diastolic sounds are normal.[16,17,19,305] One should detect S_4 in virtually all horses, and this sound may be loud in some cases. One normally auscultates isolated atrial sounds at slow resting heart rates; however, multiple isolated S_4 sounds indicate high-grade atrioventricular block. S_3 is normal and can achieve great intensity with ventricular dilation and elevated filling pressures such as occur with heart failure.

One may detect other sounds with heart disease. Systolic clicks occur uncommonly over the great vessels and are thought to be benign. A systolic click that is most audible over the left apex may be a marker for mitral valve disease or prolapse of the valve into the left atrium. Constrictive pericardial disease may lead to a loud ventricular filling sound, a ventricular knock. In addition to muffling of sounds, pericarditis often is associated with a pericardial friction rub. The clinician classically detects these rubs during three portions of the cardiac cycle (systole, early diastole, late diastole). One must distinguish these sounds from pleural friction rubs or respiratory mediated sounds, which occasionally are associated with the cardiac cycle. Pleural and pulmonary rub tend to be heard only during one or two phases of the cardiac cycle and may not correlate closely.

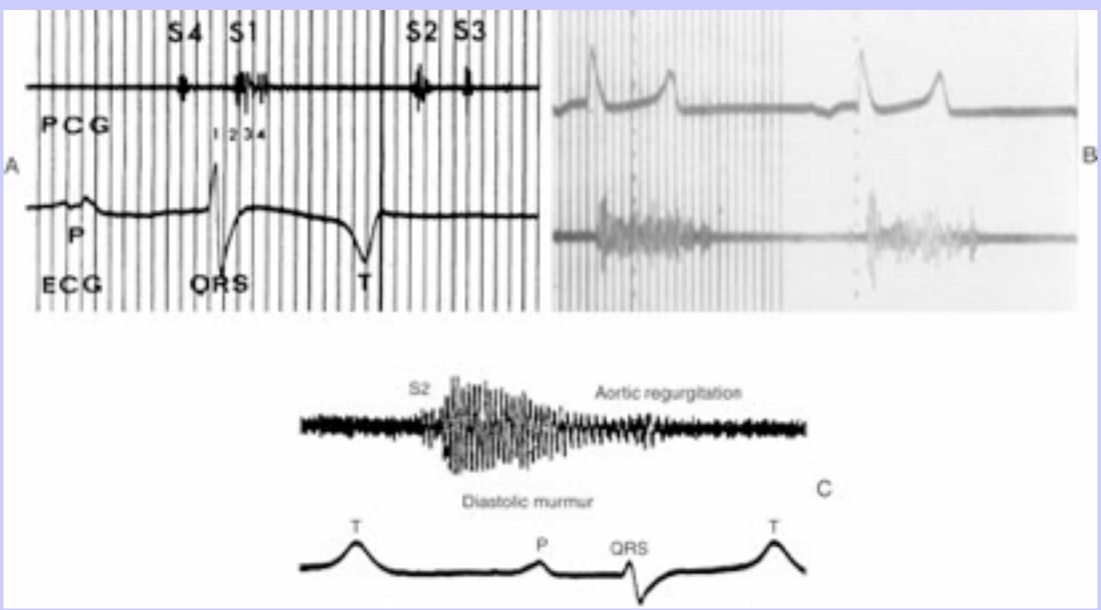
8.3.3.3 Heart Rate and Rhythm

Heart rate can change rapidly and dramatically, varying with autonomic efferent traffic and level of physical activity. Changes in the rate and rhythm are reflected in cardiac auscultation and palpation of the pulse (see Table 8-1).[299] The normal arterial pulse and cardiac rhythm is regular or cyclically irregular. Heart rate varies between 28 and 44 beats/min, although slightly slower heart rates are detectable in some fit racehorses and higher heart rates may be present in foals,[303] yearlings, Draft Horses,[309] and healthy but nervous horses. One commonly detects an elevated resting heart rate with late pregnancy, fever, pain, hypovolemia, severe anemia, infection, or shock. Persistent, otherwise unexplained tachycardia is also typical of heart failure wherein the standing heart rate usually exceeds 60 beats/min.

One must differentiate physiologic arrhythmias associated with high resting vagal tone from pathologic arrhythmias, because relaxed and fit horses have many normal bradyarrhythmias. The most common of these bradyarrhythmias is second-degree atrioventricular block (see Figure 8-3), which is reported in 15% to 18% of normal horses at rest and has been detected in up to 44% of normal horses with 24-hour continuous electrocardiographic monitoring.[172,175,177,310,311] Second-degree atrioventricular block is most common in fit 372
racehorses or in other high-performance animals and disappears with exercise or excitement or after 373
administration of atropine. Sinus arrhythmia also occurs regularly in horses and often is associated with sinus bradycardia and resting heart rates of 24 to 28 beats/min. Sinus arrhythmia may wax and wane with respiration; however, synchronization with ventilation is not a consistent finding in the horse and is more

likely related to changes in baroreceptor tone. Sinoatrial block and sinoatrial arrest occur sporadically in fit horses. Because these physiologic arrhythmias are associated with high vagal tone, the heart rate typically ranges from a low normal value to bradycardia. The clinician can undertake maneuvers that reduce vagal activity and increase sympathetic tone to ensure that the rhythm becomes normal. Successful methods include leading the horse through three or four tight circles, jogging, or lunging. One should recognize, however, that some horses redevelop physiologic atrioventricular block within 10 to 60 seconds following completion of such maneuvers. If the arrhythmia persists after exercise, or if the auscultatory findings suggest another arrhythmia (see Table 8-1), the clinician should obtain an ECG.

Figure 8-9 Phonocardiograms *(PCG)*. **A,** The four normal heart sounds (S1 through S4) are evident in this recording. (The numbers *1* through *4* indicate the various components of the first heart sound; *ECG,* electrocardiogram.) **B,** Systolic murmur recorded from a yearling with a ventricular septal defect. (P waves on the electrocardiogram are negative in this tracing.) **C,** Decrescendo, musical, holodiastolic murmur recorded from a horse with aortic regurgitation. The murmur begins in early diastole (after the T wave) and ends at the QRS complex. (A recording courtesy of D. Smetzer, R.L. Hamlin, and C.R. Smith.)



The arrhythmias associated with heart disease occur most often with normal to increased heart rates, with the exception of advanced second-degree and complete (third-degree) atrioventricular block (see Table 8-1).[*] Atrial premature beats are characterized by a regular cardiac rhythm, which is interrupted suddenly by an earlier than normal beat(s). Because the sinus node resets during an atrial premature beat, the following pause is usually incomplete or less than compensatory. The atrioventricular node also can block premature atrial

beats, leading to a sudden pause in the rhythm. A ventricular premature beat is followed by a fully compensatory pause unless the extrasystole is interpolated between two normal sinus beats. Pulse deficits (more first sounds than arterial pulses) are expected with premature beats.

Atrial fibrillation is characterized by an irregular rhythm, with beats occurring sooner than expected, and pauses in the rhythm with no consistent underlying diastolic interval. The atrial contraction and hence S_4 are 373

absent in atrial fibrillation. Infrequently, atrial fibrillation develops with recurring periodicity of cycle lengths that can cause difficulty in distinguishing this abnormal rhythm from second-degree atrioventricular block. [169,171,179] Another diagnostic pitfall can be the presence of a split S_1, because the clinician may mistake this for a closely timed S_4-S_1 complex. 374

Atrial tachycardia, however, may develop regular atrioventricular conduction and rapid regular rhythm. At rest, however, horses with atrial tachycardia generally have irregular rhythms because of frequent second-degree atrioventricular block but with heart rates that are slightly higher than normal. Auscultation of the horse in a quiet area may reveal isolated audible S_4 sounds associated with the second-degree atrioventricular block. Junctional and ventricular tachycardias usually are manifested by a rapid regular rhythm, unless the arrhythmia is multiform, in which case the heart may sound irregular, such as in atrial fibrillation. Because of associated atrioventricular dissociation or abnormal ventricular activation, the heart sounds may be split or variable in intensity.

* References 2, 3, 33, 103, 299, 312–314.

8.3.3.4 Cardiac Murmurs

Cardiac murmurs are prolonged audible vibrations developing in a usually quiet portion of the cardiac cycle. In general, murmurs are manifestations of normal (functional) or abnormal (pathologic) blood flow in the heart and blood vessels. Although many heart murmurs are functional (physiologic and innocent), other murmurs provide evidence of heart disease and may require further investigation. Most *functional* cardiac murmurs are caused by vibrations that attend the *ejection* of blood from the heart during systole or the *rapid filling* of the ventricles during early diastole.[*] Causes of abnormal murmurs include incompetent cardiac valves, septal defects, vascular lesions, and (rarely) valvular stenosis (see Tables 8-2 and 8-3). The continuous murmur of patent ductus arteriosus is considered a normal finding in full-term foals for up to 3 days after parturition but occasionally persists for almost a week after foaling.[233,298,303,318]

One of the challenges of physical diagnosis is determination of the cause and clinical significance of a cardiac murmur. With knowledge and experience the clinician can determine accurately the *likelihood* that a murmur is physiologic or pathologic and can select animals requiring additional studies efficiently. The clinician should describe the *timing, duration, quality or pitch, grade, point of maximal murmur intensity,* and *radiation* of a murmur (see Table 8-2), as well as the effect of changing heart rate on the sounds. One determines the timing relative to the heart sounds and designates murmurs as systolic, diastolic, or continuous. When the overall timing is not obvious, the clinician can palpate the apical impulse, which occurs in early systole, or the systolic pulse in the brachial or median artery to identify the timing of the murmur. Skilled auscultators subdivide the timing of murmurs into proto-, meso-, or telesystole or proto-, meso-, and telediastole (early, middle, or late, respectively) because the timing and duration of the murmur often correlate with specific flow disturbances. Shorter murmurs, especially those occurring in early systole or protodiastole, are more likely to be functional, although this is not always the case. Experience also teaches that loud murmurs are more likely to be associated with cardiac pathology, but again exceptions occur. The pitch or quality of the murmur

provides additional insight. For example, mixed frequency (harsh or blowing) murmurs are typical of cardiac disease. A brief vibratory or musical murmur is typically functional; however, a prolonged musical murmur suggests a valvular lesion. Applying the characteristics of murmurs to the clinical setting requires study, an organized scheme, and practice. When one believes a murmur to represent an underlying cardiac pathologic condition, further investigation is necessary. The overall significance of the hemodynamic abnormality should include consideration of the work history, general physical examination, presence or absence of heart enlargement or cardiac failure, and results of ancillary tests such as echocardiography, electrocardiography, and exercise performance (or testing).

Functional, innocent, or physiologic heart murmurs are common and are not attributed to pathologic conditions of the heart. Such murmurs may be related to the large size and tremendous inflow and outflow volumes of the equine heart.[315] Physiologic causes of functional murmurs include fever, high sympathetic activity (e.g., colic, exercise, and pain), moderate to severe anemia, and peripheral vasodilation. These murmurs are typically soft (grades 1 to 3 out of 6), localized, and short; they crescendo-decrescendo in time; and they are labile. Increasing heart rate usually increases the intensity and duration of a functional murmur, although in some horses the murmur becomes less intense. Functional murmurs are not holosystolic or holodiastolic; therefore the heart sounds should still be evident.

The most common functional murmur is the systolic ejection murmur heard over the aortic and pulmonic valves and into their respective arteries at the left cardiac base (see Figure 8-7).[4] The functional ejection murmur is generated by flow into the great vessels and by definition must start after S_1 and end before S_2. Nonetheless, in some horses the functional ejection murmur can achieve substantial intensity (rarely, a grade 4 or 5 out of 6), and may seem holosystolic at higher heart rates. At times a remarkable change in functional 374
murmur intensity occurs from one day to another; for example, the varying murmurs identified in some horses 375
with emergent colic. Because a loud functional murmur can radiate caudally, one may confuse the murmur with MR. In terms of diagnostic considerations, the clinician should not mistake a functional ejection murmur for that of aortic or pulmonic stenosis. Pulmonic stenosis is rare in the horse; aortic stenosis is rarer. Accordingly, one rarely entertains these valvular malformations in the differential diagnosis. The subpulmonic (subarterial) VSD may be loudest over the great vessels, but such murmurs are typically loud and usually prompt echocardiographic investigation.

Functional diastolic murmurs are also common, especially in young horses and in the Thoroughbred breed. These murmurs are generally soft and detectable over the LV or RV inlets, from the dorsal atrium to the ventricular apex. The functional protodiastolic murmur is an early diastolic murmur detected between S_2 and S_3. The murmur may be musical, vibratory, or squeaky and typically accentuates with increased heart rate.[4] The murmur most reasonably represents the rapid early filling of the ventricles. The presystolic functional murmur is audible between S_4 and S_1, may sound like a rub or long heart sound following the atrial contraction, and can be confused with an early systolic murmur or a friction rub.

The most commonly detected murmurs associated with structural heart disease are those generated by TR, MR, aortic regurgitation, and VSD. Tables 8-2 and 8-3 indicate typical causes and auscultatory features of these murmurs, which are described in detail later in this chapter. Detection of a cardiac murmur indicative of valvular disease or a congenital cardiac defect necessitates additional studies to assess the situation objectively. Certainly, the clinician should consider the history, because the horse with excellent performance and good exercise tolerance is unlikely to have serious heart disease. Auscultation, however, is insufficient to distinguish trivial from significant heart disease in the poorly performing horse or in a horse with a cardiac arrhythmia. Echocardiography is helpful in these cases to quantify heart size and objectively assess ventricular

function. One can identify or discount underlying lesions such as a valve vegetation, ruptured valve chorda tendinea, or dilated cardiomyopathy. Doppler echocardiography can document abnormal blood flow and can pinpoint the cause of a murmur. One cannot obtain this type of objective information as easily or as effectively by other noninvasive methods. Other methods of assessing the significance of heart murmurs are thoracic radiography, exercise testing, radionuclide angiography, and cardiac catheterization. Cardiac catheterization has been largely replaced by Doppler echocardiography.

Figure 8-10 Peracute pulmonary edema in a horse with a ruptured chorda tendinea. The photo was taken immediately after euthanasia. An important note is that tracheal froth is often a postmortem artifact, particularly when observed hours after death.

* References 18, 19, 32, 36, 124, 224, 289, 295, 298, 306, 315–317.

8.3.3.5

Pulmonary Auscultation

Auscultation of the lungs should reveal normal breath sounds with the horse at rest, while ventilating into a rebreathing bag, and after exercise. Decreased or absent airway sounds or large airway sounds in the ventral portions of the thorax indicate a pleural effusion, a common finding in biventricular or right-sided heart failure. Moist or bubbling (fluid) sounds or crackles (i.e., rales) are not auscultated commonly in the lungs of horses with pulmonary edema and left-sided heart failure. Instead, tachypnea associated with harsh bronchovesicular breath sounds is usually audible, because horses with chronic left-sided CHF seem to develop more interstitial than alveolar pulmonary edema. When alveolar edema does develop, respiratory distress may be severe, and free fluid may be auscultated in the trachea. On rare occasions, primarily with peracute left-sided heart failure, froth is visible at the nares and the horse will cough and expel large quantities

of pulmonary fluid (Figure 8-10). Such horses demonstrate severe respiratory distress (marked tachypnea and dyspnea), anxiety, and agitation.

8.3.4 EXAMINATION OF THE PERIPHERAL VASCULATURE

An evaluation of the peripheral vasculature is part of a cardiovascular workup and should include examination of the arteries and veins in the head, forelimbs, and hindlimbs. When possible, one also should measure arterial blood pressure. The genesis of the arterial and venous pulse has been described previously. The heart sounds should correlate with the jugular and arterial pulses. One can identify heart rate and rhythm, as well as altered 375
hemodynamic states, by palpating the facial artery pulse, which can be described as normal, hypokinetic (weak), 376
hyperkinetic, or variable. Irregularity often indicates a cardiac arrhythmia. Abnormalities of arterial pressure and perfusion may cause changes in mucous membrane color and capillary refill time. Refill time is prolonged with hypotension or peripheral vasoconstriction; conversely, it may be shortened with vasodilation.

Normal quality *arterial pulses* should be palpable in the facial, median, carotid, great metatarsal, coccygeal, and digital arteries. Thready or hypokinetic arterial pulses are detectable in CHF and in diseases associated with cardiovascular collapse, such as endotoxemia, or after profuse hemorrhage. Thready arterial pulses also may be detectable only in the hindlimbs with aortic-iliac thrombosis. Bounding, hyperkinetic arterial pulses are palpable with clinically significant aortic regurgitation, patent ductus arteriosus, and aortic to pulmonary or aortic to right-sided heart fistulae. Great variation in the intensity of the pulse usually occurs with arrhythmias, particularly atrial fibrillation and multiform ventricular tachycardia. A pulse deficit (S_1 without palpable pulse) occurs when developed LV pressure does not exceed aortic pressure. Deficits are likely to be palpable in association with arrhythmias, particularly premature beats or following short diastolic periods of tachyarrhythmias.

Palpation of the arterial pulse is a crude indication of the *arterial blood pressure,* representing the difference between peak systolic and diastolic pressures, the rate of rise of arterial pressure, and the physical characteristics of the artery and surrounding tissues. One can measure arterial blood pressure directly by arterial puncture or cannulation or indirectly using various auscultatory, Doppler, or oscillometric techniques.[68,221,237,319–344] With percutaneous placement of an arterial catheter in the facial or transverse tibial artery, frequently one can monitor pressure invasively in critically ill or anesthetized horses. Indirect methods have been used successfully to monitor pressure in the coccygeal artery; however, these methods are less sensitive in hypotensive animals and may lag in response during rapid changes in blood pressure.[322,324,326] One must direct attention to placement and diameter of the occluding cuff when using indirect methods,[336] and the optimal width of the cuff (bladder) is between approximately one quarter to one fifth of the circumference of the tail when measuring pressure in the middle coccygeal artery.[330,335] The arterial pulse wave itself varies, depending on the site of measurement, and the distal arterial systolic pressure may be higher and the diastolic pressure lower than the corresponding aortic pressures.

Arterial blood pressure monitoring includes determination of systolic, diastolic, and mean pressures. Arterial pressure is determined by the interplay between stroke volume, heart rate, and vascular resistance (see Box 8-3; Figure 8-3). Significant increases in CO, during exercise for example, lead to significant increases in arterial blood pressure with systolic pressures exceeding 200 mm Hg.[214,341,345]

Normal reported values for indirect arterial pressure are 111.8 mm Hg systolic (ranging from 79 to 145 mm Hg) and 67.7 diastolic (ranging from 49 to 106 mm Hg).[*] The values obtained at the coccygeal artery are slightly lower than central arterial pressures, but this is not a problem when monitoring an individual horse. Blood pressure is lowest in neonates and rises during the first month of life to the normal adult range.[221,324] Some

variation exists among breeds. Draft breeds tend to have lower pressures than do racehorses, and Standardbreds have lower pressures than do Thoroughbreds.[340] Arterial pressures fluctuate slightly with ventilation and significantly with positive pressure ventilation or cyclic changes in heart rate. Posture imposes a significant influence on arterial pressure, because raising the head from the feeding position necessitates a higher aortic pressure to maintain cerebral perfusion pressure. Obviously, lowering of the head minimizes the hydrostatic pressure imposed during raising of the head. Mean arterial pressure measured in the middle coccygeal artery can vary approximately 20 mm Hg with the changing head position.[337]

The left ventricle generates the systolic arterial pressure, which consequently is affected by the interplay between the stroke volume, aortic compliance, and previous diastolic blood pressure. Arterial *pulse pressure,* the difference between systolic and diastolic values, depends greatly on stroke volume and the peripheral arteriolar resistance that determines the runoff of diastolic pressure. Pulse pressure determines the intensity of the palpable peripheral arterial pulse. Ventricular failure reduces pulse pressure, whereas abnormal diastolic runoff (aortic insufficiency, generalized vasodilation) widens the pulse pressure. Diastolic and mean pressures are better estimates of perfusion pressure; arteriolar vasoconstriction or higher CO increases these variables.

One should examine the cutaneous veins for distensibility, refill, thrombosis, and estimated venous pressure. *Jugular pulsations* are normally observable in the thoracic inlet and up to the ventral one third (10 cm) of the neck. These pulsations reflect right atrial pressure changes (discussed previously) and are visible along the entire length of the jugular vein when the head is lowered below the level of the heart. The carotid arterial pulse may be transmitted through the jugular furrow and mimic an abnormal systolic pulse wave in the jugular vein if the vein 376
is distended. Occluding the jugular vein ventrally can demonstrate if the pulses are actually originating in the 377
right atrium, because light digital pressure over the vein prevents venous pulse transmission, or prominent collapse. One may observe pronounced jugular pulsations occasionally in excited but otherwise normal horses with high sympathetic tone. In some of these cases the venous collapse is the most prominent movement and the pulse is simply normal venous filling. When in doubt, one can verify this finding by simple ultrasound imaging of the ventral portion of the vein. The jugular veins in normal horses are collapsed, but the veins in the limbs and on the torso are visible and somewhat filled. Venous pressure in the jugular vein is normally less than 10 cm of water above the phlebostatic point.

Figure 8-11 Jugular venous distention in a Shire foal with biventricular congestive heart failure caused by congenital heart disease.

One can observe abnormal jugular pulses with arrhythmias causing atrioventricular dissociation, with diseases of the tricuspid valve, and with RV failure. An abnormal jugular pulse is one that extends proximally up the jugular vein for more than 10 cm in systole (with the head of the horse held in a normal position) or that demonstrates retrograde filling from the heart when one occludes the vein dorsally. Doppler studies of the jugular vein can distinguish a prominent pulse caused by retrograde flow from an apparent pulse caused by prominent collapse and normal filling. Generalized venous distention, often accompanied by subcutaneous edema, is characteristic of heart failure (Figure 8-11). Distention of only the veins cranial to the thoracic inlet should indicate a cranial mediastinal mass or abscess with obstruction of the cranial vena cava and not CHF.[94] Prolonged refill of the saphenous vein indicates the possibility of aortic-iliac thrombosis and decreased arterial supply to the affected limb.[197–202,347,348] Distended veins that are firm on palpation, with considerable distention of the more proximal veins, indicate probable venous thrombus.[349]

Figure 8-12 Ascites, ventral edema, and weight loss are evident in this mare with right-sided congestive heart failure. The distended lateral thoracic vein *(arrow)* is evident caudal to the triceps.

* References 322, 325, 326, 330, 331, 333–337, 340, 342, 346.

8.3.5

CONGESTIVE HEART FAILURE

CHF in horses is uncommon; however, one generally can make a diagnosis of this pathophysiologic state during a complete physical examination.[350] Important clinical findings include persistent tachycardia (typically heart

rate higher than 60 beats/min), jugular distention and pulsation, abnormal arterial pulses, fluid retention (ventral subcutaneous edema, pleural effusion, or pulmonary edema), and loss of body condition (Figure 8-12). LV failure following valvular or myocardial heart disease causes pulmonary venous congestion with pulmonary interstitial or, uncommonly, alveolar edema. Tachypnea or coughing associated with lung edema of left-sided CHF frequently is misdiagnosed as pneumonia or chronic obstructive pulmonary disease. One also may detect lethargy, exercise intolerance, collapse (or syncope), anorexia, depression, and weight loss with CHF or cardiac insufficiency. Heart failure is discussed more fully later in this chapter.

8.4 Diagnostic And Laboratory Studies

8.4.1 EXERCISE TESTING

Exercise testing is an increasingly important part of the complete cardiovascular evaluation.[95,288] Although the sensitivity, specificity, and specific endpoints of the exercise test (as it pertains to detection of organic heart disease) are not defined completely, exercise studies may detect subtle cardiovascular disease or unmask a serious problem such as a ventricular tachyarrhythmia. Because most horses function as athletes, one should include some form of exercise testing in the prepurchase cardiovascular examination, unless the animal is too young to perform or is to be used solely for breeding. Even in these situations, evaluation of a foal, weanling, or yearling after a period of free exercise is optimal. Furthermore, one should determine that a stallion has sufficient stamina to perform in the breeding shed. Thus the clinician should evaluate the cardiovascular system at rest and immediately after exercise and under the anticipated form of work required by the prospective owner. Increasingly, standardized treadmill testing is used to detect subtle clinical abnormalities that limit peak performance.[183,283–287,351,352] Treadmill exercise is also important when evaluating the horse with a history of collapse or syncope, because one should evaluate the cardiac response to exercise without risk to a rider.

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Changes in heart rate with exercise have been well studied.[183,283–287,351,352] The normal horse develops sinus tachycardia, shortening of conduction intervals, and significant ST-T alterations associated with exercise. The maximal heart rate achieved depends on the level of exercise performed. At heart rates of less than 100 to 120 beats/min, sinoatrial node discharge is labile and subject to psychologic influences. Once the horse commences exercise, the heart rate should accelerate and then stabilize at a level appropriate for the work being performed. As a general guideline, heart rates of 70 to 120 beats/min are normal at the trot, 120 to 150 beats/min at the canter, 150 to 180 beats/min at a hard gallop, and more than 180 beats/min when galloping. The maximal heart rate for most horses is between 210 and 240 beats/min, although some animals develop higher rates. A linear relationship exists between heart rate and the velocity or work effort of exercise when the heart rate is between 120 and 210 beats/min. Heart rate usually recovers rapidly and falls to less than 100 beats/min within 5 minutes of cessation of exercise. Many factors influence the recovery of heart rate to the resting level, including the humidity, ambient temperature, training, fitness, work performed, psychologic factors, and state of cardiovascular health. After maximal work, such as a race, an hour may pass before the heart rate completely recovers to the resting rate. An inappropriately high heart rate for a given level of exercise may simply denote a healthy but unfit horse; however, this finding also can indicate the presence of cardiovascular, pulmonary, or musculoskeletal disease. Information about the heart rate is particularly useful when a horse has the heart rate monitored routinely during exercise.

One may observe exercise-induced arrhythmias during or after exercise. During the recovery period following exercise, horses have large fluctuations in sympathetic and parasympathetic tone. Arrhythmias are common during this time, even if the heart rhythm was normal at rest and at peak exercise. One most frequently detects significant sinus arrhythmias, second-degree atrioventricular block, supraventricular ectopia, and ventricular

premature beats in the immediate postexercise period. When one detects arrhythmias during a routine postexercise examination, an exercising ECG obtained by radiotelemetry is necessary to determine if the arrhythmia is present during exercise. One may use a heart rate monitor to provide an indication of arrhythmias during exercise; however, the detection of erratic heart blips or beeps does not adequately characterize the arrhythmia. With the exception of vagally mediated arrhythmias, one should consider postexercise arrhythmias suspicious. One should consider frequent or repetitive supraventricular or ventricular premature beats detected after exercise to be abnormal.

Arrhythmias that may be induced during exercise include supraventricular premature complexes, atrial fibrillation, and ventricular extrasystoles. One should include exercise-induced (paroxysmal) atrial fibrillation in the differential diagnosis of horses with poor performance during high-intensity exercise.[39,353,354] Recognition of this arrhythmia may require ambulatory electrocardiography or radiotelemetry ECG recordings during an exercise test. Exercise-induced ventricular arrhythmias are a concern because ventricular rhythm disturbances can lead to poor performance, sudden stopping, or even falling, and have been suspected as one cause of sudden death.[59,61–64] Repetitive ventricular activity (paroxysmal ventricular tachycardia) is particularly worrisome and may indicate preexisting myocardial disease or myocardial ischemia with altered cardiac electric activity. Some horses require a maximal performance to elicit abnormal cardiac rhythms. Performance problems detected only at the peak of exercise necessitate performing a treadmill exercise test to exhaustion in an attempt to investigate whether cardiac arrhythmias are a cause of the poor performance. This is particularly important if one suspects cardiovascular disease from the history or clinical examination.

One may use exercise echocardiography in an attempt to identify myocardial dysfunction.[288,355,356] When examined within 2 to 3 minutes following high-intensity exercise and with the heart rate exceeding 100 beats/min, the normal horse demonstrates a symmetric increase in LV shortening with increase in shortening fraction and cross-sectional shortening area. One can view these changes from the short-axis tomograms and prominent thickening of the septum and LV free wall from long-axis images. Persistent abnormalities in regional myocardial contraction suggest vascular disease with ischemia, preexistent myocardial fibrosis, or localized conduction disturbance. Myocardial isoenzymes may be elevated in some cases.[288] 378

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8.4.2 ELECTROCARDIOGRAPHY

8.4.2.1 Normal Cardiac Cell Electric Activity

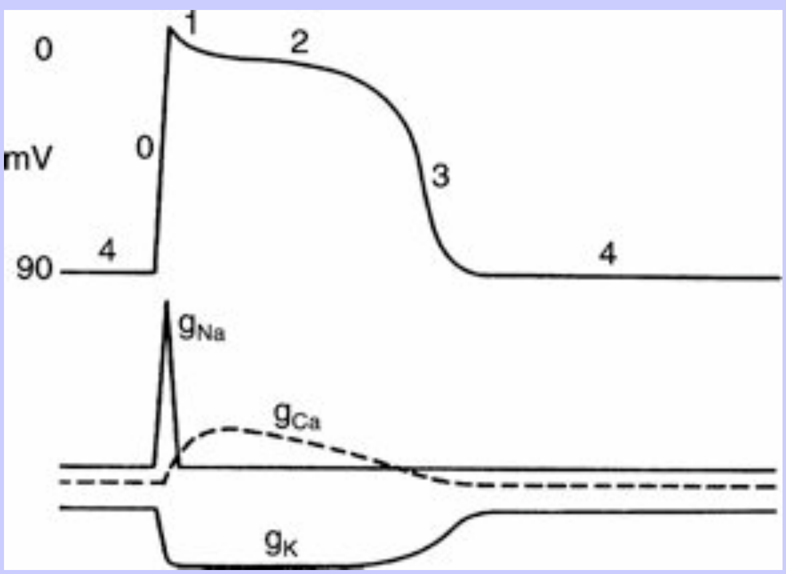
Myocytes in the atria and ventricles are excitable, and some of the specialized cardiac tissues are capable of spontaneous depolarization independent of extrinsic innervation. The processes responsible for generation of electric activity in the heart are caused by ion fluxes across the cell membrane.[70,181] The ECG represents this electric activity. The clinician requires a general understanding of cellular activity, generation, and spread of the cardiac electric impulse; effects of autonomic innervation; and electrocardiographic lead systems to interpret the equine ECG.

The partially selective nature of the cardiac cell membrane and the presence of various cell membrane pumps lead to a situation in which ions are partitioned unequally across the cell membrane.[70] This partitioning results in high potassium and low sodium concentrations intracellularly compared with the extracellular fluid. Other ions including chloride and magnesium, but most notably calcium, are important to cellular electric activity. Calcium also is essential for cardiac contraction. Sudden changes in cardiac cell membrane permeability or conductance to sodium, calcium, potassium, and chloride are responsible for the processes of depolarization,

muscular contraction, and repolarization. In turn, serum electrolyte concentration, acid-base status, autonomic traffic, myocardial perfusion and oxygenation, heart disease, and drugs affect these processes. The basic cellular processes of depolarization, calcium influx, and repolarization form the basis for, respectively, the P and QRS complexes of the ECG, myocardial contraction, and the ST-T wave of the ECG.

The resting membrane potential is determined primarily by the partitioning of potassium ions and proteins across the cell membrane and the relative impermeability of the membrane to sodium. *Depolarization* of atrial and ventricular myocytes and Purkinje fibers is caused by a fast current related to a rapid influx of extracellular sodium into the cell.[72,181,311] Phase 0 of the cardiac cell action potential recording represents depolarization (Figure 8-13). Cardiac tissues depolarized in this manner normally conduct electric impulses at high velocity. In contrast, the cells of the sinoatrial and atrioventricular nodes, as well as ischemic cells, demonstrate less negative diastolic membrane potentials. These cells depend on a slow inward current of depolarization carried mainly by calcium ions. Because of this property, cells in the sinoatrial node, atrioventricular node, and ischemic tissue conduct electric impulses slowly and can be involved in blocks, abnormal automatic mechanisms, or reentrant pathways that promote cardiac arrhythmias. Antiarrhythmic drugs act differently on tissues that are fast versus slow current dependent (see the section Cardiac Arrhythmias).

Figure 8-13 The cardiac action potential. The diagram demonstrates the phases of depolarization and repolarization as well as membrane conductance *(g)* to important ions (see the text for details). (From Berne RM, Levy MN: *Cardiovascular physiology,* St Louis, 1986, CV Mosby.)



The influx of extracellular calcium into the cell during phase 2 of the action potential triggers the release of intracellular calcium from the sarcoplasmic reticulum. Increases in cytosolic calcium cause myocardial contraction. Hypoxia, acidosis, and many drugs can affect myocardial contractility by interfering with calcium entry into cells, with the release of calcium from the sarcoplasmic reticulum, or with the binding of calcium to

contractile filaments.[70,97,181] Conversely, digitalis glycosides, dobutamine, and dopamine increase calcium influx and myocardial contractility.

Cellular *repolarization* is initiated by a decrease in the cell membrane conductance to sodium and calcium coincident with increased conductance to potassium. As intracellular potassium moves out of the cell, along its concentration gradient, phase 3 of the cardiac action potential occurs. Sympathetic stimulation also may activate an inward chloride current that facilitates repolarization at higher heart rates. Hypoxia and abnormalities in serum potassium or calcium alter this process. Hyperkalemia accelerates repolarization and shortens the ST-T wave of the ECG. This shortening occurs because hyperkalemia *increases* membrane permeability to potassium and the high intracellular potassium (exceeding 100 mmol/L) is more than adequate to drive potassium out of the cell (because extracellular potassium rarely exceeds 10 mmol/L).

Spontaneous depolarization or automaticity is a property of select cardiac tissues. Such activity is most prominent in the sinoatrial node but also may occur in cells around the atrioventricular node and in cells of the His-Purkinje network. Normal automaticity is observable during phase 4 of the cardiac action potential. 379
Spontaneous *pacemaker activity* is generated in normal pacemaker tissues by the background inward sodium 380
current and a time-related decrease in membrane permeability to potassium ion efflux.[181] Once membrane threshold is reached, short- and long-lasting calcium channels open, allowing the cell to depolarize via a slow inward current. The spontaneous depolarization process is modified greatly by vagal and sympathetic efferent traffic. With sympathetic stimulation and at higher heart rates additional inward currents, such as funny current, become activated. Depression of normal sinoatrial automaticity or increased activity in other tissues in the atria, Purkinje system, or in cells around the atrioventricular node may lead to an abnormal or ectopic cardiac rhythm. When the principal abnormality resides in depression of sinoatrial node function or blockage of the impulse in the atrioventricular node, the slow discharge of a subsidiary pacemaker is termed an escape mechanism. The normal purpose of these latent cardiac pacemakers is to rescue the heart from extreme bradycardia or asystole. However, ectopic pacemakers often are enhanced by drugs, inflammation, sympathetic activity, electrolyte disturbances, or ischemia, leading to *ectopic* rhythms that manifest as premature complexes or tachycardias. Other electrophysiologic mechanisms, including reentry and myocardial fibrillation, account for the development of other arrhythmias.

8.4.2.2 Genesis of the Electrocardiogram

The sinoatrial node is located near the right auricle; therefore initial cardiac muscle depolarization crosses the right atrium (see Figure 8-3). Activation waves spread through the atrial myocardial cells to the left atrium and also in the direction of the atrioventricular node. Specialized atrial muscle cells composed of internodal pathways and Bachman's bundle facilitate transmission of current across the atria.[8,357] These specialized pathways, as well as the sinoatrial node, differ functionally from normal atrial muscle, are resistant to high serum potassium concentrations, and still are activated during hyperkalemia.

Conduction continues across the atrioventricular tissues by first entering the atrioventricular node. Current transmission across the atrioventricular nodal cells is particularly slow because depolarization depends on slow current and is subject to physiologic blockade caused by varying vagal efferent activity.[170] Conduction proceeds at a greater velocity through the bundle of His and bundle branches. Propagation of the impulse through the ventricles is enhanced by a rapidly conducting Purkinje system that penetrates completely through the ventricular myocardium.[6,7]

Autonomic traffic affects these processes dramatically. Parasympathetic activity depresses sinoatrial nodal activity, enhances intraatrial conduction by shortening atrial action potential duration, and slows atrioventricular nodal conduction. Conversely, sympathetic efferent traffic increases heart rate and shortens atrioventricular conduction time.[181] Sympathetic activity also increases cellular excitability, predisposes to some cardiac arrhythmias, and increases myocardial oxygen consumption by augmenting the heart rate, force of myocardial contraction, and myocardial wall tension. Parasympathetic efferent traffic dominates in the resting, standing horse and frequently fluctuates with changes in blood pressure (see Figure 8-3). The pronounced sinus arrhythmia, sinoatrial block, and second-degree atrioventricular block so often encountered in the normal horse is caused by changing vagal tone and serves to regulate arterial blood pressure at rest.[68]

Figure 8-14 A normal equine base-apex lead electrocardiogram (25 mm/sec; 1 cm equals 1 mV). The waveforms are indicated. The QRS complex often lacks an R wave in this lead (QS complex). The T wave is labile and may be negative, biphasic, or positive. Increases in heart rate or sympathetic tone usually lead to positive T waves in this lead.

The ECG graphs the time-voltage activity of the heart. The average electric potential generated by the heart muscle is recorded throughout the different phases of the cardiac cycle with time displayed along the x-axis and electric potential inscribed vertically (Figure 8-14). The normal waveforms are the P wave (atrial depolarization), P-R interval (mainly caused by atrioventricular nodal conduction), QRS complex (ventricular depolarization), and ST-T wave (ventricular repolarization). A prominent atrial repolarization wave (Ta wave) often is noted in the P-R segment of the equine ECG, particularly at faster heart rates. The QT interval represents total electric activation-repolarization time.

8.4.2.3 Clinical Electrocardiography

The clinical application of electrocardiography to the horse has been studied extensively.[*] The principles of recording and interpreting the equine ECG are similar to those used for human beings, dogs, and other species.[103,363] The lead systems used are identical, although some modified leads, such as the base to apex lead, have 380
been found to be useful in monitoring the cardiac rhythm of the horse. A number of semiorthogonal lead 381
systems have been evaluated experimentally but rarely are used in clinical practice. The modified Einthoven's lead system consisting of leads frontal planes I, II, III, aV_R, aV_L, and aV_F and lead V_{10} is applicable for ECG studies of horses (Box 8-7). The base-apex lead or chest leads most often are used for rhythm analysis.

The value of continuous, ambulatory (Holter) ECG monitoring is obvious for identification of infrequent rhythm disturbances, quantifying the severity of an arrhythmia, or objectively evaluating drug therapy (Figure 8-15). In one study, many horses that were historically and clinically without evidence of cardiac disease were observed to have supraventricular or (less often) ventricular arrhythmias.[310] The clinician can perform Holter monitoring with contact electrodes using a bipolar lead system similar to that used for the equine heart rate monitors. One usually places electrodes over the left saddle area and sternum and keeps them moist and in contact with the horse using a surcingle and padding material. This type of continuous 24-hour rhythm monitor can be useful to evaluate the horse with a history of syncope or an arrhythmia but in which one cannot induce an arrhythmia during resting, exercise, and postexercise ECG examinations.

8.4.2.3.1

BOX 8-7 ELECTROCARDIOGRAPHIC LEADS

8.4.2.3.1.1

Frontal Plane Leads

Bipolar leads

- Lead I = Left foreleg [+] – right foreleg [–]
- Lead II = Left hindleg [+] – right foreleg [–]
- Lead III = Left hindleg [+] – left foreleg [–]

Unipolar augmented limb leads

- Lead aV_R = Right foreleg [+] – left foreleg and left hindleg [–]
- Lead aV_L = Left foreleg [+] – right foreleg and left hindleg [–]
- Lead aV_F = Left hindleg [+] – right foreleg and left hindleg [–]

8.4.2.3.1.2

Precordial Leads

Base-apex monitor lead

- Positive electrode over the left apex compared with the negative electrode over the right jugular furrow[*]

Precordial V leads[†]

- Positive electrode over the selected precordial site; the lead is named based on the location of the exploring (V or C) electrode

Lead V_3: near the left apex

Lead V_{10}: over the dorsal spinous processes (interscapular)

* The most common method of obtaining the base-apex lead is to place the left foreleg electrode over the left apex, the right foreleg electrode over the jugular furrow (or at the top of the right scapular spine), and select lead I on the electrocardiograph. This lead is an excellent choice to monitor the cardiac rhythm.

† Precordial leads other than V_{10} (over the dorsal spine) are not commonly used.

Telemetry-based ECG recordings commonly are used in exercise testing (see the previous section, Exercise Testing) and in monitoring of critical patients in hospital settings. The lead systems used are often similar to those used for Holter ECG recordings; however, the degree of artifact that occurs during exercise is considerable. Those interested in consistent recordings must experiment with various electrode positions and lead systems.

The orientation, amplitude, and duration of the ECG waveforms depend on many factors, including the age of the horse,[303,386] the lead examined, the size of the cardiac chambers, the degree of training, and even the phase of ventilation.[367,368] The principal use of the ECG is to diagnose the heart rhythm, because the ECG is less sensitive for detecting cardiomegaly, especially in horses with mild to moderate heart enlargement. A 381
normal ECG does not exclude heart disease; moreover, the ECG is not a test of myocardial function. One 382
should undertake a systematic approach to ECG analysis and compare the results with reference values (Table 8-4).

Figure 8-15 Examples of extrasystoles. Atrial *(P' at top)* and ventricular *(bottom)* extrasystoles in a mare with heart failure, sinus tachycardia, and premature beats. The recordings are from a 24-hour tape-recorded (Holter) electrocardiogram. Two transthoracic leads recorded simultaneously. The waveforms are indicated. The dark circles indicate the premature complexes. The P-R interval of the atrial premature complex is longer because of physiologic refractoriness in the atrioventricular node. The ventricular ectopic follows the sinus P wave (late diastolic) but discharges the ventricle before the sinus impulse can cause a normal QRS complex. The T wave of the ventricular extrasystole is abnormal (secondary T wave change). Paper speed is 25 mm/sec.

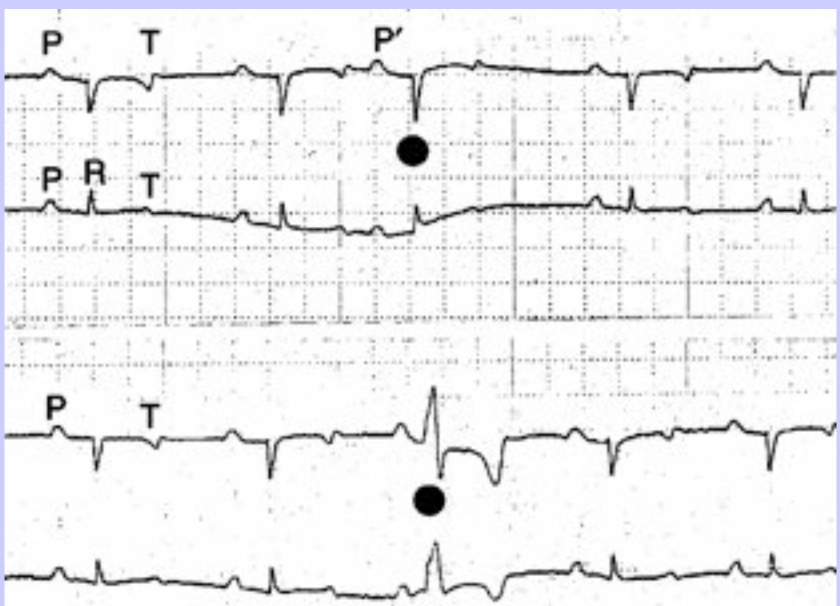


TABLE 8-4 Electrocardiography: Approximate Limits for Normal Frontal Plane Leads

VARIABLE	MAXIMAL VALUE	COMMENTS
Heart rate	40 beats/min	Resting mature horses; higher in foals, colts, and ponies
Cardiac rhythm	—	Sinus mechanisms, sinus arrhythmia, vagal-induced first- and second-degree atrioventricular block*
P wave	0.16 seconds	Atrial repolarization (Ta wave) may make measurement difficult.
P-R interval	0.48 seconds	Varies; longer when vagal tone is high.
QRS complex	0.14 seconds	May vary with the size of the heart (often longer in the base-apex lead).
QT interval	0.575 seconds	Inversely related to the heart rate
R wave lead I	0.85 mV	
R wave leads 2, aV_F	2.3 mV	
Frontal axis	0–100 degrees	Axis varies in foals and yearlings.

Technical aspects: paper speed, calibration, and lead(s)
Artifacts: electric, motion, twitching, and muscle tremor
Heart rate per minute: atrial and ventricular
Cardiac rhythm: atrial, atrioventricular conduction sequence, and ventricular conduction
Arrhythmias
Site or chamber of abnormal impulse formation, myocardial fibrillation, or conduction disturbance
Rate of abnormal impulse formation
Conduction of abnormal impulses
Patterns or repeating cycles
P wave: morphology, duration, amplitude, and variation
P-R interval: duration, variation, and conduction block (of P wave)
QRS: morphology, duration, and amplitude
Frontal plane mean electric axis
ST segment: depression or elevation
T wave: changes in morphology or size; QT interval
Miscellaneous: electrical alternans, synchronous diaphragmatic contraction

* When evaluating the electrocardiographic rhythm, one should consider the following points:

Atrial depolarization generates the P wave. Normal activation proceeds from right to left and craniad to caudad, leading to positive P waves in left-right lead I and also in craniocaudal leads II and aV_F.[8,11] The normal P wave is notched or bifid; however, one may encounter single-peaked, diphasic, and polyphasic P waves in normal horses. A negative/positive P wave often is recorded if the focus of pacemaker activity shifts to the caudal right atrium near the coronary sinus (Figure 8-16). The initial peak of the common bifid P wave reportedly is caused by depolarization of the middle and caudal one third of the right atrium.[6] The second peak represents activation of the atrial septum and the medial surface of the left atrium. The P wave peaks can be subdivided with P_1 reported to be as high as 0.25 mV (mean of 0.14) and the second peak, P_2, reported to be as high as 0.5 mV (mean of 0.28).[363] Subtle differences exist even among breeds of horses. The P wave 382
morphology can change cyclically with waxing and waning of vagal tone during sinus arrhythmia. During 383
tachycardia, the P wave shortens, becomes more peaked, and is followed by a prominent atrial repolarization (Ta) wave that deviates the PQ segment downward. Such features make the diagnosis of atrial enlargement by electrocardiography difficult.

Figure 8-16 P waves of the horse are demonstrated. On the left is a normal, bifid P wave morphology with two distinct peaks (designated as *P1* and *P2*) and also a physiologic second-degree atrioventricular block *(arrow)*. The center panel demonstrates a negative/positive P wave of coronary sinus origin. This is a normal variation. The right panel shows increased amplitude P waves recorded in a horse after conversion from atrial fibrillation. The second peak is particularly large and may indicate atrial enlargement; however, such voltage criteria correlate poorly with cardiomegaly in horses. Echocardiography is a more accurate method for evaluating atrial size. Lead 2 paper speed is 25 mm/sec.

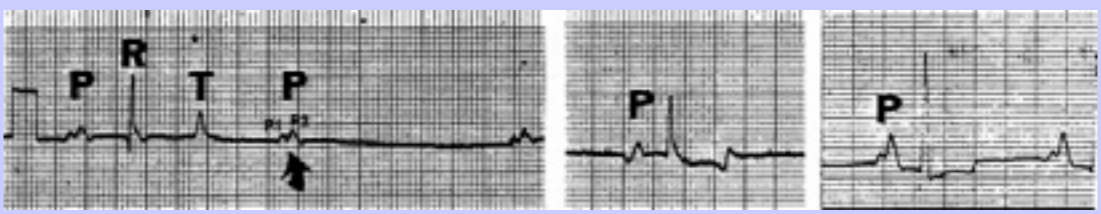
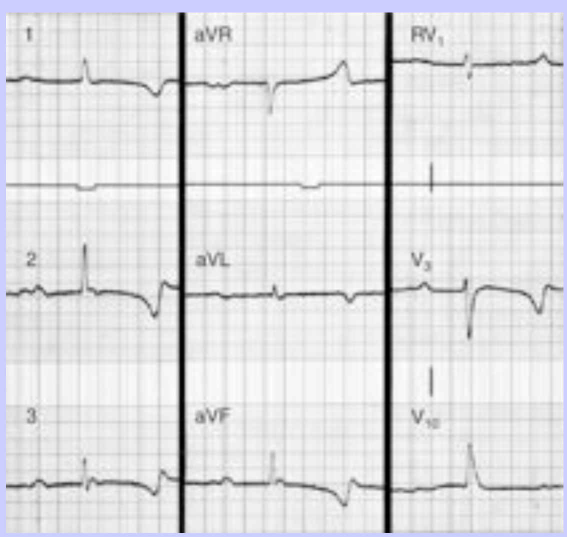


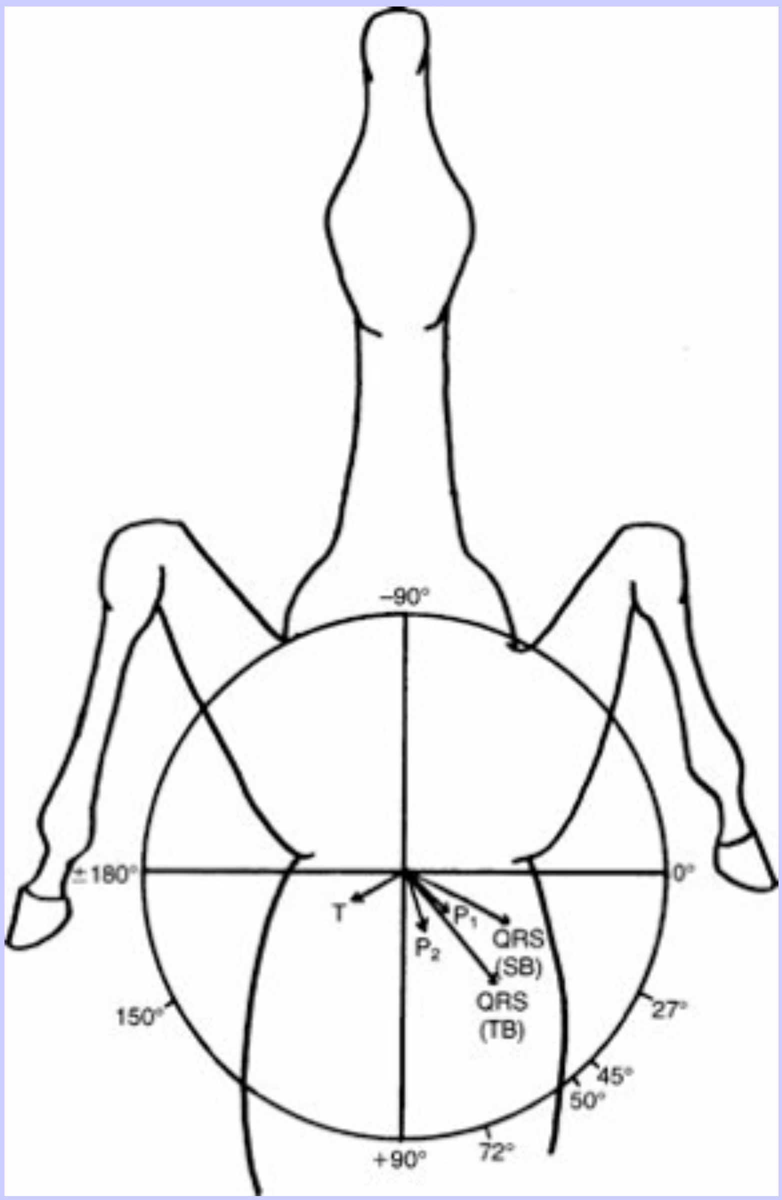
Figure 8-17 Multiple lead recordings in a normal horse. The varying configuration of the P-QRS-T complexes across the leads is notable.



One estimates the time required for conduction across the atrioventricular node and His-Purkinje system by measuring the P-R interval. Because physiologic atrioventricular block is so common, significant variation exists in the P-R interval even within the same horse; thus normal maximal values for the P-R interval are difficult to state. Values that persistently exceed 0.5 seconds are probably abnormal. Variation in the P-R interval usually is not related to changes in ventilation[42] but often is correlated with changes in blood pressure and baroreceptor activation.[68]

The morphology of the QRS complex varies. The complete penetration of the conduction system into the free walls of the ventricle causes these chambers to be activated simultaneously with a burst of depolarization, which cancels much of the divergent electromotive forces.[6,7] Consequently, the normal electric axis may vary and the amplitude of the QRS complex can be small in the frontal plane leads. A substantial dorsally oriented vector causes a prominent positive terminal deflection in lead V_{10}, whereas the (negative electrode) right base to (positive electrode) left apex lead exhibits a prominent S-wave (Figure 8-17). The normal slur in the ST segment makes determination of QRS duration difficult in many horses.[35,393] The mean amplitude for the R wave in lead II for normal racehorses is about 0.8 to 1.1 mV.[*] Clinical experience suggests that R wave amplitudes exceeding 2.2 mV in lead II or 1.7 mV in lead I are often abnormal. But occasionally the R wave amplitude in a normal horse exceeds even these limits.

Figure 8-18 Diagram of the normal frontal plane lead axis. The predominant vectors for the QRS complex in Standardbred *(SB)* and Thoroughbred *(TB)* horses are indicated. The orientation of the two components of the normal P wave (*P1* and *P2*; see Figure 8-16) differ slightly. (From Fregin GF: The cardiovascular system. In Mansmann RA, McAllister ES, Pratt PW, editors: *Equine medicine and surgery,* Santa Barbara, Calif, 1982, American Veterinary Publications.)



One can inspect the frontal plane leads to estimate the mean electric axis of depolarization, the average wave of depolarization (Figure 8-18). This determination usually is reported by direction using a quadrant orientation (right or left; cranial or caudad) or is stated in degrees (lead I is 0 degrees; lead II is 60 degrees; lead aV_F is 90 degrees; lead III is 120 degrees; lead aV_R is minus 150 degrees; and lead aV_L is minus 30 degrees). One can estimate the general direction of the QRS axis quickly by surveying the height of the R waves in the frontal leads and selecting the lead with the greatest net-positive QRS complex. The axis in foals and yearlings varies and frequently is oriented craniad, whereas the frontal axis in most mature horses is 383 directed left-caudad. Abnormal axis deviations have been observed with cardiomegaly, cor pulmonale, 384 conduction disturbances, and electrolyte imbalance.[389]

Following the ventricular activation, the electrocardiograph records repolarization of the ventricles. This period is measured from the end of the QRS complex (the J point) and extends to the end of the T wave.[35,362,394] The T wave vector most often is directed toward the right caudal quadrant, resulting in a positive T wave in lead III and a negative or isoelectric T wave in lead I in resting horses.[363] Although some clinical surveys have suggested that abnormalities of the ST-T indicate cardiac dysfunction in performance animals, significant deviation of the ST segment and increased amplitude of the T wave are anticipated even in normal horses during exercise or even with excitement-induced tachycardia. Simply no unanimity of opinion exists regarding T waves in horses, nor does compelling evidence that T-wave abnormalities can be interpreted consistently. Progressive J point or ST segment deviation in the horse with hypovolemia or shock may indicate myocardial ischemia, whereas enlargement of the T wave may develop with myocardial hypoxia or hyperkalemia.

Diagnosis and management of cardiac arrhythmias are discussed later in this chapter.

* References 1, 3, 6–8, 11, 30, 35, 40, 43, 44, 69, 72, 103, 166, 172, 173, 221, 292, 299, 311, 358–392.

* References 1, 3, 103, 311, 363, 364, 389.

8.4.3 THORACIC RADIOGRAPHY

The use of thoracic radiography in the evaluation of the equine heart has severe limitations because of the large size of the mature horse and the ability to obtain only a standing lateral thoracic radiograph in all except the smallest neonatal foals.[105] Although one can obtain recumbent lateral, and on occasion ventrodorsal or dorsoventral, projections on small neonatal foals, the stress of restraint or requirement for heavy sedation can contraindicate such positioning. Thoracic radiographs may be useful to identify areas of pulmonary or pleural disease and assist in the differential diagnosis of respiratory problems.

One may detect gross changes in cardiac size and shape in a lateral thoracic radiograph of a foal or horse with significant cardiomegaly.[105,297,395–397] A normal thoracic radiograph does not indicate necessarily that the heart is normal in size. Mild to moderate increases in the size of the cardiac chambers may go undetected, particularly in adult horses. Generalized enlargement of the cardiac silhouette occurs in cases of significant pericardial effusion or with CHF (Figure 8-19). One may detect dorsal displacement of the trachea in some horses with left atrial (LA) and LV enlargement. In some horses with LA enlargement, the caudodorsal border of the cardiac silhouette bulges caudally. One may detect increased contact between the ventral border of the heart and the sternum with RV enlargement, but this is usually difficult to appreciate. A 50% decrease in the spinotracheal angle (the angle between the dorsal border of the trachea and the ventral border of the adjacent thoracic vertebrae) was demonstrated in young horses with cardiomegaly caused by congenital cardiac disease.[105]

Evaluation of the pulmonary vasculature and pulmonary parenchyma is also difficult and depends on radiographic technique. Occasionally one can observe enlarged pulmonary vessels and increased radiolucency of the pulmonary parenchyma associated with pulmonary overcirculation (as with septal defects); the opposite is also true in congenital right-to-left shunts. Pulmonary edema causes generalized increased radiopacity, particularly in the hilar regions; the characteristic air bronchograms are more readily identifiable in the hilar regions when alveolar edema is present.

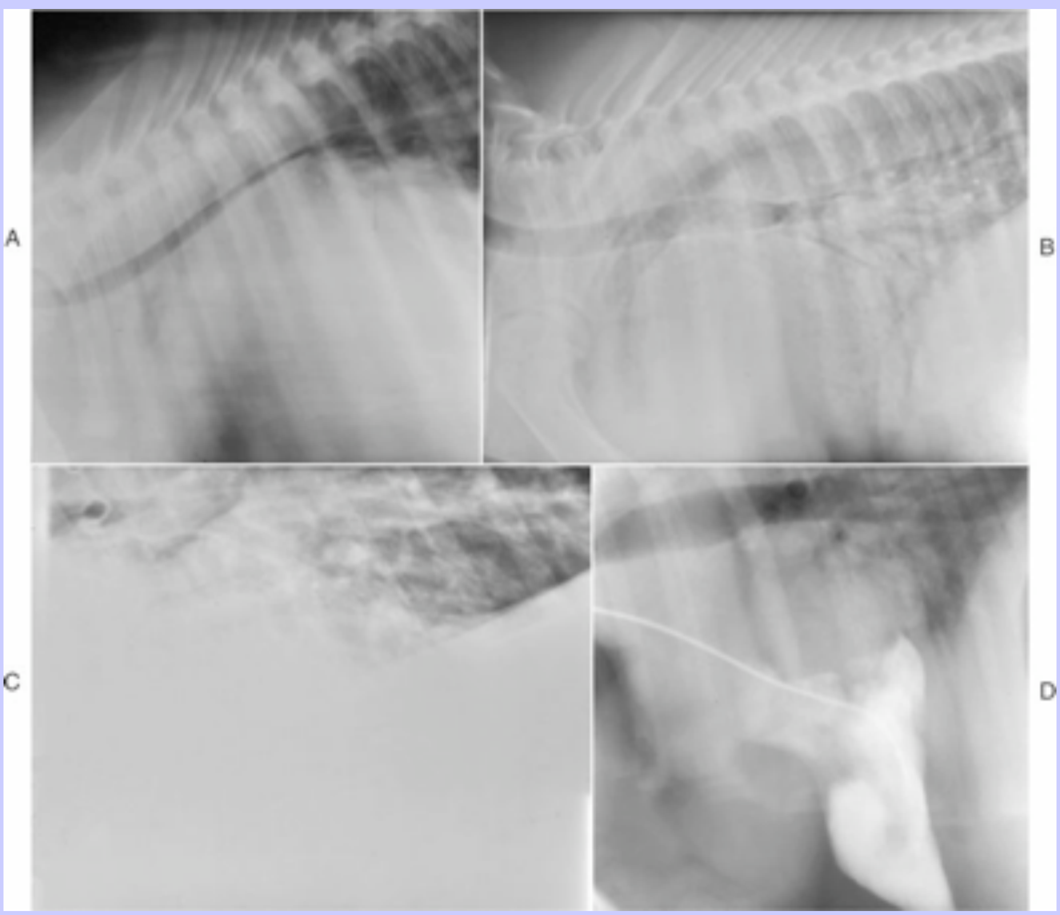
Angiocardiography is only practical in foals less than 250 lb and usually requires general anesthesia, which may be contraindicated in the foal with a severely compromised cardiovascular system. Selective and nonselective positive contrast angiocardiograms have been performed in foals and adult horses (see Figure 8-19),[146,395,398] but these techniques have been replaced almost exclusively by echocardiography and Doppler studies.

Nuclear medicine imaging has been developed in some equine referral institutions and is likely to become more widespread in the future because it provides another method of assessing cardiac function. First pass nuclear angiocardiography is most widely used and permits the visualization of the cardiac chambers during sequential phases of the cardiac cycle.[105] Application of first pass studies may be useful for identifying cardiac shunting, but again this diagnosis is attained more simply by echocardiography.

8.4.4 ECHOCARDIOGRAPHY

Ultrasound is the mechanical vibration of sound waves within a medium at frequencies greater than 20,000 cycles/sec. These inaudible sound waves follow the laws of optics, can be transmitted through tissue, and also can be refracted and reflected. This last property permits the clinician to image the heart and other organs.[399] Echocardiography is the application of ultrasound diagnostics to the heart. When combined with Doppler studies,[400] echocardiography has evolved to become the most important diagnostic study currently available for evaluation of the equine heart.[*] Successful examination of the equine heart requires the use of crystals that vibrate at frequencies between 2 to 5 MHz, a detailed knowledge of ultrasound anatomy, thorough appreciation 384
of equine cardiac diseases, and technical skills with imaging and instrumentation. Ultimately, a thorough 385
echocardiographic examination yields information about the presence and nature of heart lesions, the size of the heart and great vessels, ventricular function, valvular function, blood flow, and the relevant hemodynamics about a cardiac lesion.

Figure 8-19 Thoracic radiography. **A,** Significant cardiomegaly in a Quarter Horse foal with complex congenital heart disease, including multiple ventricular septal defects. **B,** Cardiomegaly and alveolar pulmonary edema in a filly with mitral regurgitation and left-sided congestive heart failure. **C,** Increased pulmonary density and pleural effusion in a Standardbred gelding with atrial fibrillation, atrioventricular valvular regurgitation, myocardial failure, and biventricular congestive heart failure. **D,** Angiocardiogram obtained from an Arabian foal with pulmonary atresia and a large ventricular septal defect (pseudotruncus arteriosus). Contrast medium was injected in the left ventricle, and this medium opacifies a dilated, overriding aorta. Right-to-left shunting across the ventricular septal defect results in dilution of contrast medium.



The essential principle of echocardiography is straightforward: When one directs ultrasound into the chest and toward the heart, some of the sound energy reflects back to the transducer. This occurs because the echo beam encounters tissue interfaces with different acoustic impedance, such as collagen and blood, that reflect the ultrasound. A handheld transducer acts to send and receive ultrasound.[409,449] The echocardiograph computer is capable of determining the spatial orientation and distance of the returning echoes, processing the signals, and displaying an image of the heart. The image usually is displayed, by convention, with that part of the heart 385
closest to the transducer at the top of the display and adjacent to the transducer artifact. Two imaging modalities, 388
the M-mode (motion) and two-dimensional (B-mode or cross-sectional) formats are in general use. If Doppler studies are included, one can detect blood flow relative to the two-dimensional and M-mode images (Box 8-8, Figure 8-20; see also Figure 8-6).[139,266] One can use M-mode, two-dimensional, contrast echo, and Doppler studies to identify pathologic conditions, quantify the heart size and mass,[49,50,403] estimate ventricular function, and identify effects of training.[446,447] One can apply the technique to assess the fetal heart.[450]

8.4.4.1 BOX 8-8 INTERPRETATION OF DOPPLER AND ECHOCARDIOGRAPHIC STUDIES

8.4.4.1.1 Cardiac Rhythm

Arrhythmias alter cardiac motion and velocity signals.

8.4.4.1.2 Identification of Atria, Cardiac Septa, Ventricles, Inlet and Outlet Valves, Great Vessels, Veins

Presence, absence, hypoplasia, or atresia of anticipated structures.

Identification of cardiac mass lesions and thrombi.

Identification of obvious lesions (e.g., septal defect, thick valve).

8.4.4.1.3 Cardiac Mensuration

Ventricular dilation or hypertrophy

Atrial enlargement

Aortic or pulmonary artery dilation or attenuation

Abnormally small chamber or great vessel

8.4.4.1.4 Global Left Ventricular Systolic Function

Subjective analysis of radial shortening (short-axis view).

M-mode fractional shortening (normal usually between 32% and 55%).

Reduced fractional shortening: Rule out dilated cardiomyopathy, chronic valvular heart disease, myocarditis, and myocardial necrosis (e.g., monensin); atrial fibrillation (mild depression); and prolonged ventricular tachycardia. General anesthesia and sedative drugs can decrease fractional shortening.

Increased fractional shortening: Rule out volume overload (especially mitral regurgitation or aortic regurgitation), sympathetic activity, and recent exercise.

8.4.4.1.5
Mitral and Tricuspid Valves

Identification of valve cusps, support apparatus, and motion during cardiac cycle.

Reduced opening or leaflet separation (M-mode E-F slope): Rule out stenosis, vegetation, decreased atrioventricular flow, and aortic regurgitant jet-striking septal mitral leaflet.

Increased echogenicity: Rule out vegetation, degenerative thickening, and dysplasia.

Diastolic mitral fluttering: Rule out aortic insufficiency.

Diastolic tricuspid valve fluttering: Rule out ventricular septal defect and ruptured aortic sinus aneurysm.

Systolic mitral fluttering: Rule out atrioventricular insufficiency.

Chaotic motion: Rule out arrhythmias and ruptured chordae tendineae.

Prolapse into the atrium: Rule out degenerative disease, ruptured chordae tendineae, connective tissue disorder, bacterial endocarditis, and malformation.

Premature (diastolic) closure: Rule out severe semilunar valve insufficiency or long P-R interval.

Delayed (systolic) closure: Rule out LV[*] failure (high end-diastolic pressure).

Increased mitral E point to septal distance: Rule out LV dilation, cardiac failure, and aortic regurgitant jet-striking valve.

Cleft: Rule out endocardial cushion defect.

8.4.4.1.6
Aortic/Pulmonic Valves

Identification of valve leaflets and motion during cardiac cycle.

Narrowing of the LV or RV outflow tracts: Rule out stenosis and hypertrophy.

Diastolic fluttering: Rule out semilunar valve insufficiency.

Systolic fluttering: normal or high-flow state.

Increased echogenicity (focal or diffuse): Rule out bacterial vegetation, degeneration, and congenital lesion.

Prolapse into ventricle: Rule out degenerative (connective tissue) disease, endocarditis, and subaortic septal defect.

Lack of systolic separation: Rule out low cardiac output, arrhythmia, stenosis, and endocarditis.

Premature (midsystolic) closure: Rule out outflow tract obstruction and ventricular septal defect.

Systolic doming: Rule out congenital valve fusion/stenosis and endocarditis.

8.4.4.1.7
Left Ventricular Wall and Chamber

Hypertrophy: Rule out hypertension, aortic or subaortic stenosis, hypertrophic cardiomyopathy, myocardial infiltration, and volume depletion (pseudohypertrophy).

Dilation: Rule out causes of volume overload, cardiomyopathy, valvular disease, left-to-right shunts, and atrioventricular fistula.

Hypokinesis: Rule out myocardial failure, anesthesia, myocarditis, myocardial necrosis, and ischemia or fibrosis (regional wall motion defect).

Hyperkinesis: Rule out mitral or aortic insufficiency, volume overload (e.g., left-to-right shunts), sympathetic stimulation, and hypertrophic cardiomyopathy, with or without stenosis.

Dyskinesis: Rule out cardiomyopathy, ischemia, infarct, thrombotic disease, and toxic insult (e.g., ionophore).

Giant papillary muscle: Rule out hypertrophy, and atrioventricular valve dysplasia.

Hypo- and hyperechogenicity: normal (variation), infarct, fibrosis, dissection, septal defect.

8.4.4.1.8
Ventricular Septal Wall

Hypertrophy: Rule out aortic or pulmonic stenosis, pulmonary or systemic hypertension, tetralogy of Fallot, and truncus arteriosus.

Hyperkinesis: As per LV chamber.

Hypokinesis: As per LV chamber. Rule out RV pressure or volume overload.

Paradoxic or flattened motion: Rule out moderate to severe RV volume or pressure overload, such as atrioseptal defect, tricuspid regurgitation, and pulmonary hypertension.

Echo “dropout”: Rule out septal defect and aneurysm.

Discontinuity of septum and aortic root: Rule out septal defect, tetralogy of Fallot, pulmonary artery atresia, and truncus arteriosus.

8.4.4.1.9
Right Ventricular Wall and Chamber

Hypertrophy: Rule out pulmonary hypertension, pulmonic stenosis, and tetralogy of Fallot.

Dilation: Rule out tricuspid insufficiency, chronic RV pressure overload, and atrial septal defect.

8.4.4.1.10
Left Atrium

Decreased size: Rule out right-to-left shunting and hypovolemia.

Dilation: Rule out mitral regurgitation or stenosis, cardiomyopathy, left-side heart failure, and left-to-right shunts.

Increased density: Rule out thrombus or tumor.

8.4.4.1.11 Right Atrium

Dilation: Rule out tricuspid regurgitation or stenosis, cardiomyopathy, right-side heart failure, and atrial septal defect.

Increased density: Rule out tumor and thrombus.

8.4.4.1.12 Atrial Septal Wall

Significant bowing: Rule out volume overload of one atrium.

Echo “dropout”: Rule out septal defect.

8.4.4.1.13 Pulmonary Artery

Absence: Rule out atresia.

Dilation: Rule out pulmonic stenosis, intracardiac left-to-right shunt, patent ductus arteriosus, pulmonary hypertension from any causes including left-sided congestive heart failure.

8.4.4.1.14 Aorta

Decreased diameter: Rule out low cardiac output.

Dilation: Rule out subaortic or aortic stenosis, tetralogy of Fallot, pulmonary artery atresia, patent ductus arteriosus, systemic hypertension, truncus arteriosus, and aortic insufficiency.

Aneurysm: Rule out congenital lesion, or endocarditis or vascular disease as cause.

8.4.4.1.15 Pericardium

Rule out pericardial effusion, constriction, fibrinous reaction or fronds, or mass lesion.

Rule out pulmonary or mediastinal abscess.

8.4.4.1.16 Doppler Flow Studies (Color Coded, Pulsed Wave, Doppler, Continuous Wave)

Estimation of cardiac output (flow-velocity integral × vessel cross-sectional area × heart rate).

Estimation of ventricular function (from aortic and pulmonary artery acceleration time or from tissue Doppler imaging).

Estimation of intracardiac pressures (from continuous wave velocity measurements across incompetent, stenotic valves or septal defects).

Detection of valvular regurgitation (abnormal direction, turbulent flow signals).

Detection of valvular stenosis (abnormal high-velocity, turbulent flow signals).

Detection of intracardiac shunting (abnormal turbulent flow signals)

* *LV,* Left ventricle; *RV,* right ventricle.

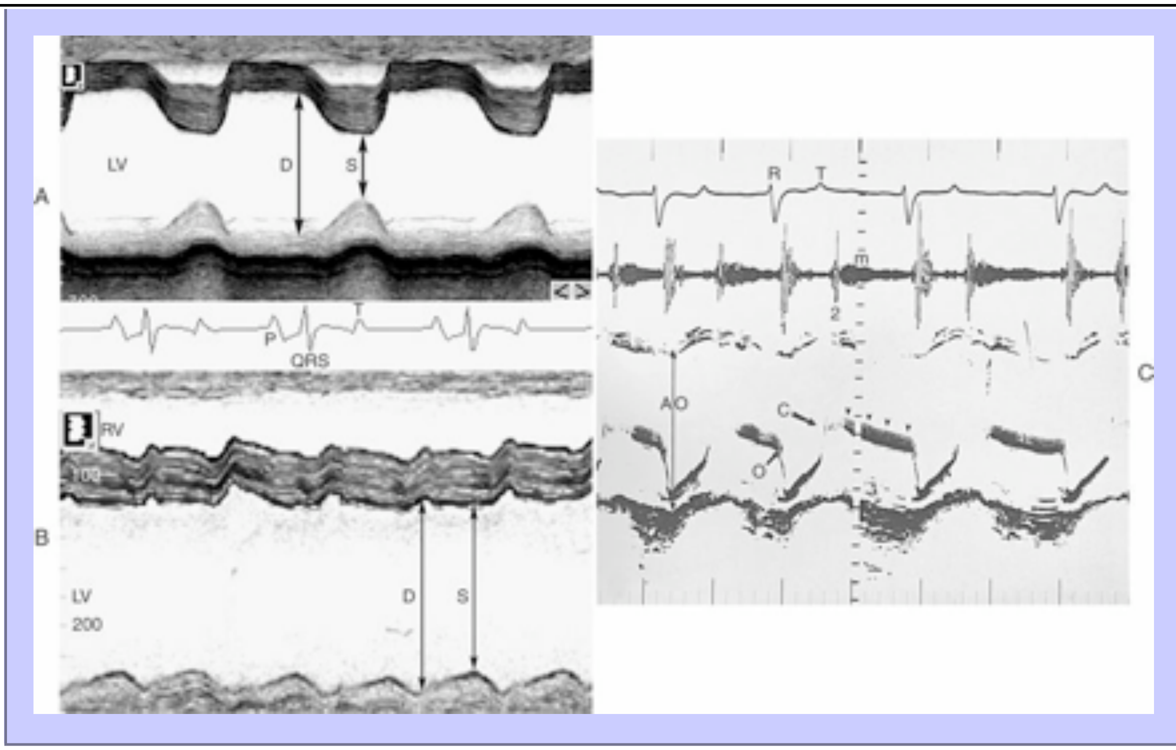
Modified from Bonagura JD, Herring DS, Welker F: Echocardiography, *Vet Clin North Am Equine Pract* 1:311, 1985.

The M-mode echocardiogram is a single-crystal ice pick image of the heart (see Figure 8-6).[13,14,53,451] The movement of the cardiac structures (vertical axis) is displayed over time along the horizontal axis. The ECG waveforms provide a timing reference, and the depth of the cardiac structures from the transducer (y-axis) is displayed in centimeters. Visualization of the characteristic movements of cardiac structures permits the experienced viewer to evaluate and quantify cardiac anatomy and function (see Figure 8-20). The high sampling rate of the M-mode study makes it excellent for visualizing rapidly vibrating structures, such as the oscillating mitral leaflet in aortic regurgitation.[128]

The two-dimensional echocardiogram generates a cardiac field by mechanically or electrically sweeping one or more piezoelectric crystals across the heart (Figure 8-21).[*] In conventional transthoracic echocardiography, the operator directs the imaging probe by hand to achieve a suitable tomographic plane. The pie-shaped image obtained has depth and breadth but has no significant thickness. Accordingly, the operator must use different image planes to interrogate the three-dimensional heart. These imaging planes are designated long-axis (sagittal), short-axis (coronal), apical (when the transducer is near the left apex), and angled (hybrid) views. The two-dimensional field is updated constantly to visualize cardiac motion in real time and occurs at typical sampling rates of 20 to 40 frames/sec with the update rate inversely related to penetration depth and angle of field. Fully digital echocardiographs can display much faster frame rates, often exceeding 60 frames/sec. In most cases the two-dimensional study is watched in real-time and also recorded on videotape or digitally for subsequent playback and analysis. Human interpretation generally is limited to 32 frames/sec, so that recordings made at higher frame rates require slow motion playback for detailed analysis. Transesophageal echocardiography has been used in horses[448] but requires specially made endoscope-mounted transducers and is most practical in anesthetized or sedated horses because of the fragile (and expensive) nature of the probe.

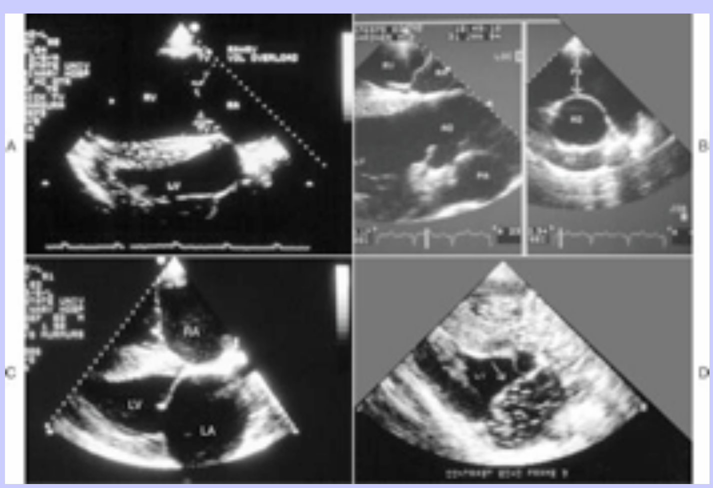
Doppler echocardiography relies on the Doppler principle to measure the direction and velocity of red blood cells in the heart (Figure 8-22; see also Figure 8-6).[*] In Doppler echocardiography, a portion of the ultrasound emitted by the transducer strikes moving red blood cells. These targets reflect ultrasound to the transducer. Because the red blood cells constitute a moving source of (reflected) ultrasound, the returning sound waves attain a frequency that is slightly different from that originally transmitted (the carrier frequency). When the echocardiograph unit records the Doppler frequency shift, calculation of red blood cell velocity and direction relative to the transducer is possible. The information is displayed as a Doppler spectrum showing time along the horizontal axis, flow direction relative to the transducer as above (toward) or below (away from) a zero baseline, 388
and calculated red blood cell velocity along the y-axis. Thus pulsed Doppler methods measure direction and 389
velocity of red blood cells within a discrete area of the heart or circulation. Disturbed flow, as might be recorded across a regurgitant orifice or VSD, results in a high velocity and broadened velocity spectrum.

Figure 8-20 M-mode echocardiography. **A,** M-mode tracing demonstrating a hyperdynamic left ventricle *(LV)* in a horse with acute mitral regurgitation. The significant change in left ventricular dimensions from diastole *(D)* to systole *(S)* is notable and is typical of volume overloading with preserved ventricular function. Furthermore, the reduced resistance to ejection of blood into the left atrium enhances ventricular shortening. Depth calibration in millimeters is shown on the left. **B,** Recording through the ventricles demonstrating decreased contractility with a reduced left ventricular shortening fraction. This pattern of contraction can be caused by myocarditis, myocardial injury (e.g., monensin), idiopathic dilated cardiomyopathy, chronic volume overload, protracted ventricular tachycardia, or administration of negative inotropic drugs. Depth calibration in millimeters is shown on the left. (*RV,* Right ventricle.) **C,** Recording from a horse with aortic regurgitation and atrial fibrillation, demonstrating fine diastolic fluttering of an aortic valve leaflet *(small arrows)*. The aortic root *(AO),* valve opening *(O)*, and valve closing *(C)* are indicated. The murmur (m) of aortic regurgitation is evident in the phonocardiogram above. First *(1)* and second *(2)* heart sounds are labeled. Diastolic fluttering of the mitral valve (most common), aortic valve, ventricular septum, or walls of the aortic root may be observed in horses with this hemodynamic abnormality. The M-mode sampling rate (approximately 1000 pulses/sec) is ideal for detecting these high-frequency events.



Color-coded Doppler imaging is a more refined example of pulsed wave technology whereby flow toward the transducer is coded in red and flow away is represented in blue. Calculated velocity is displayed in relative shades of these colors and green or yellow are added to the flow mapping to identify turbulence. Color coding permits superimposition of flow information onto the two-dimensional image (Figure 8-23). Although substantial technical challenges exist in terms of penetration and frame rate, color Doppler imaging is useful in horses because one can screen a large area of the heart for flow disturbances. For example, finding a jet of MR using color Doppler imaging is much easier than with other Doppler methods. A pivotal limitation of color Doppler regards temporal resolution. Depending on the system used, the frame rates of interrogation can be slow 389
(often <10 frames/sec). Thus timing of the flow event is mandatory using spectral Doppler or by invoking the M- 390
mode cursor and recording the event by color M-mode echocardiography. Timing prevents the clinician from misinterpreting normal backflow signals related to valve closure or diagnosing a diastolic flow event as systolic.

Figure 8-21 Two-dimensional echocardiograms. **A,** Long-axis image from the right thorax in a 12-year-old Quarter Horse gelding demonstrating significant right ventricular and right atrial dilation following tricuspid regurgitation and elevated pulmonary artery pressure. In this case a tumor that obstructed flow in the main pulmonary artery caused the pulmonary hypertension. The ventricular septum is flat and bulges slightly into the left ventricle. **B,** Images of the ascending aorta and pulmonary artery obtained from the right hemithorax *(left panel)* and left cranial hemithorax. An evaluation of the pulmonary artery diameter relative to the aorta is useful when identifying pulmonary hypertension. **C,** Biatrial dilation in a Thoroughbred colt with mitral regurgitation, pulmonary hypertension, atrial fibrillation, and congestive heart failure. Both atria are rounded and appear turgid. The cause of mitral disease in this case was idiopathic lymphocytic-plasmacytic mitral valvulitis. (*RA,* Right atrium; *LV,* left ventricle; *LA,* left atrium.) **D,** Contrast echocardiogram demonstrating right-to-left shunting at the level of the foramen ovale in a foal with severe respiratory disease. The saline generates echocontrast, opacifies the right atrium and right ventricle and visibly fills the left atrium *(arrows)* although the left ventricle has not yet been opacified. This technique is easy and practical for demonstrating right-to-left shunts across the cardiac septa.

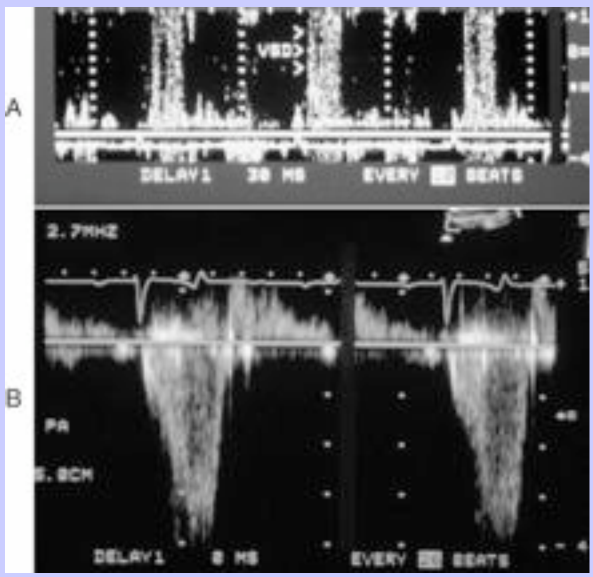


Pulsed wave spectral and color Doppler techniques can provide accurate information about the location of flow disturbances but cannot measure high-velocity flow faithfully. High-velocity flow occurs as red blood cells are ejected from higher to lower pressure zones across incompetent valves, stenotic valves, and intra- and extracardiac shunts (provided that a pressure difference exists across the defect). In general, once velocities exceed about 2.5 m/sec in either direction, part of the returning signal is displayed in the incorrect direction. This problem is called signal aliasing[225] (see Figure 8-22, *A*). To quantify high-velocity flow, one must invoke a third modality, high pulse-repetition-frequency Doppler or continuous wave Doppler. Continuous wave Doppler has virtually unlimited ability to record high velocity but does not provide the spatial discrimination found in pulsed 390
wave Doppler modalities. Recording disturbed flow accurately has clinical value. The appearance of a flow 391
disturbance can provide qualitative information about the severity of a lesion (see later in this chapter). Furthermore, one can estimate the pressure difference between the source and the sink of a high-velocity jet by the modified Bernoulli equation, in which pressure drop in millimeters of mercury equals $4V^2$ (where maximal velocity, V, is measured in meters per second by continuous wave Doppler). If, for example, a peak velocity of 4.8 m/sec is recorded across a VSD, and if the systolic systemic arterial blood pressure is determined noninvasively to be 125 mm Hg, the estimated RV systolic pressure would be calculated as follows:

$$\begin{gathered}\text{Pressure drop} = 4 \times 4.8^2 = 92 \text{ mm Hg}\\ \text{Estimated LV systolic pressure} = 125 \text{ mm Hg}\\ \text{Estimated RV systolic pressure} = 125 - 92 = 33 \text{ mm Hg}\end{gathered}$$

These findings indicate a restrictive septal defect, a lesion unlikely to cause difficulties for the horse except at highest levels of performance.

Figure 8-22 Spectral Doppler echocardiograms. **A,** Pulsed wave Doppler study from a horse with a ventricular septal defect. The baseline of zero flow has been shifted to the bottom. A high-velocity, turbulent systolic jet is recorded but cannot be quantified faithfully. The signal wraps around the baseline (signal alias). Calibration dots are 20 cm/sec. **B,** A continuous wave Doppler study from a filly with tetralogy of Fallot. The high-velocity flow pattern across the stenotic pulmonary artery is recorded as a negative signal below the zero baseline. Calibration dots are 1 m/sec. The jet velocity exceeds 4 m/sec.



Echocardiography is most useful in evaluating congenital heart disease, heart murmurs, pericardial diseases, heart failure, and cardiac arrhythmias. Ultrasound also can identify other lesions of the lung, pleural space, or mediastinum that may masquerade as heart disease.[94,455] One can determine accurately the source of a pathologic cardiac murmur, the size and function of the heart, and the overall effect of a cardiac lesion by a complete echocardiographic and Doppler examination. A normal echocardiogram and Doppler study in a horse with a cardiac murmur is a favorable finding, suggesting a functional basis for the murmur. Conversely, identification of an abnormal flow pattern with associated cardiomegaly or abnormal ventricular function may indicate a high risk or limitation for work. As with all diagnostic studies, results can be ambiguous, particularly regarding the tricuspid valve, because physiologic TR, silent to auscultation, is detectable by Doppler studies in many clinically normal horses. Contrast echocardiography[456], whereby saline is used to delineate the path of blood flow, is especially useful for the detection of right-to-left shunts in foals, including patent foramen ovale (see Figure 8-21). Reference values for ventricular chamber size, wall thickness, and LV shortening fraction

have been published,[51,206,220,266–268,426] and Table 8-5 gives some representative data.[220,266] Clinical applications of echocardiography are illustrated later in this chapter.

* References 14, 49, 51, 72, 80, 82, 87, 88, 91, 102, 117, 119, 120, 127–129, 132, 133, 135, 139, 140, 153, 163, 179, 205, 206, 208, 210, 219–222, 225, 230, 240–242, 245–248, 266–271, 297, 355, 356, 399–448.

* References 50, 51, 87, 266, 351, 452, 453.

* References 139, 140, 208, 225, 247, 407, 415, 454.

8.4.5 CARDIAC CATHETERIZATION

A large body of literature is derived from catheterization studies of the standing, exercising, anesthetized, and diseased horses.[*] In the clinical setting, however, cardiac catheterization has been replaced largely by Doppler echocardiography. The clinician can obtain anatomic, flow, and pressure estimates noninvasively, safely, and more readily with ultrasound studies. Some indications for cardiac catheterization in clinical patients remain, particularly when one requires accurate measurement of pulmonary artery pressure or entertains a diagnosis of occult constrictive pericardial disease.

Hemodynamic variables one can measure or calculate by catheterization include arterial blood pressure, pulmonary arterial and pulmonary artery occlusion (capillary wedge) pressure, intracardiac pressures, central venous pressure, CO, systemic and pulmonary vascular resistances, and arteriovenous oxygen difference. Normal values for these depend on the methods used for measurement, the head and body position of the horse, and the effects of administered tranquilizers, sedatives, or anesthetic agents. One should appreciate some general principles of hemodynamics. 391

Figure 8-23 Black-and-white representations of color Doppler echocardiograms demonstrating mapping of blood flow through the heart. **A,** Perimembranous ventricular septal defect in a foal *(arrow)*. The flow pattern was coded red and aliased as the velocity increased across the defect. **B,** Wide spray of mitral regurgitation with flow moving from left ventricle *(LV)* into the left atrium *(LA)* in a horse with mitral valve prolapse. (*RV,* Right ventricle; *RA,* right atrium.) **C,** Color M-mode study from a horse with tricuspid regurgitation. The bright systolic flow pattern, following the QRS of the electrocardiogram, was mapped as turbulence in the color study. This is an excellent method for timing abnormal flow events.

Pressures on the left side of the circulation include systemic arterial, LV, and LA pressures. Systemic arterial pressure is linked closely to LV function, impedance to blood flow in the aorta, systemic vascular resistance, and heart rate. Measurement of arterial blood pressure has been described previously. Peak systolic pressures in the aorta and left ventricle should be essentially equal, and a substantial difference between these values would indicate an obstruction to outflow, a most rare condition in horses. LV diastolic pressure reflects diastolic function and filling and the ability of the ventricle to empty its stroke volume. The reported diastolic pressure is usually the end-diastolic pressure (typically 12 to 24 mm Hg in standing animals), which is higher than the early

(often subatmospheric) minimal diastolic LV pressure. Horses and ponies have higher ventricular end-diastolic pressures than do human beings or dogs.[21,24,214,468] Exercise is a physiologic cause of increased LA and ventricular end-diastolic pressures.[278,459,462,481–484] Elevation of resting LV end-diastolic pressure generally indicates reduced myocardial contractility, ventricular failure, LV volume overload as with mitral or aortic regurgitation, myocardial infiltration (lymphoma, amyloid), or pericardial constraint or increased ventricular wall stiffness.

One can obtain the pulmonary arterial pressures by percutaneous placement of a flow-directed catheter from the jugular vein to the pulmonary artery. Inflating the balloon tip to occlude the pulmonary artery transiently and wedge the distal catheter tip may be possible. One can use such pulmonary capillary wedge pressures to estimate pulmonary capillary and venous pressures as well as LA pressure.[233,462,464,482,485] The pulmonary artery pressures in standing mature horses are considerably lower than are those of the aorta because of the lower resistance encountered in the pulmonary vascular tree. Mean pulmonary artery pressure is higher in the newborn foal and decreases significantly during the first 2 weeks of life because of decreased pulmonary arteriolar 392

393 resistance.[233] Normal pulmonary arterial pressures are 35 to 38 mm Hg systolic and 14 to 22 mm Hg diastolic. Pulmonary pressures increase dramatically with increased CO as encountered during exercise.[341,459,462,464,482] The pressure that must develop in the pulmonary artery depends not only on pulmonary arteriolar resistance but also (unlike the systemic circulation) on the pulmonary capillary resistance. Pulmonary function can influence both of these variables. Alveolar hypoxia and acidosis cause reactive pulmonary arterial vasoconstriction, raising pulmonary pressures.[486] This reaction may be particularly important in newborn foals in which vascular resistance is already high.[233] LV function directly influences pulmonary artery pressure because elevation of LA and pulmonary venous pressures places a direct burden on the pulmonary artery and right ventricle.[214] LV failure leads invariably to secondary pulmonary hypertension that can be severe, often with pulmonary pressures exceeding 100 mm Hg. Presumably, other factors such as reactive vasoconstriction (from lung edema) or anatomic changes in the pulmonary vascular tree must develop to sustain such high resistances. In other cases the cause of pulmonary hypertension may not be evident.[487] Accordingly, one must assess elevated pulmonary artery pressures in light of pulmonary wedge (or LV end-diastolic) pressure[272,273] and CO. When pulmonary artery pressure increases, RV and right atrial (RA) pressures must be high to overcome the pressure load. If pulmonary hypertension is chronic, cardiac enlargement, TR, and right-sided heart failure can ensue. The pulmonary occlusion or wedge pressure is an estimate of LV filling pressure and should approximate LV end-diastolic pressure, provided that no obstructions exist in the pulmonary veins or at the mitral valve. The pulmonary artery diastolic pressure also can estimate pulmonary artery wedge pressure provided that the heart rate is normal and pulmonary arterial vasoconstriction (as might occur with hypoxia) is minimal. The pulmonary artery diastolic pressure does not fully equilibrate with the wedge pressure during tachycardia or if pulmonary vasoconstriction and a significant pressure gradient exist between the pulmonary artery diastolic and wedge pressures.[482,485] The wedge pressure is reduced in hypovolemia and is increased during exercise,[482,484] left-sided heart failure,[273,421] severe MR, or after overinfusion of crystalloid or depression of LV function.

TABLE 8-5 Reference Values for Equine Echocardiography

MEASUREMENT	MEAN[*] (cm)	SD[*]	MEAN[†] (cm)	SD[†]
Left ventricle (diastole), right/left	11.90/12.25	0.71/0.72	11.06	1.34
Left ventricle (systole)	7.35	0.72	6.11	0.91
	7.71	0.52	—	—
Left ventricular wall (diastole)	2.39	0.26	—	—
Left ventricular wall (systole)	3.96	0.93	—	—
Ventricular septum (diastole)	3.02	0.39	3.06	0.6
Ventricular septum (systole)	4.55	0.55	4.81	0.71
Right ventricle (diastole)	3.83	0.91	—	—
Right ventricle (systole)	2.71	1.00	—	—
Right ventricular wall (diastole) from the left side	1.44	0.24	—	—
Aorta (diastole)	8.50	0.51	7.31	—
Left ventricular shortening fraction, right/left	38.76/37.01	4.59/3.91	44.1	6.4
Left atrial diameter (systole)[‡]	2.8	0.78	—	—
Aortic sinotubular junction (diastole)[‡]	7.45	0.39	—	—
Body mass (kg)	517	—	445	—

SD, Standard deviation.

* Data from Long KJ, Bonagura JD, Darke PG: Standardised imaging technique for guided M-mode and Doppler echocardiography in the horse, Equine Vet J 24:226, 1992. (26 Thoroughbred horses).

† Data from Lescure F, Tamzali Y: Reference values for echocardiography applied to sport horses (English thoroughbreds and French riding horses), Rev Med Vet 135:405, 1984.

‡ Left atrial and aortic two-dimensional data from Patteson MW, Gibbs C, Wooton PR et al: Echocardiographic measurement of cardiac dimensions and indices of cardiac function in normal adult thoroughbred horses, Equine Vet J Suppl 19:18–27, 1995.

The RV systolic pressure is lower than that of the left ventricle and usually varies from 40 mm Hg up to 60 mm Hg in standing horses. One often can measure a small gradient (usually 10 to 15 mm Hg) between the ventricular apex and pulmonary artery during systole in normal animals. RV end-diastolic pressure is usually between 10 and 14 mm Hg. The RA and central venous pressures influence RV end-diastolic pressure and preload. Hydrostatic venous blood pressure becomes higher or lower, causing corresponding changes in the RV end-
diastolic pressure if the head of the horse is raised or lowered.[336] As with the left ventricle, depression of 393
contractility reduces the rate of systolic pressure development.[272] Elevated RV systolic pressure is recorded in 394
pulmonary hypertension from any cause, with large VSDs, and with RV outflow obstruction such as that caused

by pulmonic stenosis, tetralogy of Fallot, or large pulmonary valve vegetation.[125] One encounters pathologic elevations in RV diastolic pressure with pericardial disease, pulmonary hypertension, severe right-sided valvular disease, and CHF (Figure 8-24).

Figure 8-24 Recording of intravascular pressure during cardiac catheterization. Right ventricular pressure recordings from a Thoroughbred yearling with atrial fibrillation, pulmonary hypertension, and biventricular congestive heart failure. The pressure waveforms vary because of ventilation and the arrhythmia. Peak pressures exceed 70 mm Hg. In the absence of pulmonic stenosis or a large ventricular septal defect, this indicates pulmonary hypertension. The ventricular end-diastolic pressure also is elevated and is compatible with heart failure.

Mean RA and central venous (vena caval) pressures estimate RV filling pressure and represent a balance between blood volume, venomotor tone, body position, and heart function.[328] Central venous pressure increases significantly in recumbent horses, especially during general anesthesia. Values frequently double from the standing preanesthetic measurement and central venous pressure determinations of 20 to 30 cm of water are common.[466,488] A single measurement of central venous pressure or of the RA pressure profile is of little value. Trends are most important in assessing the volume status and cardiac function of the animal. The x descent of the RA pressure waveform may be replaced by a positive c-v wave in the setting of significant TR; this pressure wave corresponds to a prominent jugular pulsation observed on inspection of the neck.

One can measure CO by thermodilution techniques, arteriovenous oxygen difference, Doppler studies, lithium dilution, or other methods.[*] One most often determines CO while monitoring the effects of fluid and drug therapy on the circulation in critical care situations because CO represents a key variable in oxygen delivery to tissues. Reported values range from 72 to 88 ml/min/kg in standing horses or ponies. One can estimate CO noninvasively using Doppler echocardiographic methods. One can use the mixed venous (pulmonary artery) oxygen content (milliliters of oxygen over deciliters of blood) as an indirect estimate of CO. Mixed venous

samples obtained from pulmonary arterial catheters are superior to venous samples obtained from the jugular or peripheral veins.[384] As CO increases, the tissues extract less oxygen from each aliquot of blood; consequently, the venous oxygen content increases.[235] As CO decreases, tissue extraction of oxygen increases, oxygen content decreases, and the systemic-venous O_2 difference thus widens. One also can use oximetry determinations to detect intracardiac or extracardiac shunting, for example, to identify a left-to-right shunting VSD.

One cannot measure systemic and pulmonary vascular resistances in the intact animal but can calculate these using a variation of Poiseuille's law. The general formula for calculation of resistance is as follows:

$$\text{Vascular resistance} = \frac{\text{Mean arterial pressure} - \text{Mean atrial pressure}}{\text{Cardiac output}}$$

where one measures CO in liters per minute by thermodilution. The pressures used are mean aortic pressure and mean RA pressure for the systemic vascular resistance and mean pulmonary arterial pressure and mean pulmonary wedge pressure for the pulmonary vascular resistance. One may add correction values to convert resistance to centimeter-gram-second units.[490] Because mean arterial pressure is similar between horses of different size, calculated vascular resistance is normally higher in smaller horses and ponies. Mechanisms that increase systemic vascular resistance include sympathetic activation, activation of the renin-angiotensin system, and the release of other vasoactive hormones into the blood, including arginine vasopressin (antidiuretic hormone) and epinephrine.[70] The pulmonary vascular resistance is tied to pulmonary vascular anatomy, age, total lung capillary resistance, LA pressure, and degree of pulmonary vascular constriction. The latter is controlled by the tension of alveolar oxygen and local mediators including nitric oxide and endothelin.

* References 37, 42, 46, 47, 68, 192, 226, 228, 229, 233–235, 237, 238, 240, 241, 243, 245–248, 251, 272, 275–278, 319, 323, 327, 328, 332, 337, 344, 422, 457–480.

* References 228, 229, 233–235, 240, 241, 243–248, 459, 461, 463, 479, 489.

8.4.6 LABORATORY STUDIES

Cardiovascular disorders may develop as a result of systemic or metabolic diseases such as electrolyte disturbances or septicemia. Conversely, circulatory failure or cardiovascular infections may alter routine laboratory tests. Prerenal azotemia and hyponatremia, for example, may be detectable in the horse with CHF, 394
especially after diuretic therapy. Myocardial enzymes or proteins such as troponin may be released in the 395
circulation from primary or secondary cardiac muscle injury, as might occur with hypotension and ischemia.

Box 8-4 gives an overview of the laboratory studies useful in assessing and managing cardiovascular diseases. A routine complete blood count and fibrinogen, biochemical profile, and urinalysis are indicated in horses with arrhythmias or heart failure or with clinical evidence of endocarditis, pericarditis, vasculitis, or pleural effusion. Additional studies including blood cultures, blood gas tensions, other electrolytes (e.g., magnesium), oxygen saturation, cytologic examination and culture of pericardial effusates, serum troponin I concentrations, and myocardial isoenzymes of creatine kinase or lactate dehydrogenase are indicated in selected cases. Monitoring of serum or plasma drug concentrations, especially quinidine and digoxin, are appropriate when using these drugs to treat patients with heart disease. Application of these studies is discussed under specific diseases.

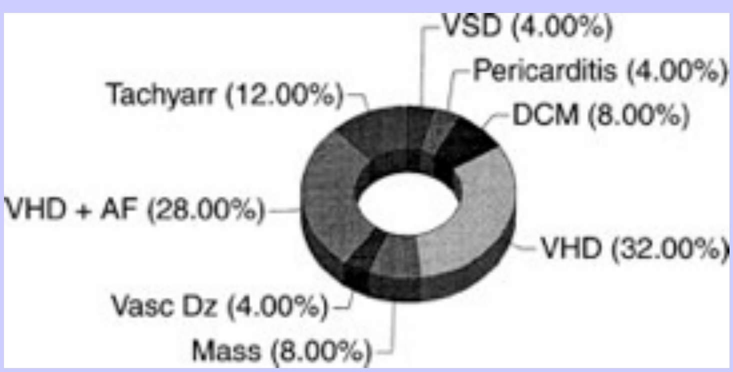
8.5 Congestive Heart Failure

8.5.1 CAUSES

Heart failure is a clinical syndrome characterized by limited CO, increased neurohormonal activity, renal sodium retention, edema in tissues, and transudation of fluid into serous body cavities. The neurohormonal and renal abnormalities that characterize heart failure have not been studied extensively in horses but are probably similar to those reported in other species.[98] Probably the most characteristic clinical feature of heart failure, short of exercise intolerance, is the increase in venous pressures identified by physical examination or by cardiac catheterization. One recognizes the typical horse with cardiac failure when heart failure progresses to the overtly congested phase, with fluid accumulation.

Most cardiac lesions in horses are not severe enough to lead to CHF, and this syndrome is not common in equine practice. Nevertheless, CHF does develop in foals and mature horses as a result of diverse disorders, including congenital malformation, severe degenerative valvular disease, valvulitis, bacterial endocarditis, dilated cardiomyopathy, myocarditis, myocardial necrosis, pericardial disease, vascular rupture, pulmonary hypertension, artery obstruction, or persistent tachyarrhythmia.[104,491,492] The most common cause of CHF in horses is valvular heart disease, which often is complicated by atrial fibrillation (Figure 8-25). In addition to structural lesions of the heart and blood vessels, primary electric disturbances, particularly sustained atrioventricular dissociation caused by junctional or ventricular tachycardia, can reduce myocardial function, decrease CO, and lead to CHF.[493] This is especially likely when the heart rate exceeds 100 beats/min for many days. Resolution of CHF in such cases may be possible if antiarrhythmic therapy is successful.

Figure 8-25 Graph demonstrating the overall causes of congestive heart failure in 25 horses at a referral teaching hospital as a percentage of the total number of cases of equine congestive heart failure. *Tachyarr,* Tachyarrhythmia; *VSD,* ventricular septal defect; *DCM,* dilated cardiomyopathy; *VHD,* valvular heart disease; *Mass,* mass lesion; *Vasc Dz,* vascular disease; *VHD + AF,* valvular heart disease and atrial fibrillation. (Data from Ohio State University Veterinary Hospital.)



8.5.2 CLINICAL RECOGNITION

CHF may develop suddenly or gradually. Peracute heart failure can occur following rupture of mitral valve chordae tendineae or consequent to acute bacterial endocarditis of a cardiac valve. Heart failure may progress rapidly in a foal with a large VSD as the pulmonary vascular resistance falls in the weeks following birth. Chronic valvular regurgitation may, after many years, lead to CHF, although only a small number of horses with degenerative valvular disease develop CHF. Some lesions, which might otherwise be well tolerated, can cause heart failure if demands for CO increase. Examples of such demands include strenuous work, severe anemia, or persistent fever. Pregnancy is another example: the volume expansion and increased demands for CO in the latter stages of gestation may precipitate CHF in a mare with previously compensated heart disease. Development of atrial fibrillation in a horse with underlying structural disease, such as dilated cardiomyopathy or MR, can precipitate CHF. Such cases are distinguished from the more typical case of atrial fibrillation by the presence of persistent, resting tachycardia and identification by echocardiography of structural lesions and cardiomegaly.

The clinical features of CHF are easily recognizable,[350] and determination of the underlying cause of heart disease is not difficult if one combines careful auscultation with available diagnostic methods, particularly 395
echocardiography and ultrasound examination of body cavities.[*] Presenting clinical signs can vary but may 396
include exercise intolerance, poor recovery after exercise, weight loss, coughing, tachypnea, ventral edema, and even colic-like signs.

The clinical signs of right-sided CHF include resting tachycardia and prominent S_3 along with generalized ventral, preputial, pectoral, and limb edema. Isolated limb edema, which is so common in hospitalized horses, is not a sign of CHF. Nor should ventral edema, in the absence of generalized venous distention, suggest a diagnosis of CHF. This finding should prompt consideration of other disorders including vasculitis. Scrutiny of the jugular and other superficial veins generally makes the recognition of right-sided CHF straightforward, because affected horses demonstrate elevated venous pressure and pathologic jugular pulses and filling. The most common causes of isolated right-sided CHF are tricuspid or pulmonic valve lesions as with valvular endocarditis, diffuse pulmonary vascular disease, and pericardial disease. In most cases a prominent murmur of TR is evident over the right thorax. When pulmonary hypertension underlies right-sided heart failure, the tricuspid murmur may be especially loud and the pulmonic component of S_2 tympanic. Because right-sided heart failure often develops consequent to pulmonary hypertension caused by left-sided heart failure, left-sided murmurs are common. Another common association of right-sided CHF is persistent cardiac arrhythmia, such as atrial fibrillation, superimposed on structural heart disease of any form. One must distinguish cranial mediastinal lymphosarcoma and cranial thoracic masses from RV failure.[508] Examination with two-dimensional echocardiography and thoracic ultrasonography facilitates diagnosis.

Recognizing isolated left-sided heart failure, which causes pulmonary venous congestion and pulmonary edema, can be more difficult. In these cases, heart sounds and murmurs may be difficult to evaluate because of loud airway sounds or crackles. If a cardiac murmur is present but missed, one may entertain an erroneous diagnosis of pneumonia. Again, resting tachycardia and loud S_3 are typical in these cases, and echocardiographic examination of the horse is useful. Typical findings in left-sided CHF are dilation and rounding of the left atrium and ventricle and often dilation of the pulmonary artery caused by pulmonary hypertension. Careful examination may demonstrate anatomic lesions of the aortic root, ventricular septum, left heart valves, or chordae tendineae. Fulminant left-sided CHF can lead to coughing and expectoration of edema—a grave sign in most horses.

Biventricular heart failure most commonly occurs when severe left-sided heart disease results in chronic CHF. The clinical signs of advanced biventricular heart failure include resting tachycardia (usually >60 beats/min), loud ventricular filling sound, subcutaneous edema, tachypnea (from pulmonary edema or pleural effusion), varying amounts of ascites and pleural effusion, a small pericardial effusion, jugular distention, and abnormal jugular pulsations. In most cases a systolic murmur of TR and often murmurs typical of mitral or aortic valvular regurgitation or a VSD are present. Chronic biventricular CHF frequently is characterized by lethargy and also by loss of body condition.

One must understand that *chronic* left-sided CHF in the horse is likely to cause interstitial lung edema and pulmonary hypertension. The magnitude of pulmonary hypertension is often impressive, with systolic pulmonary artery pressures exceeding 80 mm Hg in many cases. The mechanisms by which the lung accommodates such severe and chronic elevations in pulmonary venous pressures without development of alveolar edema are only speculative to date, but pulmonary vascular resistance likely increases out of proportion to the increase in wedge pressure (as occurs in some human patients with chronic left-sided heart failure). This accommodation might be related to anatomic or remodeling changes in pulmonary veins or arteries induced by chronic elevation of LA pressure. Thus the clinician should anticipate signs of right-sided CHF even when the primary lesion can be identified on the left side of the heart. This finding can be attributed to RV dilation and TR, which are caused by increased pulmonary vascular resistance. Furthermore, generalized fluid retention is a characteristic of chronic CHF, and some of this retained fluid undoubtedly pools in systemic venous beds and third spaces.

Clinical findings of severe pulmonary hypertension caused by left-sided CHF include a loud, tympanic, pulmonary component of S_2 (heard cranioventral to the aortic valve area), a systolic murmur and jugular pulse of tricuspid insufficiency, and dilation of the pulmonary artery, which can be identified by two-dimensional echocardiography. Further scrutiny allows one to identify LA dilation and a lesion of the left heart valves, aorta, or left ventricle. Investigation of the exact cause of CHF involves integration of physical examination findings with routine diagnostic studies such as electrocardiography and echocardiography. Thoracic radiographs may be helpful and can demonstrate increased pulmonary vascularity, pulmonary infiltration near the hilus, pleural effusion, and rounding or enlargement of the cardiac silhouette. Echocardiography is essential to identify structural lesions, quantify cardiomegaly, and measure systolic ventricular function. Doppler echocardiography can document abnormal flow patterns and predict pulmonary artery pressures (using the Bernoulli relationship), provided that one can record the velocity of any TR or pulmonary insufficiency jets faithfully. The ECG is required to evaluate cardiac arrhythmias.

* References 84, 85, 98, 102, 104, 127, 136–138, 142, 164, 257, 262, 263, 272, 297, 428, 487, 491, 492, 494–507.

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8.5.3 THERAPY

Therapy for CHF is realistic for potentially reversible disorders such as pericarditis or sustained ventricular tachyarrhythmia. Therapy also may be feasible for valuable breeding stallions and mares, mares that develop CHF during gestation, or horses and ponies kept as pets.[136,350,509] Before therapy can commence, one needs an accurate diagnosis. For example, pericardiocentesis (not cardiac drugs) would be appropriate initial management of cardiac tamponade; thereafter, one could consider surgical drainage of the effusion (using drainage tubes) or a pericardiectomy.[83,84,91,510,511] Antibiotics would be essential for treating bacterial endocarditis or infective pericarditis. Sustained junctional and ventricular tachyarrhythmias may lead to low CO and a potentially reversible dilated cardiomyopathy. Antiarrhythmic therapy with quinidine, magnesium sulfate solution,

lidocaine, propafenone, or procainamide may be effective for treating some of these arrhythmias, and with resumption of normal rhythm, CHF may be reversed.

Furosemide,[257,499,512,513] digoxin,[*] and vasodilators or angiotensin-converting enzyme (ACE) inhibitors[145,156] are the mainstays for short- and long-term management of CHF (Table 8-6). One must titrate the diuretic dosage to mobilize edema and prevent its recurrence and improve respiratory effort when pulmonary edema is evident, and one should control the dosage to prevent prerenal azotemia or electrolyte disturbances. One eventually may discontinue diuretic therapy in some horses. Depending on urgency and ability to monitor ECG rhythm, one can administer digoxin initially as a loading dose or by using an intravenous or oral maintenance regimen. The reported elimination half-life of digoxin has not been consistent (7.2 to 28 hours) and probably relates to the clinical state of the animals studied. As expected, oral doses of digoxin are higher because of lower bioavailability (20%).[263] Long-term digoxin therapy involves once or twice daily oral administration, usually mixed with molasses and some grain. One should monitor chronic digitalization by measuring serum digoxin concentration and evaluating a blood sample (drawn between 8 and 12 hours after the previous dose). Therapeutic values for the trough plasma level generally are considered to be between 1 and 2 ng/ml. Periodic cardiac examinations, measurements of serum biochemistries (creatinine, electrolytes), and recordings of ECG rhythm strips are warranted during any long-term course of therapy.

Published experience using vasodilators and ACE inhibitors in horses is limited. One might consider hydralazine therapy[236] in the initial management of severe MR caused by ruptured chordae tendineae or endocarditis, because systemic arterial dilation can reduce the mitral regurgitant fraction greatly. ACE inhibitors have been prescribed for treatment or prevention of CHF in horses with apparent clinical success. Clinical improvement has occurred in many horses with enalapril[516] administered at 0.5 mg/kg orally twice daily and is affordable for clients with the generic formulation. Although the cost may be prohibitive, ramipril also has been tolerated in some cases.[517]

For horses with cardiomyopathy or valvular disease or in pregnant mares with CHF, the development of atrial fibrillation may precipitate CHF. Furosemide and digoxin can control CHF effectively in many of these animals. The use of ACE inhibitors is contraindicated in pregnant mares, but one can use hydralazine without any demonstrable ill effects on the fetus. Quinidine generally is contraindicated in such cases, although horses sometimes may convert after the resolution of CHF by medical therapy. One may achieve reasonable long-term control of CHF, thus permitting a comfortable existence for the horse and, in the case of breeding animals, continued reproductive service.

Prognosis in CHF always is guarded when irreversible structural heart disease is the cause of failure; thus the long-term outcome for life is poor. Of course, the horse with CHF requires rest to reduce demands on the heart and should never be worked or ridden because of the risk of pulmonary artery rupture or syncope. Valuable horses may be used for breeding; however, pregnant mares are likely to be difficult to control in the later stages of gestation, probably related to the volume expansion that accompanies the later stages of pregnancy.

* References 136, 249, 250, 252, 253, 257, 258, 260, 262, 314, 500, 509, 514, 515.

8.6 Congenital Heart Disease

The prevalence of congenital heart disease in the overall equine population is unknown. In one survey of causes for neonatal death or euthanasia in 608 cases, the prevalence of congenital heart disease was 3.5%.[150] Congenital heart disease often is considered when a foal, weanling, or immature horse is identified with a prominent cardiac murmur, cyanosis, or signs of CHF.[316] A variety of cardiac malformations have been identified.[*] Theoretically, a

great number of cardiac malformations could occur, including anomalies of venous drainage, atrial situs or septation, atrioventricular connection, atrial or ventricular development (including formation of the two 397
atrioventricular valves), ventricular outflow tracts, semilunar valves, and great vessels.[547,548] Furthermore, 399
abnormal segmental connections might occur leading to discordance in the path of systemic or pulmonary venous return relative to the pulmonary artery or aorta. These abnormalities include transposition of the great vessels and double-outlet ventricle, in which both great vessels exit the right or LV cavity. However, practically speaking, the most common cardiac malformations in horses involve shunting of blood at the atrial or ventricular regions.

TABLE 8-6 Drug Therapy of Heart Disease

DRUG	FORMULATION	DOSAGE FORMS	INDICATIONS	USUAL DOSE
Digoxin	Lanoxin	Lanoxin 0.25 mg/ml, 2-ml vials; Lanoxin tablets 0.5 mg	CHF,* atrial tachyarrhythmias, control of rapid ventricular response in atrial fibrillation/flutter	IV maintenance dose for treatment of CHF: 0.22 mg/100 kg body mass, q12h
				Oral maintenance dose for chronic therapy of CHF or for control of ventricular rate response in atrial fibrillation: 1.1–1.75 mg/100 kg body mass, q12h
				IV maintenance dose for control of ventricular rate response in atrial fibrillation (neither CHF nor renal failure is present): 0.22–0.375 mg/100 kg body mass, q12h
				IV loading dose: 0.44–0.75 mg/100 kg body mass, q12h for two doses (not commonly used)
Procainamide	USP	100 or 500 mg/ml for injection; 500-mg capsules	Ventricular arrhythmias, atrial arrythmias	25–35 mg/kg q8h per os
	Pronestyl			1 mg/kg/min IV to a maximum of 20 mg/kg
Lidocaine	Xylocaine without epinephrine	2% solution, 20 mg/ml concentration	Ventricular tachycardia	0.5–1.5 mg/kg slowly IV; can repeat in 15 minutes
				Constant IV infusion of 25–50 g/kg/min
Diltiazem	USP Cardizem	5-mg/ml vial; USP powder	Supraventricular tachycardia	0.5 mg/kg IV every 30 minutes up to 1.25 mg/kg total dose
Propranolol	USP Inderal	1-mg vials	Unresponsive tachyarrythmias	0.03 mg/kg IV

Propafenone	Rhythmol	150- and 300-mg tablets	Sustained ventricular tachycardia; atrial fibrillation	2 mg/kg per os q8h
Bretylium tosylate	Bretylium	500-mg/10-ml vials	Life-threatening or refractory ventricular arrhythmias	3–5 mg/kg IV
Furosemide	Lasix Furosemide USP	5% solution, 50 mg/ml	Edema	1–2 mg/kg as needed
Dobutamine	Dobutrex	20-mg vials for infusion	Cardiogenic shock, hypotension, complete atrioventricular block (emergency therapy)	1–5 μg/kg/min
Hydralazine	Apresoline Hydralazine USP	50-mg tablets	Mitral regurgitation	0.5–1.5 mg/kg q12h
Quinidine sulfate (oral); quinidine gluconate (IV)	USP Cardioquin Quinaglute	50-g jars of quinidine sulfate powder, USP; tablets; quinidine gluconate for injection, 80 mg/ml	Atrial arrhythmias, ventricular arrhythmias	22 mg/kg per os 1–10 mg/kg IV, total dose (see Box 8-11 for details)
Magnesium SO_4	USP (filter)	Dissolve 20–25 g in sterile water; filter	Ventricular tachycardia	IV infusion at 1 g/min to effect, up to a maximum of 25 g
Dexamethasone	Azium Azium SP	Injection, 4 mg/ml	Ventricular tachycardia, complete atrioventricular block	0.1 mg/kg to 0.22 mg/kg IV or IM
Glycopyrrolate or atropine	Robinol Atropine USP	0.2 mg/L 0.4 mg/L	Sinus bradycardia, vagal-induced arrthythmias	0.005 to 0.01 mg/kg IV
Sodium bicarbonate	Bicarbonate sodium, USP	Approximately 1 mEq/ml, available in ampules or bottles	Hyperkalemia, atrial standstill; quinidine toxicosis	1 mEq/kg IV; can be repeated
Phenylephrine HCl	Neo-Synephrine	10 mg/ml	Arterial hypotension, excessive vasodilation, quinidine toxicosis	0.01 mg/kg to effect

* CHF, Congestive heart failure; IV, intravenous(ly); IM, intramuscularly.

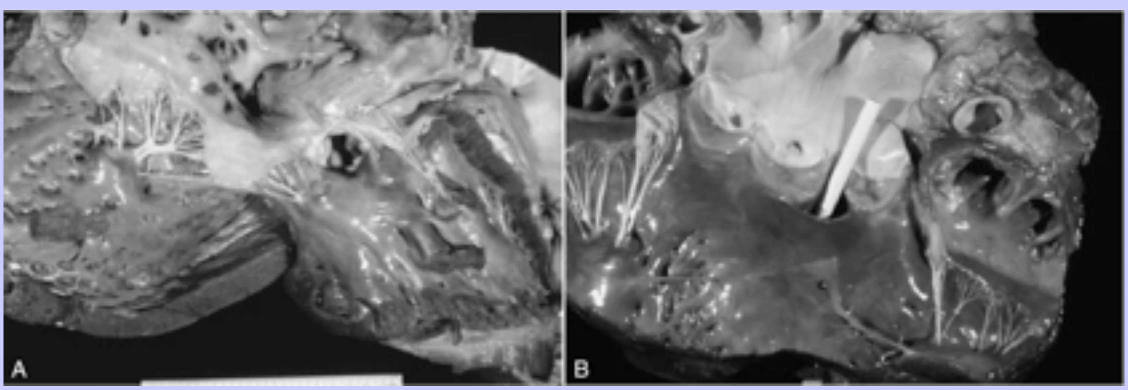
Although some defects are lethal to the neonate, others are compatible with life but limit peak performance or reproductive value. Most cardiac malformations involve shunting of blood, with the VSD most often recognized (Figures 8-26 to 8-28). Isolated malformations leading to valvular stenosis or incompetency are uncommon. Rare lesions such as subaortic stenosis,[530] double-outlet right ventricle, transposition of the great vessels,[147,155] bicuspid

pulmonary valve, pulmonary stenosis,[158] persistent fetal circulation,[549] hypoplastic left heart syndrome,[148,149] mitral valve malformation,[439] endocardial fibroelastosis,[60,550] total anomalous pulmonary venous return, and aortic origin of the pulmonary artery are not discussed further. Some of the more frequently recognized defects[297] are described later.

8.6.1 PATHOGENESIS

The underlying genetic factors guiding normal development of the heart and those leading to cardiac malformation are understood incompletely. Cardiac morphogenesis is complicated, but understanding elementary aspects of cardiac development is helpful, especially as these pertain to congenital heart disease.[551] Among these fundamentals are the septation of the atria, the anatomic components forming the ventricular septum, the separation of the great vessels, and the normal fetal circulation.

Figure 8-26 Pathologic evaluation of ventricular septal defects. **A,** Opened right ventricle from a mare demonstrating a perimembranous septal defect opening just beneath the septal leaflet of the tricuspid valve. Aortic valve cusps are visible through the defect. Congestive heart failure occurred late in life, after the development of atrial fibrillation. **B,** A large ventricular septal defect in a horse. A probe runs through the defect. The dorsal location immediately beneath the right and the noncoronary cusps of the aortic valve are notable. Ostia of both coronary arteries are also evident.

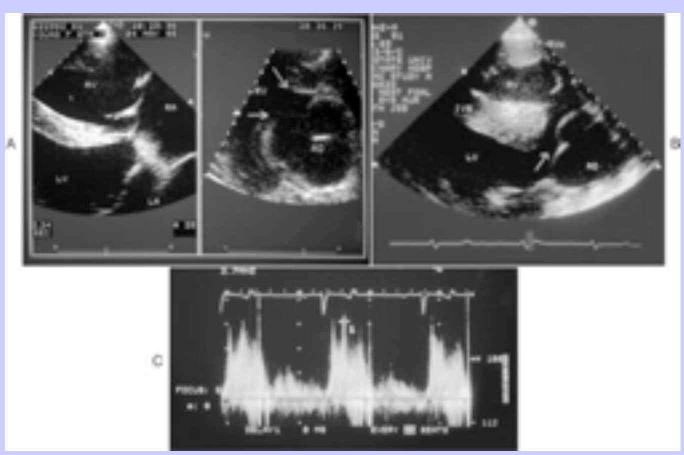


The right and left atria are separated by incorporation of the right horn of the sinus venosus and through development and fusion of two prominent membranes, septum primum and septum secundum. The endocardial cushions close the gap between the atrial and ventricular septa. These tissues also contribute to the atrioventricular septum, that segment filling the gap between the mitral valve septal insertion on the left and the more ventral tricuspid valve insertion on the right. The foramen ovale, a normal atrial structure, is located approximately in the middle of the atrial septum and creates a passageway for blood to flow from right to left

atrium in the normal fetus. The equine foramen ovale resembles a fenestrated finger cot and is visible with echocardiography as a mobile septal membrane even in healthy, full-term foals. This interatrial path may persist in foals with pulmonary hypertension and elevated RA pressures. Failure of normal development in any of these tissues can lead to an atrial septal defect (ASD), which is typically designated by the location of the defective membrane (Figure 8-29).

The ventricular septum is a complicated partition that includes a small membranous portion located opposite the aortic root and craniodorsal to the tricuspid valve, an inlet septum below the septal tricuspid leaflet, an apically 399
located muscular or trabecular septum, and a dorsal outflow segment that separates the subaortic and the 400
subpulmonic infundibulum (see Figure 8-28). The ventral atrial septum connects to the dorsal ventricular septum by growth and differentiation of endocardial cushions. These swellings also form the atrioventricular valves. Insufficient development of any embryonic component of the ventricular septum can lead to a VSD, the most common cardiac malformation in horses. Defective differentiation of the endocardial cushions causes various combinations of an ostium primum (ventral) ASD, an inlet VSD, malformation of the atrioventricular valves, or common atrium with a single atrioventricular valve.

Figure 8-27 Echocardiograms demonstrating ventricular septal defects (see also Figures 8-22, *A;* 8-23, *A;* and 8-31). **A,** This subaortic ventricular septal defect is not evident from the long-axis image *(left panel)* but is visible in the short-axis tomogram *(arrows)*. This Quarter Horse gelding also had pulmonic stenosis. The elevated right ventricular pressures have led to significant right ventricular enlargement with bulging of the septum toward the left ventricle. **B,** Malalignment and a perimembranous septal defect associated with prolapse of an aortic valve leaflet across the defect *(arrow)*. (*RVW,* Right ventricular wall.) **C,** A continuous wave Doppler study recorded from a filly with a large, unrestrictive ventricular septal defect. The maximal velocity of 3.1 m/sec predicts a pressure difference of less than 40 mm Hg.



The aorta and pulmonary artery begin as a single vessel in the conus arteriosus. This common vessel, the truncus arteriosus eventually is partitioned by migration of the conus and development of the conotruncal and spiral septa. Twisting of the spiral septum produces appropriate alignment (concordance) of the great vessels with their respective ventricular chambers. The descending aorta and pulmonary artery are connected by the ductus arteriosus, which carries fetal blood from pulmonary artery to descending aorta. Maldevelopment of conotruncal or spiral septal tissues leads to complicated congenital heart defects in the horse, including persistent truncus arteriosus[552] and double-outlet right ventricle.[520] Persistent patency of the ductus arteriosus is rare (Figure 8-30).

An understanding of the fundamental circulatory patterns of the fetus is helpful. Two circulations exist, one serving the embryo and the other communicating with the placenta. Functionally, two right-to-left shunts exist: one across the foramen ovale and the other across the ductus arteriosus. The fetal lungs are collapsed, pulmonary 400
vascular resistance is high, and pulmonary blood flow is minimal. Desaturated blood returning from the fetal 401
tissues is collected in the cardinal venous system and enters the sinus venosus and right atrium. This blood is largely earmarked for the right ventricle and pulmonary artery. Most pulmonary arterial flow is diverted through the ductus arteriosus to the descending aorta and placenta to be oxygenated. Well-saturated blood returning across the umbilical veins is delivered by the caudal vena cava to the right atrium where it preferentially crosses the foramen ovale to enter the left atrium, left ventricle, and ascending aorta. These patterns change dramatically with foaling. As the lungs expand, pulmonary vascular resistance falls, and pulmonary blood flow increases. The resultant increase in LA pressure functionally closes the foramen ovale within the first 24 to 48 hours of life. Similarly, inhibition of local prostaglandins leads to functional closure of the ductus arteriosus within 72 hours in 401
most full-term foals. Persistence of the right-to-left shunts, especially at the level of the foramen ovale, can occur 402
in premature foals or those suffering from severe pulmonary disease with associated pulmonary hypertension. In these cases, shunting across the foramen ovale represents an additional mechanism for arterial desaturation and tissue hypoxia.

Figure 8-28 Pathophysiology of ventricular septal defects *(VSD)*. See text for details. *LA,* Left atrium; *RA,* right atrium; *LVDP,* left ventricular diastolic pressure; *RV,* right ventricle, *RVH,* right ventricular hypertrophy. (From Bonagura JD: Congenital heart disease. In Bonagura JD, editor: *Cardiology,* New York, 1987, Churchill Livingstone.)

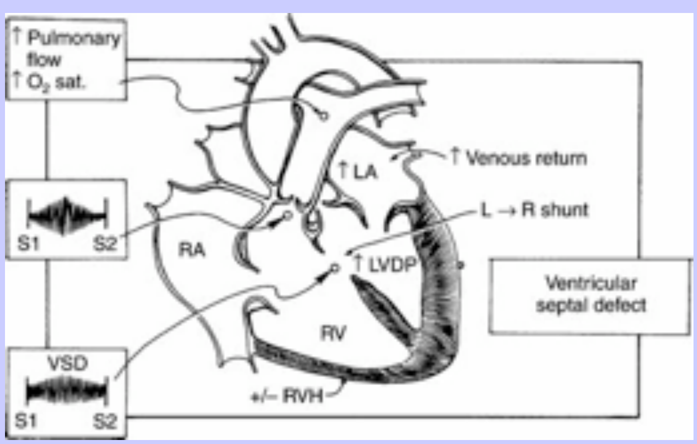


Figure 8-29 Echocardiogram demonstrating a primum atrial septal defect in a foal with complex congenital heart disease that included a common *(left)* ventricle, rudimentary *(right)* ventricle, and double-outlet ventricle. The septal defect is evident between the four cardiac chambers. The dorsal secundum septum *(right)* is present as well as the apical ventricular septum *(left)*.

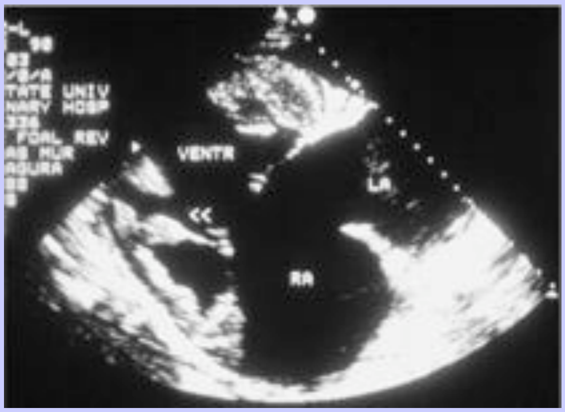


Figure 8-30 A postmortem demonstration of the ductus arteriosus *(arrow)* between the descending aorta and the pulmonary artery in a foal with complex congenital heart disease.

* References 3, 14, 27, 28, 60, 87, 103, 104, 115, 116, 121, 124, 135, 141, 145–159, 233, 276, 290, 297, 318, 395, 401, 402, 404, 410, 428, 456, 497, 498, 502, 503, 505, 518–546.

8.6.2 CLINICAL PATHOPHYSIOLOGY OF SHUNTS

Fundamental to understanding cardiac malformations in the horse is an appreciation of shunt physiology and the potential responses of the heart and circulation to a shunt.[518] Shunting can be defined as abnormal deviation of blood flow between the systemic (left) and pulmonary (right) sides of the circulation. Possibilities include left-to-right, right-to-left, and bidirectional shunting.

Systemic-to-pulmonary (left-to-right) shunting is the expected result of an ASD, VSD, or patent ductus arteriosus (PDA) so long as systemic pressures and resistance exceed that on the right side. Even in cases of abnormal ventricular-arterial development, as with persistent truncus arteriosus, double-outlet right ventricle, or univentricular heart, the clinical findings of a left-to-right shunt may predominate unless obstruction to blood flow occurs at the pulmonary valvular or arteriolar levels. The actual shunt volume carried to the lungs depends on the caliber (or restrictive nature) of the lesion orifice and the relative resistances between the systemic and pulmonary circulations. Shunting may not be significant after foaling because pulmonary vascular resistance is still high and systemic arterial and LV pressures are low for a number of weeks after birth. Eventually left-to-right shunts increase pulmonary arterial flow and augment pulmonary venous return. The horse easily handles small shunts through mild left-sided dilation and hypertrophy. When the pulmonary to systemic flow ratio exceeds about 1.8:1, the shunt is usually considered clinically significant. The result is more noticeable volume overload of the left atrium and left ventricle. The greater the shunt, the higher the potential for left-sided or biventricular CHF from chronic ventricular systolic or diastolic dysfunction. Pulmonary hypertension can occur in the setting of left-to-right shunting from combinations of increased flow, inadequate development of pulmonary arterioles, and LV dysfunction. Thus consequences of significant left-to-right shunting may include any of the following: exercise intolerance, tachypnea, pulmonary edema, respiratory distress, pulmonary hypertension, atrial fibrillation, pleural effusion, jugular venous distention, or ventral edema. The foal may be smaller than expected and may have a history of antibiotic therapy for presumed bouts of pneumonia.

Right-to-left shunting stems from a different pathophysiologic state and produces another clinical presentation. When a shunt is complicated by a right-sided obstruction adjacent to or downstream from the defect, right-to-left shunting develops as right-sided pressures exceed those on the left side. This can occur in the neonate, as in tricuspid valve atresia with ASD or pulmonary valve atresia with VSD. Conversely, elevated right-sided resistance can develop more chronically from pulmonary vascular disease. For example, a large left-to-right shunt can induce medial hypertrophy and intimal thickening of pulmonary arterioles elevating pulmonary vascular resistance and decreasing the left-to-right shunt. Though uncommon, the resultant pulmonary hypertension may become severe (Eisenmenger's physiology) and reverse the shunt to right-to-left. In these cases the left-side heart chambers are small and the right ventricle is hypertrophied to achieve systemic blood pressure.

The entrance of desaturated blood from the right to left side of the circulation causes hypoxemia. Thus the consequences of a significant right-to-left shunt may include low arterial Po_2, tissue hypoxia, cyanosis, exercise intolerance, mild to moderate polycythemia, hyperviscosity of blood, and stunting of growth. Congestive heart failure is rare, but sudden death can occur, presumably from arrhythmia. The degrees of hypoxemia and cyanosis in a right-to-left shunt depend on overall pulmonary blood flow; this is a pivotal concept. If pulmonary flow is diminished, as with tetralogy of Fallot, tricuspid atresia, and pulmonary atresia with restrictive pulmonary flow, the contribution of the left ventricle to aortic flow (and thus oxygenation) is low. In some instances, right-to-left shunting develops in the setting of increased pulmonary blood flow, as with truncus arteriosus or double outlet

ventricle. These lesions cause less hypoxemia because the amount of oxygenated blood reaching the left ventricle is normal to increased. In such situations cyanosis from right-to-left shunting may be negligible and the clinical condition of left-sided or biventricular CHF may predominate. If the increased pulmonary flow is sufficient to minimize the arterial hypoxemia but not cause CHF, survival beyond 5 years of age is possible.

8.6.3 VENTRICULAR SEPTAL DEFECTS

VSD is the most important congenital heart disease of horses.[*] A genetic basis is likely in the Arabian breed. In the authors' experience, VSD also occurs regularly in Standardbred horses and in Quarter Horses. The VSD often accompanies more complicated malformations.

The location of a VSD depends on the embryogenesis of the lesion and influences the designation and even the clinical manifestations of the defect. The nomenclature of VSDs is confusing, but one may recall it best by 402
considering the main components of the normal ventricular septum (see Figures 8-26 to 8-28). In most cases a 403
VSD is located dorsally (high) on the ventricular septum, below the right or noncoronary cusp of the aortic valve on the left side, and craniodorsal to the septal tricuspid leaflet on the right.[428] Such defects generally are termed *perimembranous* or perhaps more correctly *paramembranous*. Most of these holes are also subcristal because the VSD is located caudoventral to the supraventricular crest that muscle separates the right ventricular inlet and outlet. However, a large paramembranous defect may extend under the tricuspid valve toward the inlet septum or advance across the supraventricular crest toward the outlet septum. The (conotruncal) septal defects associated with tetralogy of Fallot (Figure 8-31) and with pulmonary atresia are usually large and fall into the latter appellation. Sometimes the aortic root is displaced ventrocranially and straddles the defect, creating a malalignment VSD that is characteristic of the tetralogy of Fallot but also can occur with large paramembranous defects. Malalignment is clinically significant because the right or noncoronary cusp of the aortic valve is likely to prolapse into the defect, leading to aortic regurgitation (see Figure 8-27, *B*). A less common location for a VSD is immediately ventral to the septal tricuspid valve within the muscular septum. Such inlet VSDs are typical of an endocardial cushion defect and may be related to a primum ASD or even a persistent common atrioventricular canal, which creates a gap between all four cardiac chambers. A subaortic VSD that communicates with the outlet portion of the ventricular septum directly below the pulmonary valve is variably termed an *outlet, supracristal, subpulmonic, subarterial,* or *doubly committed* VSD. This lesion also places the aortic valve at risk for prolapse. Finally, apical muscular (trabecular) defects or multiple VSDs are rare but have been observed in horses. Some of these are small, whereas others have been enormous.

Figure 8-31 A two-dimensional echocardiogram from a filly with tetralogy of Fallot. A large ventricular septal defect is present between the right ventricular outflow tract *(RVOT)* and the subaortic region (*Ao,* aortic valve cusp observed in the oblique plane). The pulmonary artery eventually widens into a poststenotic dilation *(PSD)*. Shunting is bidirectional in this case.

Many VSDs close spontaneously in human beings, but whether this is common in horses is unknown. However, imposition of a cardiac valve can diminish the flow across a VSD. For example, scar tissue that ensnares the septal tricuspid leaflet may occlude the rim, or even a major portion of a VSD, by rendering the defect functionally smaller and possibly creating a hyperechoic aneurysm on the right septal surface. Large defects associated with malalignment of the ascending aorta to the upper border of the remaining septum often are associated with prolapse of an aortic valve leaflet (or of the aortic root) into the defect. Aortic prolapse effectively can close even a large VSD, but at the risk of permitting chronic aortic valve insufficiency over time. 428

The pathophysiology of the uncomplicated VSD is that of a left-to-right shunt as described previously (see Figure 8-28). Much of the shunt volume pumped by the left ventricle is ejected immediately into the pulmonary artery. As pulmonary flow increases, venous return to the left atrium and left ventricle increases, causing LV dilation and hypertrophy recognizable by echocardiography. Thus the left (not the right) ventricle performs most of the extra volume work. Shunting is more severe if the aortic valve prolapses with aortic regurgitation or if MR develops because of LV enlargement. If the shunt is large and pulmonary arteriolar resistance does not increase significantly, LV failure can develop. Failure is most likely to occur early in life, as the high fetal pulmonary vascular resistance declines, but late cases of CHF (with atrial fibrillation) also occur. The degree of RV hypertrophy and enlargement varies, depending on the location and size of the septal defect and pulmonary vascular resistance. Large nonrestrictive defects cause the two ventricles to behave as a common chamber, allowing ventricular pressures to equilibrate and leading to severe RV hypertrophy and pulmonary hypertension.

The clinical features of VSD vary.[428,518] Clinical signs may be absent, and one may identify the defect as an incidental finding. A mature horse may be presented for poor performance or with atrial fibrillation. Foals may be symptomatic for pulmonary edema or biventricular heart failure. Most commonly, a murmur is detectable

incidentally during the physical examination for another problem or during a prepurchase examination. Because most defects communicate near the RV inlet septum, the most consistent physical examination finding is a harsh, holo- or pansystolic murmur that is loudest just below the tricuspid valve and above the right sternal border. An ejection murmur (one to two grades softer) of relative pulmonic stenosis (from increased flow) is usually evident over the left base. The S_2 may be split more widely than normal because of disparate ventricular ejection times; the pulmonic component of S_2 may be more tympanic if pulmonary hypertension is present. In contrast, the murmur of a subarterial (subpulmonic or supracristal) VSD is loudest over the left cranial base as the high-velocity flow enters just below the pulmonary artery. When VSD is associated with complex cardiac malformation, the murmur is likely to be loud over each side of the thorax. One cannot judge the severity of the defect based on murmur intensity. In some cases a small defect may be loud; whereas a large, less restrictive defect may cause a murmur related entirely to pulmonic stenosis. If significant LV volume overload occurs, the mitral valve may become incompetent and a holosystolic murmur of MR may be evident over the left apex. The rare trabecular (muscular) VSD also may create a systolic murmur over the left or right apex. If significant cardiomegaly develops, atrial and ventricular premature complexes, or even atrial fibrillation, may occur. A holodiastolic murmur of aortic regurgitation indicates prolapse of an aortic cusp and the likelihood that the lesion is large. Substantial aortic regurgitation is associated with a hyperdynamic arterial pulse. The VSD associated with pulmonary atresia or persistent truncus arteriosus may not create a substantial murmur, but the increased flow through the dilated single vessel usually creates a loud ejection murmur over each side of the chest.

Diagnostic studies confirm the lesion and importantly assess the hemodynamic burden. Noninvasive studies are sufficient for diagnosis in almost every case, and cardiac catheterization has become unnecessary except in rare instances. The performance history is a useful overall indicator of effect, and the horse with an excellent work history is unlikely to have a large defect. The ECG is unreliable for diagnosing cardiomegaly in horses but is indicated in the setting of an arrhythmia. Thoracic radiography can be useful in foals to demonstrate cardiomegaly (see Figure 8-19, *A*), the pulmonary circulation, and the lungs and pleural space. Two-dimensional echocardiography and color Doppler imaging establish the diagnosis.[400] M-mode studies and spectral Doppler examinations are useful for assessing the hemodynamic burden of the defect.

Two-dimensional echocardiography successfully delineates the VSD in virtually all cases, provided one obtains a sufficient number of imaging planes (see Figures 8-22, 8-23, and 8-27). Collecting long-axis images of the LV outflow tract and aortic valve is important, as well as collecting short-axis images at the level of the LV outflow tract, just below the aortic leaflets. The typical paramembranous defect appears under the aortic valve and adjacent to the septal leaflet of the tricuspid valve. A true defect is characterized by an echogenic tissue interface; whereas an area of false echo dropout tends to be gradual. One can image most defects in orthogonal (long-axis/short-axis) planes. The right coronary artery of the horse is large and may be confused with a subarterial (supracristal) VSD. One also should note that an inlet VSD (ventral to the septal tricuspid valve) might not be easily visible in standard planes. Tipped or oblique views that show both atrioventricular valves may be required. Similarly, finding a muscular, apical, or small subarterial defect requires more imaging experience and is greatly assisted by color Doppler studies.

One should attempt to identify the largest diameter of the defect in complementary planes, and compare this with the size of the aorta, because orifice size is an important prognostic factor. Although two-dimensional sizing of the VSD has limitations, a defect exceeding 2.5 cm in diameter or a VSD/aortic root diameter of greater than 0.4 cm identifies a large defect with greater likelihood of clinical signs. Furthermore, two-dimensional or M-mode evidence of left-sided cardiac dilation, right ventricular enlargement, or significant dilation of the main pulmonary artery suggest a hemodynamically significant VSD and one more likely to affect performance or survival.

Color Doppler studies are best for identifying shunting across a VSD. Typically, high-velocity, turbulent flow enters the right ventricle during systole with low-velocity, uniform color shunting noted during diastole. Color Doppler imaging is helpful for identifying a VSD with atypical location and also may identify aortic regurgitation in some horses. Continuous wave Doppler is used to estimate the pressure difference between the two ventricles, for velocity (in meters/second) is proportional to the instantaneous pressure difference across the ventricles ($\Delta P = 4V^2$). A small VSD is restrictive to flow, and the peak shunt velocity generally exceeds 4.5 m/sec, assuming proper alignment to shunt flow. Should pulmonary hypertension develop related to increased pulmonary flow, left-sided heart failure, or pulmonary vascular injury, the velocity of left-to-right shunting will be lower, and one may identify a high-velocity jet of TR (>3.5 m/sec).

Potential outcomes of the isolated VSD include tolerance of the lesion; partial or complete closure of a VSD by adherence of the septal tricuspid leaflet, fibrous tissue, right ventricular hypertrophy, or aortic valve prolapse; progressive aortic regurgitation; atrial fibrillation; left-sided or biventricular CHF; pulmonary hypertension (with left-to-right shunting); or reversal of the shunt with development of arterial hypoxemia and cyanosis. The latter 404
would be caused by severe pulmonary vascular disease (Eisenmenger's physiology) or fibromuscular obstruction 405
in the right ventricular outlet leading to subpulmonic stenosis. The horse with a small-diameter paramembranous defect, high-velocity left-to-right shunt, mild cardiomegaly, normal right ventricular cavity, and normal heart rhythm probably has a restrictive VSD that will be well tolerated. Most of these animals can perform sufficiently in the show ring, as a hunter-jumper, or even as an endurance or racehorse. Large defects that are associated with echocardiographic evidence of moderate to severe cardiomegaly, right ventricular hypertrophy, aortic root prolapse, or Doppler evidence of pulmonary hypertension are prone to complications and carry a less favorable prognosis for performance or life regardless of current clinical signs.

Definitive therapy for VSD involves cardiopulmonary bypass surgery and is impractical. Surgical banding of the pulmonary artery elevates right ventricular pressures and reduces left-to-right shunting; however, this procedure also limits CO and is not advisable. Should CHF or atrial fibrillation develop, one can consider medical therapy in selected cases, but even if the response to treatment is good, the owner should not use the horse. Treatment involves daily administration of digoxin and furosemide (with or without an ACE inhibitor if economics are not an issue) as discussed previously. In the authors' view, one generally should discourage breeding of affected animals, especially in Arabian horses.

* References 121, 135, 141, 147, 152, 155, 401, 404, 428, 456, 503, 518, 525, 531, 537–541.

8.6.4 ATRIAL SEPTAL DEFECTS

ASDs, including endocardial cushion defects, are uncommon in foals.[*] As indicated previously, an ASD may involve different portions of the atrial septum. Abnormal atrial septal patency is more likely to be observed with complex congenital cardiac defects, particularly with tricuspid or pulmonary atresia. An isolated ASD may be clinically insignificant, with no significant murmur or clinical signs. Moderate to good exercise capacity may be expected because left-to-right shunting decreases as the systemic to pulmonary vascular resistance ratio declines with exercise. In the case of a large ASD, left-to-right shunting leads to right-sided volume overload and pulmonary overcirculation. Such defects are visible with echocardiography (see Figure 8-29), and Doppler echocardiography can confirm the direction of the shunt and estimate its severity. Atrial fibrillation may occur along with ASD.

Complete endocardial cushion defects rarely occur, but are serious, usually leading to CHF or atrial fibrillation at an early age. This developmental defect typically consists of a large ASD of the primum and atrioventricular

septum, a common atrioventricular valve leaflet, and a defect in the inlet portion of the ventricular septum. The ventricles may be partitioned normally, unequally with one rudimentary ventricular chamber, or not at all, creating a single ventricle. In the most severe cases a common atrioventricular canal, single large atrioventricular valve, and common single ventricle exist from which both great vessels exit. The clinical signs of a complete endocardial cushion defect vary. The foal with an unobstructed outlet to the pulmonary arteries is hemodynamically similar to one with a large VSD. When a common ventricle is present, one may observe varying degrees of cyanosis. A systolic murmur is typical and may reflect flow across the VSD, left-to-right shunting (relative pulmonary stenosis), or atrioventricular valve regurgitation. An echocardiographic evaluation reveals an ASD involving the primum portion of the atrial septum; a VSD involving the muscular, inlet segment of the interventricular septum; and malformed atrioventricular valves. Doppler echocardiography reveals the intracardiac shunts and atrioventricular valvular regurgitation. CHF may supervene. The prognosis is poor.

* References 121, 149, 151, 153, 456, 505, 537.

8.6.5 PATENT DUCTUS ARTERIOSUS

PDA is a rare congenital cardiac defect in foals and is detected most frequently in combination with other, more complex congenital cardiac defects (see Figure 8-30).[*] The ductus arteriosus is a fetal vessel, derived from the left sixth aortic arch, that permits shunting from the pulmonary artery to the descending aorta in the fetus. At birth the ductus arteriosus normally constricts, in response to increased local oxygen tension and inhibition of prostaglandins. The ductus arteriosus is functionally closed 72 hours after birth in the majority of foals. If the ductus arteriosus does not close, a left-to-right shunt from the aorta to pulmonary artery occurs. Although some hereditary predisposition to the development of this congenital lesion in other species may occur, this lesion is so rare as an isolated congenital defect that this is not a significant concern. Premature foals, foals with persistent pulmonary hypertension, and foals with dams that have been given prostaglandin inhibitors may be more susceptible to developing a PDA.

The clinical signs depend on the magnitude of the shunting through the PDA, which is determined by its resistance and that of the pulmonary circulation. Physical examination findings (with a left-to-right PDA) include a continuous machinery murmur and thrill, usually loudest over the main pulmonary artery (craniodorsal to the aortic valve area), and bounding arterial pulses. 405

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Echocardiography reveals pulmonary artery, LA, and LV volume overload. The severity of these findings depends on the magnitude of the shunt. Direct visualization of the PDA is not always possible by two-dimensional echocardiography, because the ductus arteriosus may be hidden from view by the overlying lung. One can best identify ductal flow from the left hemithorax by Doppler echocardiographic examination of the main pulmonary artery. Doppler studies demonstrate continuous high-velocity, turbulent flow, toward the main pulmonary artery. One may detect cardiac enlargement and increased pulmonary vascularity in neonatal foals with a PDA and may identify radiographic evidence of pulmonary edema if the foal has developed CHF. Cardiac catheterization reveals elevated pulmonary artery and capillary wedge pressures and increased pulmonary arterial oxygen saturation. On diagnosing PDA, the clinician should evaluate the foal carefully for other congenital cardiac disease before attempting surgical correction, because complex congenital malformations are the rule in foals with a PDA, and not the exception. Late complications of this lesion include rupture of the pulmonary artery.

* References 146, 149, 155, 158, 233, 318, 502, 519, 524.

8.6.6 TETRALOGY OF FALLOT

The tetralogy of Fallot is one of the more common congenital cardiac anomalies in foals responsible for right-to-left shunting with arterial desaturation and cyanosis.[146,152,157,456] The four lesions are large paramembranous VSD, right ventricular outflow tract obstruction, cranial and rightward (dextro-) positioning of the aorta with overriding of the septal defect, and right ventricular hypertrophy. Outflow obstruction can be caused by subvalvular fibromuscular obstruction, valvular pulmonic stenosis, or hypoplasia of the pulmonary artery. Ventricular hypertrophy is caused by right ventricular outflow obstruction and the large, unrestrictive, VSD that functionally creates a common ventricle. Blood leaves the heart along the path of least resistance, and pulmonary flow depends on the severity of right ventricular outflow tract stenosis. As previously discussed, the degree of cyanosis and severity of clinical signs depends on the volume of blood traversing the lungs. In some horses a PDA is also present (pentalogy of Fallot), and this defect reduces signs by increasing pulmonary flow, left-side heart filling, and systemic arterial hemoglobin saturation.

Affected foals are usually smaller than normal, lethargic, and intolerant of exercise. Cyanosis is most evident after exercise but is variably present at rest. Arterial blood gas analysis demonstrates hypoxemia with normal or reduced P_{CO_2}. Auscultation typically is characterized by a loud systolic murmur over the pulmonic valve area on the left side caused by (sub-)pulmonic stenosis. The S_2 is usually unremarkable. Although polycythemia can be significant, it is usually mild, even when arterial oxygen tensions fall to 50 to 70 mm Hg.

Echocardiographic evaluation is diagnostic and reveals a large, unrestrictive VSD, right ventricular outflow tract obstruction, malalignment and overriding of the aortic root, and right ventricular hypertrophy (see Figure 8-31). One can identify shunting by color Doppler or saline contrast echocardiography. An injection of agitated saline into the jugular vein results in a positive contrast echocardiogram and similar to the color Doppler study discloses the right-to-left or bidirectional shunt. One can use conventional spectral Doppler studies to delineate the shunt (typically bidirectional, low-velocity flow of <2 m/sec) and right ventricular outflow obstruction (high-velocity flow exceeding 4 m/sec).

Although horses possibly may live for a number of years with tetralogy of Fallot, most affected animals are destroyed humanely because of the poor prognosis for life. Owners should not use or breed affected horses if they survive to maturity. One must distinguish tetralogy of Fallot from other causes of cyanotic heart conditions, including tricuspid atresia, pulmonary atresia with VSD, D-transposition of the great vessels, and double-outlet right ventricle with pulmonary stenosis.

8.6.7 PULMONARY ATRESIA WITH VENTRICULAR SEPTAL DEFECT

Pulmonary atresia with VSD is rare, having been observed most often in Arabian foals.[146,540,545,553] This malformation represents in some ways the exaggerated form of tetralogy of Fallot, with the following findings:

1. Atresia of the right ventricular outlet
2. Hypertrophy of the right ventricle
3. Presence of a large malalignment VSD (in most cases)
4. Partitioning of the fetal truncus arteriosus so unequal that the aorta is greatly dilated and the pulmonary trunk is atretic or severely hypoplastic

Without careful ultrasound studies (or necropsy dissection) of the pulmonary circulation, one may mistake the dilated aorta for a persistent truncus arteriosus, hence the moniker *pseudotruncus arteriosus*. Because of the atretic pulmonary valve, pulmonary blood flow must be derived from a PDA or the aorta. In the latter instance, the systemic collaterals are usually from bronchial arteries. Pulmonary atresia with intact ventricular septum has been diagnosed rarely.[546]

The diagnosis of pulmonary atresia usually is stimulated by clinical findings of cyanosis, cardiac murmur, and stunting in a foal or weanling. Echocardiography confirms the diagnosis. Careful imaging can identify the main lesions: concentric hypertrophy of the right ventricle, unrestrictive VSD, dilated malaligned great vessel (the aorta), and inability to identify the pulmonary valve in the rudimentary right ventricular outflow tract (though a small pouch may be visible). Careful ultrasound examination of the ascending aorta and aortic arch from the right and left sides of the thorax will fail to reveal a normal origin for the pulmonary trunk; however, one usually can find the bifurcation of the pulmonary artery from a cranial imaging position and can document continuous flow into the vessel by pulsed wave Doppler echocardiography. The origin of pulmonary flow is typically from the ductus arteriosus or a large collateral systemic artery.

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8.6.8 TRUNCUS ARTERIOSUS

The failure of the fetal truncus arteriosus to partition into the aorta and pulmonary artery represents a rare anomaly of the equine heart.[154,539] In this condition the fetal truncus never partitions and both ventricles continue to develop, communicating with the truncus arteriosus across a large VSD. Systemic, coronary, and pulmonary arterial flows each arise from the truncus, which is guarded by a truncal valve (which can be incompetent or stenotic). Pulmonary blood flow originates from one or more pulmonary arteries, which arise directly from the truncus arteriosus in one of three general ways (types I, II, and III).

The pathophysiology and clinical findings of this malformation depend largely on the magnitude of pulmonary blood flow. If the pulmonary artery origins are not stenotic and if pulmonary vascular resistance remains low, the clinical condition resembles a left-to-right shunt, except for right-to-left mixing of blood across the VSD. However, the degree of arterial hypoxemia may not be severe, and cyanosis may not be obvious. In such cases, development to maturity is possible provided CHF does not occur from unrestricted pulmonary flow and volume overload of the left ventricle. Conversely, high pulmonary vascular resistance in truncus arteriosus is associated with diminished pulmonary flow, arterial desaturation, and findings more similar to pulmonary atresia as discussed previously.

Clinical examination usually indicates a systolic cardiac murmur. The mucous membranes may be pink or cyanotic. If left-to-right shunting is significant, CHF may occur. With careful ultrasound examination, one can identify the truncus and origin of the pulmonary arteries, thus distinguishing the condition from pulmonary atresia with VSD. Furthermore, in some cases an abnormal truncal valve (with four leaflets) suggests the diagnosis. Management is best accomplished by consultation with a cardiac specialist.

8.6.9 TRICUSPID ATRESIA

Another differential diagnosis for cyanotic heart disease is atresia of the tricuspid valve.[141,146,153,155,553–555] This malformation dictates right-to-left shunting of systemic venous blood at the atrial level. The atrial shunt may be across a true ASD or a patent foramen ovale. Because all venous return must mix in the left atrium, this malformation generally causes significant hypoxemia with cyanosis. Affected foals rarely survive to weanling

age unless a VSD and functional right ventricular outflow tract exist. Otherwise, pulmonary flow must come from a ductus arteriosus or systemic collaterals (e.g., bronchial arteries). Most foals are stunted, nurse poorly, and exhibit severe exercise intolerance and cyanosis at rest. Arterial oxygen tension can be low (40 to 60 mm Hg). Echocardiography reveals a dilated right atrium and coronary sinus, atretic tricuspid valve, and rudimentary right ventricle (larger if a functional left-to-right shunting VSD is present). One must observe atrial shunting that allows systemic venous return to empty into the left atrium; a VSD may be present. One can verify abnormal flow patterns by contrast or color Doppler echocardiography. Prognosis is grave.

8.7 Acquired Valvular Heart Disease

Healthy cardiac valves maintain normal antegrade flow in the heart and prevent significant regurgitation of blood. Diseased cardiac valves, which can be stenotic or incompetent, limit CO and place an increased workload on the heart. Stenotic valvular lesions in horses are typically congenital and are rare.[115,146,147,158] However, acquired valvular incompetency (also called valvular insufficiency or valvular regurgitation) is common.[*] The majority of valvular diseases in horses are degenerative.[†] Bacterial endocarditis, ruptured chordae tendineae, and inflammatory valvulitis are infrequent causes of valvular disease. Box 8-2 summarizes important causes of valvular dysfunction. Degenerative valvular disease and bacterial endocarditis are the valvular problems most often encountered by the equine practitioner and are the focus of this section.

The clinical significance of a valvular lesion depends largely on the severity of regurgitation across the valve. Many horses clearly adapt to trivial or mild valvular regurgitation with no apparent effect on performance. The severity of valvular regurgitation is related to the cross-sectional area of the regurgitant orifice, the pressure gradient driving blood across the valve, and the time allowed for regurgitation. Regardless of the volume, the movement of blood from a high to low pressure chamber is associated with a high-velocity jet proportional to the pressure drop measured from source to sink. The production of high-velocity jets leads to disturbed flow (with 407
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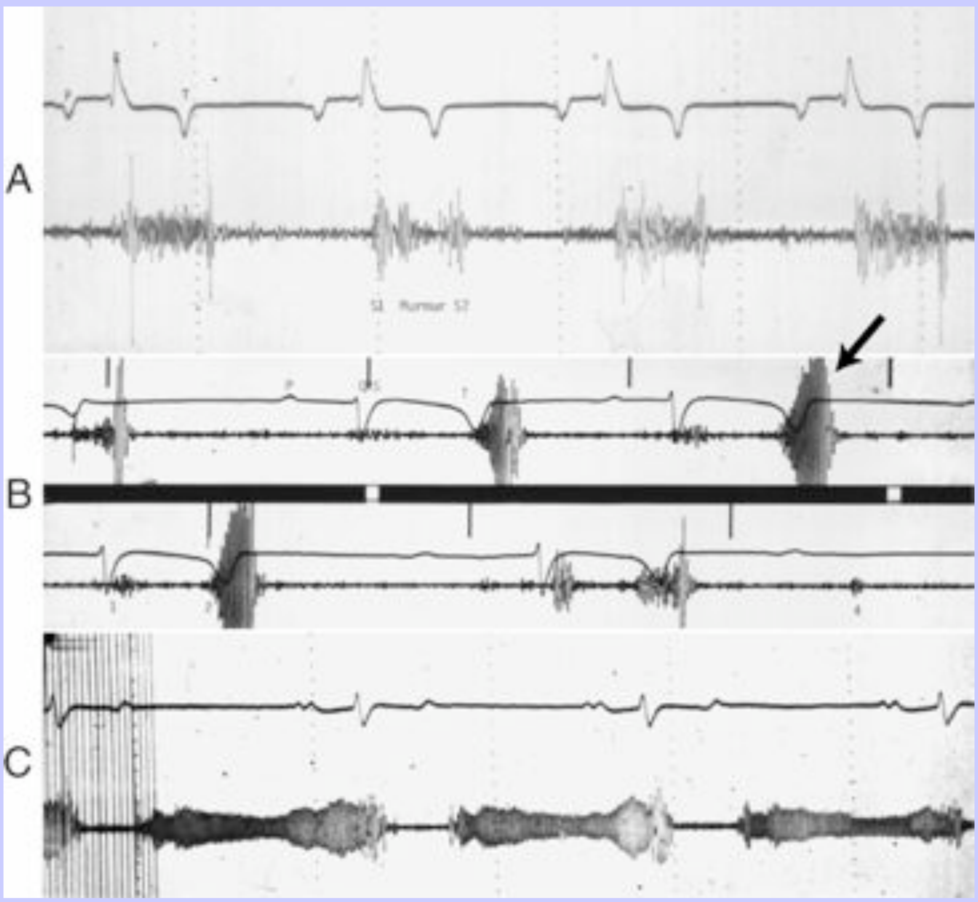
turbulence) and in many cases to a cardiac murmur, the hallmark clinical feature of valvular heart disease (Figure 8-32).

Figure 8-32 Phonocardiograms of cardiac murmurs caused by valvular heart disease. **A,** A holosystolic murmur of mitral regurgitation in a horse with chronic valvular degeneration. **B,** A variable, late systolic murmur of mitral regurgitation related to mitral valve prolapse. The murmur has a crescendo and peaks at end systole *(arrow)*. Heart sounds are indicated. The murmur obscures the second sound. **C,** A holodiastolic, vibratory murmur of aortic regurgitation with presystolic accentuation. The accentuation probably is related to atrial contraction, altered ventricular volume and pressure, and an incremental increase in regurgitant volume.



One may diagnose valvular incompetency by auscultation or with Doppler echocardiography.[119,430,437] Auscultation is the most clinically important method for identifying clinically significant valvular disease. Doppler studies are so sensitive for identification of valvular regurgitation that color Doppler imaging represents the clinical gold standard for identifying valvular dysfunction. Many horses with normal auscultation findings demonstrate

varying degrees of valvular regurgitation by Doppler examination, however, and the point deserves emphasis. Most cardiologists consider these silent valvular leaks as normal,[454] especially those observed on the right side of the heart. No doubt some cases of silent regurgitation do represent the earliest signs of degenerative valvular disease, but clinical benefit to screening horses by Doppler is not established, and the approach is not practical or necessarily predictive of future outcome.

Although examiners usually discount silent regurgitation, one also must place the clinical significance of *audible* valvular regurgitation in context. For example, examination of high-performance athletes demonstrates murmurs of tricuspid, mitral, or aortic valvular insufficiency in many of these horses. These findings are readily verifiable by Doppler imaging. In one study of 2-year-olds,[437] the prevalence of tricuspid and mitral regurgitant murmurs increased significantly over a 9-month training period to an incidence of 25.5% and 21.8%, respectively. The causes of these changes and the interpretation of these findings are problematic, especially when referenced to the clinical benchmark of normal. Possibly the high-level training induces changes in ventricular geometry or in valvular thickness related to cardiovascular work, elevations of blood pressure during training, or other factors. Nevertheless, whether these murmurs are actually normal or not, clearly one must interpret the *clinical significance* of a regurgitant murmur in a horse with caution and certainly in light of training, performance history, and clinical findings.

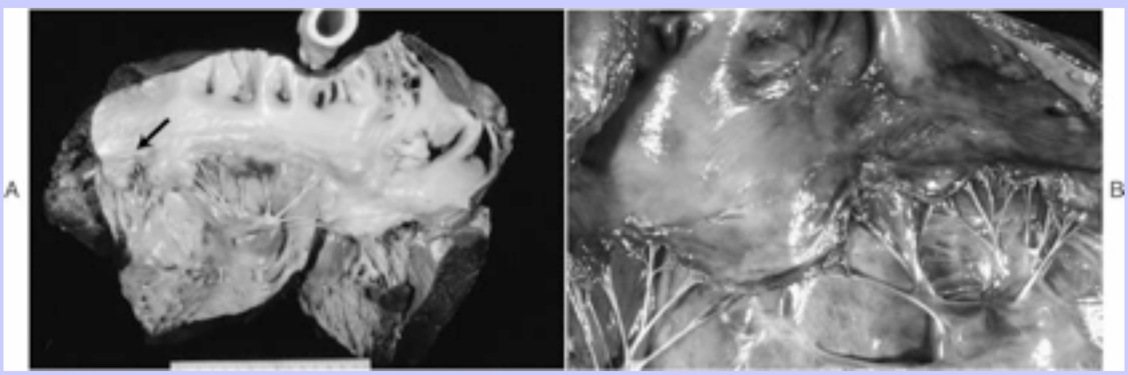
Assessing the significance of a cardiac murmur involves scrutiny of the work history, the physical examination findings, and the results of echocardiography. Other studies such as electrocardiography and exercise testing provide further information by which to judge the importance of a murmur. As a general rule, significant regurgitant murmurs tend to be loud and long. However, the intensity of an insufficiency murmur is related not only to the regurgitant volume but also to the driving pressures of blood and the physical characteristics of the thorax. Therefore although the examiner can grade the intensity of a heart murmur (see the foregoing discussion in this chapter), grading the severity of regurgitation by auscultation alone is not possible. Loud murmurs may be associated with regurgitant volumes that are inconsequential to an individual horse, especially when the murmur is high-pitched, vibratory, or musical. Thus although clinically significant valvular heart disease is best *identified* by auscultation (see Table 8-2), one must assess the *clinical relevance* of a valvular incompetency in other ways.[*] This approach is emphasized as the commonly detected acquired valvular conditions are considered in this section.

8.7.1 MITRAL REGURGITATION

MR is one of the more common murmurs detected in horses. The etiopathologic basis of mitral valve incompetency can be any of the following: degenerative thickening, idiopathic disease, prolapse of the valve, ruptured chordae tendineae, bacterial endocarditis, noninfective valvulitis, primary or ischemic myocardial 408
disease, and congenital malformation of the valve (Figures 8-33 and 8-34; see also Tables 8-2 and 8-3).[*] 409
Degenerative, fibrotic thickening of the mitral valve has been observed at necropsy in mature horses and is probably the basis for most cases of mild to moderate MR, including those with normal two-dimensional echocardiographic imaging. The basis of mitral valve prolapse is uncertain but could involve connective tissue disease of the leaflets, stretched chordae tendineae, minor chordal ruptures, or ischemic injury to a papillary muscle. Ruptured mitral valve chordae tendineae with flail mitral leaflet can occur in animals of any age, including foals. Chordal ruptures, which often involve the accessory mitral cusps, may lead to severe MR with fulminant CHF. Necropsy findings often show degenerative thickening of the ruptured strand; endocarditis rarely is involved. The mitral valve is one of the main valves affected in bacterial endocarditis of horses. Although uncommon, mitral valve infection can lead to ulceration, vegetation, or chordal injury and substantial MR (see the following discussion). The authors have observed MR caused by severe mitral scarring and thickening in weanlings and young horses. The cause of these lesions is unknown, but the nonsuppurative valvulitis identified

might be related to an immune-mediated process. Cardiomyopathy, myocarditis, or myocardial infarction can lead to valvular insufficiency through dilation of the mitral annulus or loss of papillary muscle support.

Figure 8-33 Postmortem lesions of mitral regurgitation (see also Figure 8-40, A). **A,** The opened left ventricle and left atrium are viewed from the caudal perspective. Mild mitral valve fibrosis has led to thickening and incompetency. A jet lesion is evident *(arrow)* as well as modest left-sided heart enlargement. **B,** Chronic suppurative valvulitis caused by chronic endocarditis has led to scarring, thickening, and distortion of the mitral valve. This horse developed severe left-sided congestive heart failure. Noninfective valvulitis also is recognized sporadically in horses, particularly in younger animals.

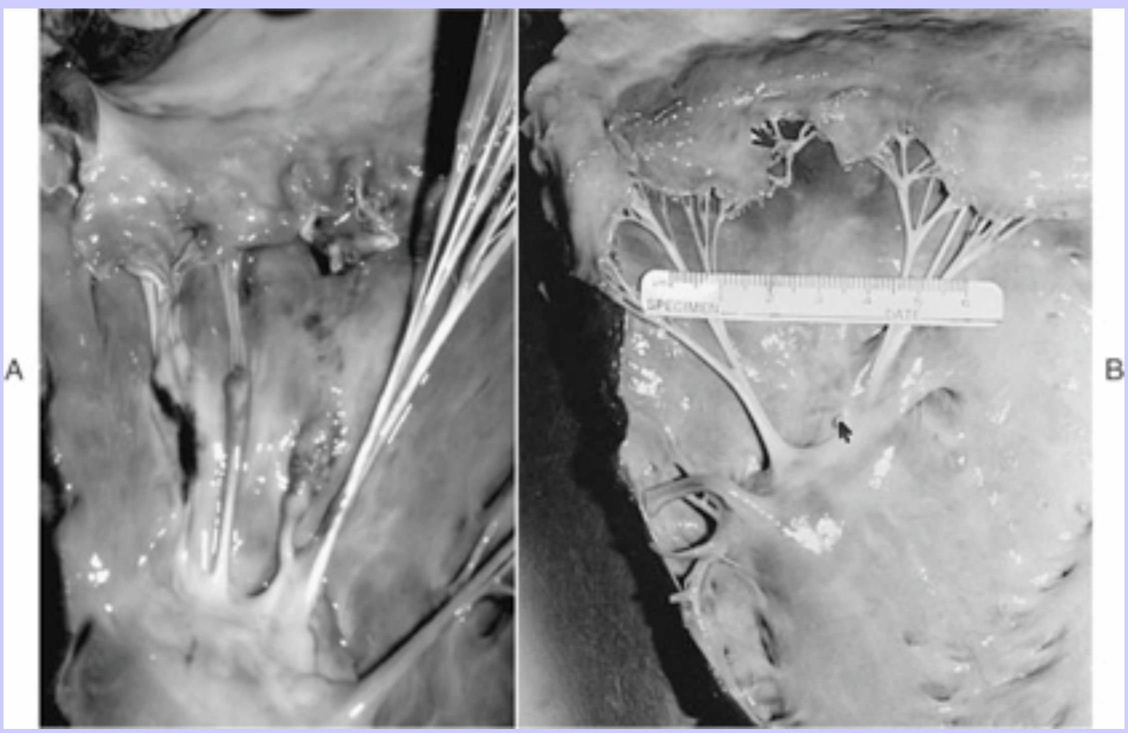


The *clinical presentation* of the horse with MR varies. MR is often an incidental finding detected during a routine examination. In other situations, one may identify the MR in a horse with suboptimal performance or overt clinical signs of heart failure. As indicated previously, soft murmurs of MR are common in high-performance horses.[437] When MR is only mild to moderate, significant LA dilation and LV volume overload are not evident, and the horse often performs satisfactorily. With moderate to severe MR, clinical signs are likely to include poor performance, exercise-induced pulmonary hemorrhage, or overt CHF. Tolerance of the lesion depends largely on whether the horse is used for vigorous work. Some horses with MR also develop atrial fibrillation, which can impair CO further. On identifying a murmur of MR within the setting of fever, weight loss, polyarthritis, or systemic inflammation, one should consider bacterial endocarditis. MR related to chordal rupture is a rare but well-recognized cause of severe CHF with fulminating pulmonary edema (see Figure 8-10). Chronic, hemodynamically significant MR from any cause can lead to pulmonary hypertension, atrial fibrillation, and biventricular CHF, with clinical signs of right-sided CHF dominating the clinical presentation (see the previous discussion in this chapter).[48,102]

The *physical examination* of the horse with MR typically reveals a grade 2 to 5 out of 6 holosystolic murmur. The murmur is most intense at or dorsal to the palpable left apical impulse and over the mitral valve area (see Figures 8-7 and 8-32). Often the murmur is loud at the aortic valve, probably related to the proximity of the septal mitral leaflet to the aortic valve or to cranial projection of the regurgitant jet. Loud MR murmurs often 409 410

project dorsally and to the right. The typical murmur of MR is long (holosystolic or pansystolic), extending into the S_2,[38] and may cause the listener to misinterpret S_3 as S_2, thus preventing a full appreciation of the significance of the murmur. When S_3 is loud, the clinician should suspect significant volume overload or increased LV diastolic pressure. Because many horses with MR also have concomitant TR, one may need echocardiographic examination and Doppler studies to differentiate bilateral atrioventricular valve insufficiency from isolated MR with radiation to the right. Finally, the murmur of MR can be modified by atrial fibrillation or premature beats.

Figure 8-34 Rupture of the mitral valve chordae tendineae. **A,** Acute rupture of a chorda tendinea in a horse with lymphocytic plasmacytic valvulitis. The flail mitral cusp actually has twisted because of loss of support. The ventral portion of the tear is obvious adjacent to intact chords. **B,** Chronic rupture of a chorda tendinea in a horse. The contraction of the scarred segments *(arrow)* is notable.

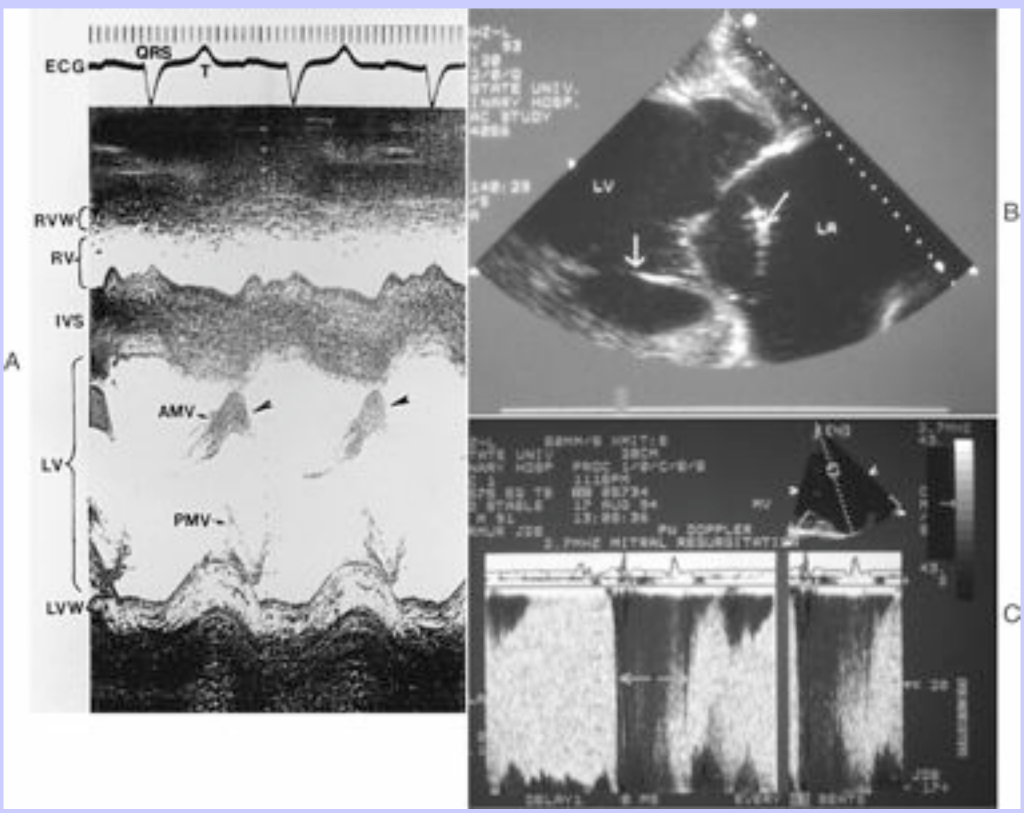


Two variants of holosystolic MR murmur are the protomesosystolic decrescendo murmur and the mid to late systolic crescendo murmur (see Figure 8-32). One may detect a decrescendo murmur with mild MR because coaptation of the leaflets may occur as the ventricular volume decreases during late systole. One easily may confuse this type of murmur with a functional ejection murmur, unless the point of maximal murmur intensity is centered near the left apex. Conversely, severe MR with CHF conceivably could cause a decrescendo murmur because atrial and ventricular pressures may equilibrate in late systole. However, such a murmur is not a common finding even in severe MR. The other variant of MR is the mid to late systolic crescendo murmur (see Figure 8-32, *B*). Presumably this murmur is caused by mitral valve prolapse. Whereas LV dilation worsens the

typical murmur of MR, the murmur of mitral valve prolapse starts *after* the left ventricle has begun ejection. Decreasing LV volume allows a mitral cusp to prolapse causing midsystolic regurgitation that builds through S_2. The resultant murmur can be harsh or musical, and the novice often confuses this flow event with an early diastolic murmur.

Echocardiography and Doppler examinations play a pivotal role in assessing the horse with MR[*] (Figure 8-35) and are indicated to examine the valve anatomy; estimate the severity of MR; measure the size of the atria, ventricles, and great vessels; and quantify LV systolic function. The underlying cause of MR may be obvious from the echocardiographic examination. Mild to moderate valvular thickening, although admittedly subjective, is compatible with degeneration or nonbacterial valvulitis. Prolapse of the mitral valve cusps has been observed in horses with MR, but the limits of normal prolapse require further definition. Lesions caused by vegetative endocarditis cause the valves to appear irregularly thickened or shortened. In cases of acute endocarditis, one 410
usually finds evidence of valve thrombus, and a high-frame rate real-time examination may show oscillation of 411
this tissue. In chronic endocarditis, the valve may be more echodense, or even appear calcified. Chordal rupture is recognizable by observing chaotic flutter of a mitral structure (a flail leaflet), prolapse of a large portion of the valve into the atrium, or the chordal remnant (it usually is contracted) flipping into the atrium during systole (see Figure 8-35). High-frequency systolic vibrations of the mitral valve may be visible on the M-mode study in horses with a musical murmur of MR. Pulsed or continuous wave or color Doppler studies can identify the mitral regurgitant jet. In most horses one can perform this examination best from the left cardiac windows. High-velocity or turbulent jets may be difficult to find without a complete and thorough examination of the mitral valve that involves multiple image planes. When color flow Doppler demonstrates a wide *origin* of the regurgitant jet and a pattern of diffuse distribution of turbulence deep into the left atrium, the likelihood of hemodynamically significant MR is greater. Quantitation of cardiac size is instrumental in assessing severity. With severe MR and LV volume overload comes rounding of the LV apex and increased end-diastolic dimension (see Table 8-5). Global LV function may appear normal to exuberant because the incompetent valve decreases ventricular afterload (see Figure 8-5, *B*). However, when MR is severe and chronic or if the underlying basis for MR is cardiomyopathy, the ventricular shortening fraction is decreased. The left atrium often assumes a more circular, almost turgid, appearance when MR is hemodynamically significant, and the two-dimensional 411
echocardiographic measure of internal atrial dimension often exceeds 13.5 to 14 cm. With acute or chronic MR, the lobar and main pulmonary artery may be dilated as a result of pulmonary hypertension, presumably related to increased LA pressure, interstitial lung edema, pulmonary vascular remodeling, or other factors. 412

Figure 8-35 Echocardiograms recorded from horses with mitral regurgitation. **A,** M-mode study demonstrating significant thickening of the anterior mitral valve *(AMV)* leaflet *(arrows)*. This would be compatible with endocarditis or severe valvulitis. (*RVW,* Right ventricular wall; *RV,* right ventricle; *IVS,* intraventricular septum; *LV,* left ventricle; *LVW,* left ventricular wall.) **B,** Flail mitral leaflet *(right arrow)* in a horse with multiple chordae ruptures. The prolapsed portion of the mitral valve forms a curved echodense line in the dilated left atrium, whereas the other valve portions are to the left of this echocardiogram. A normal chord is evident *(left arrow)*. **C,** Pulsed wave Doppler study recorded from the left atrium demonstrating a turbulent, high-velocity, aliased systolic jet of mitral regurgitation in a Thoroughbred horse. The duration of the event is shown *(arrows)*.



The *prognosis* for horses with MR varies and, as discussed previously, is related to clinical findings, work history, and results of echocardiographic studies. One should consider abnormalities observed during

echocardiography—including lesions of the mitral valve leaflets, the degree of LA and LV volume overloading, global LV function, and Doppler findings—when formulating the prognosis. Certainly, when MR is associated with CHF, atrial fibrillation, endocarditis, chordal rupture, significant cardiomegaly, severe valvular thickening, dilated cardiomyopathy, or pulmonary hypertension, the prognosis for life or performance is poor. The detection of pulmonary artery dilation indicates significant pulmonary hypertension and the possibility of pulmonary artery rupture; therefore such a horse should not be ridden or driven because of the low but concrete risk of sudden death. Fortunately, the majority of horses with MR appear to perform well, indicating that MR in most cases is not clinically important. Whether progressive exercise intolerance will develop in a particular case depends on the use of the horse and on the progression of the underlying lesion. Generally, when valve degeneration causes MR and the heart size is normal, the progression is gradual, the prognosis for life is favorable, and the horse maintains performance. When MR is detected in an untrained colt or filly, the prognosis is less encouraging. The presence of even mild to moderate cardiac dilation in a case of MR recommends a more guarded prognosis, though this is made best by serial examinations. The significance of mild MR in the high performance horse or racehorse is uncertain. In some animals, treadmill exercise is normal, whereas others may demonstrate a higher heart rate for a given level of work than might otherwise be expected. The latter finding may suggest a cardiac limitation to performance.

Regardless of cause or severity of the condition, the horse diagnosed with MR merits follow-up examinations to evaluate progression of the hemodynamic burden and to detect the development of CHF, pulmonary hypertension, or cardiac arrhythmias. Treatment of MR involves management of complications such as heart failure, endocarditis, or arrhythmias. These topics are covered elsewhere in this chapter.

* References 5, 18, 19, 38, 45, 48, 113, 116, 117, 120, 122, 131, 139, 162–164, 317, 405, 419, 538, 556–558.

† References 31, 87, 88, 102, 114, 125, 128, 129, 132–134, 137, 138, 160, 161, 523, 559–564.

* References 119, 120, 135, 247, 301, 430, 437, 454.

* References 3, 38, 45, 48, 72, 102, 103, 113, 114, 117, 120, 122, 128, 129, 131, 133, 134, 137, 138, 164, 317, 405, 558.

* References 119, 120, 429, 430, 437, 439.

8.7.2 AORTIC REGURGITATION

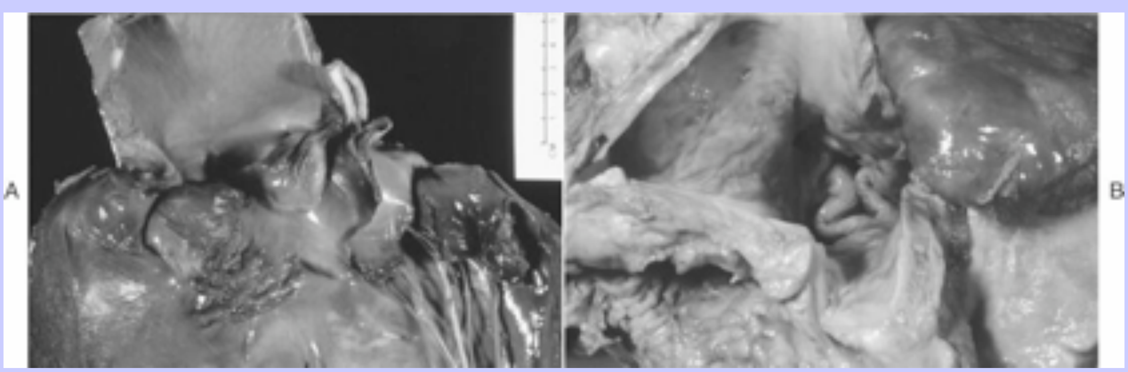
Aortic regurgitation (AR) is a common valvular insufficiency. Degeneration of the aortic valve is by far the most common reason for AR, although other potential causes include idiopathic prolapse, bacterial endocarditis, congenital valvular disease, VSD (see the previous discussion in this chapter), noninfective valvulitis, and ruptured aortic sinus aneurysm (see Tables 8-2 and 8-3).[*] The degenerative nodular lesions and fibrous bands responsible for AR in older horses have been well described by Bishop, Cole, and Smetzer[5] (Figure 8-36). Prolapse of the aortic valve is a common echocardiographic finding and probably represents another variant of connective tissue degeneration. Small fenestrations of the valve have been identified at necropsy but have uncertain clinical significance.

In most horses AR is an incidental finding detected during a routine physical, prepurchase, or insurance examination. Most horses with this murmur are older than 10 years of age, and the murmur is common in aged horses, a testament to the degenerative nature of the lesion. Careful auscultation in a quiet area may help one identify a soft diastolic murmur in a younger horse. Silent AR also may be considered physiologic, and identifying a small jet of AR by Doppler studies in horses with no identifiable diastolic murmur is common.[454] When one identifies a loud murmur of AR in a younger animal, one should consider other causes. Poor

performance is an infrequent presenting complaint in horses with AR, because most horses continue at their prior performance level, provided that no other clinical or cardiac abnormalities are present. Intermittent fevers, weight loss, or lameness should prompt consideration of endocarditis. CHF occurs infrequently with isolated AR but may develop in the horses with combined AR, mitral valvular disease, or atrial fibrillation. Statistical evidence from Marr and colleagues indicates that in the United Kingdom, older horses with AR have a shorter survival and are more prone to developing CHF and ventricular arrhythmias or dying suddenly.

One can identify clinically important AR by cardiac auscultation, which reveals a holodiastolic murmur, with the point of maximal intensity over the aortic valve area and good radiation to the right and toward the left cardiac apex. The murmur may vary greatly in intensity. The quality is typically harsh and decrescendo with a blowing nature, but presystolic accentuation may occur (see Figure 8-32, *C*). In some cases the murmur is vibratory, musical, cooing, or buzzing. One can palpate a precordial thrill over the aortic valve area when the murmur is loud. A variant of the typical AR murmur is that associated with rupture of the right aortic sinus into a cardiac 412
chamber or into the ventricular septum. In this instance the holodiastolic murmur may be louder on the right side 413
of the thorax. However, in the majority of horses with an aortic-cardiac fistula, a continuous machinery murmur is audible. Atrial fibrillation, atrial premature depolarizations, and ventricular extrasystoles infrequently have been associated with AR. The quality of the arterial pulses is a good indicator of the severity of the isolated AR.[128] Bounding arterial pulses indicate significant LV volume overload and moderate to severe AR. A systolic ejection murmur is often present, particularly when the regurgitant volume is large, and is explained by ejection of a large stroke volume across the aortic valve. A point of emphasis is that no evidence indicates that the degenerative aortic valve is anatomically stenotic.

Figure 8-36 Postmortem lesions of aortic regurgitation (see also Figure 8-40, *B*). **A,** A segment of the left ventricular outflow tract and the ascending aorta. Linear bands are evident on the two valves shown, and a large jet lesion also is visible below the valves. The lesion is typical in aged horses. **B,** The aortic valve viewed from the ascending aorta. Noninfective valvulitis and scarring have caused severe thickening.

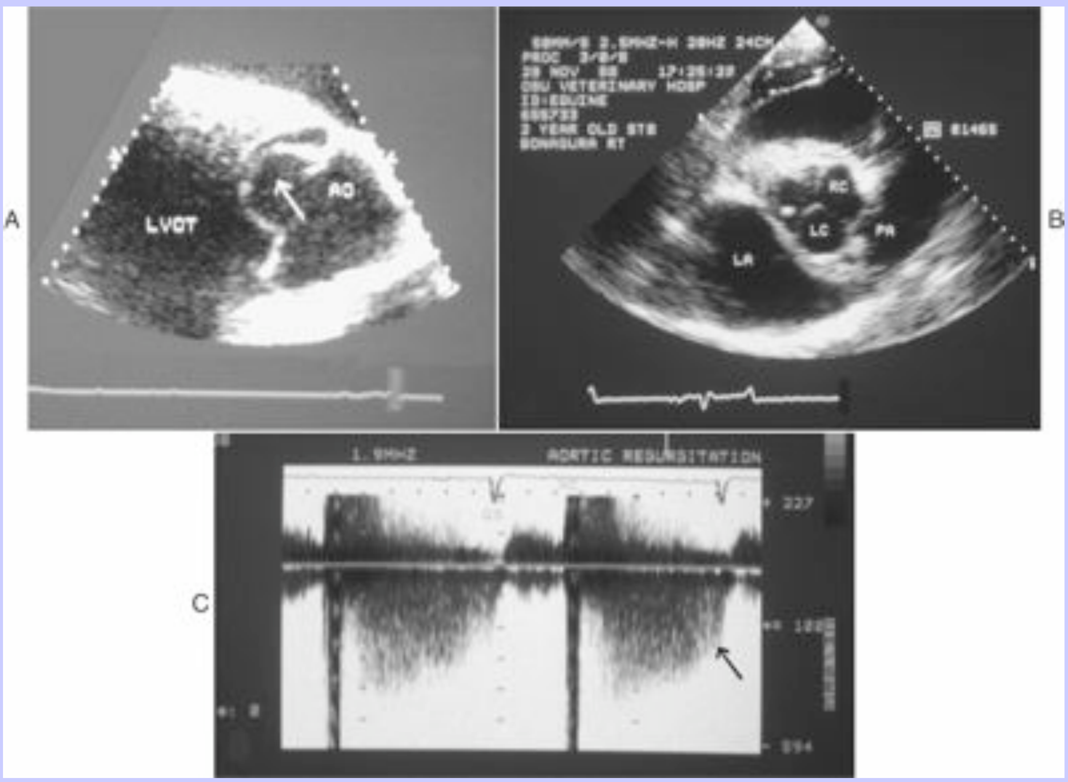


A complete echocardiographic examination including Doppler echocardiography is useful for further evaluation of horses with AR, particularly when the arterial pulse is abnormal or cardiac-related clinical signs are suspected. One may identify a number of echocardiographic abnormalities in horses with AR (Figure 8-37).[*] Subjective

thickening of the aortic valve leaflets and prolapse of one or more leaflets are common observations. In cases of endocarditis, the leaflets may appear thickened, irregular, and more highly echogenic. The valve may appear to oscillate if a fresh thrombus is within the vegetation. Fenestrations of the aortic valve leaflets, flail aortic leaflet, aortic sinus aneurysm, and aortic root prolapse into a VSD are detected rarely. Dilation of the aortic root (exceeding 10 cm) may occur in some horses with AR. The flow disturbance of AR may be evident from Doppler or M-mode studies. The Doppler examination shows one or more central or eccentric diastolic jets of AR. One can verify the timing of the flow disturbance by spectral studies or color M-mode. The traditional M-mode study in horses with AR often reveals high-frequency diastolic vibrations of the septal mitral valve leaflet related to the high-velocity regurgitant jet entering the LV outflow tract. One also may detect similar vibrations on the intraventricular septum when the regurgitant jet is directed cranially. High-frequency fluttering of the aortic valve leaflets or aortic walls may be detectable especially in horses with musical murmurs. Increased mitral valve E-point to septal separation may result from the regurgitant jet impinging on the valve or may indicate cardiac dilation and failure. In the latter case, other markers of severe insufficiency will be evident, including ventricular dilation; rounding of the LV apex; a strong, long, and wide origin Doppler signal of AR; and a short pressure half-time. An exaggerated or swinging septal motion represents another subjective observation of volume overload on real-time two-dimensional echocardiographic examination. Premature (presystolic) mitral closure is another sign of severe AR indicating elevated ventricular end-diastolic pressure. The LV volume overload that develops with AR leads to increased ventricular internal diameter at end-diastole, increased shortening fraction, and decreased free wall thickness. If myocardial failure develops, the end-systolic dimension may be increased, despite normal shortening fraction.

The clinical significance and prognosis of AR is based most accurately on the history, physical examination, and echocardiogram. Because most cases of AR are associated with a slow degeneration of the aortic valve leaflets, 413
and this occurs in older horses without other cardiac problems, the prognosis for life and performance is usually 414
good. Such animals typically have minimal echocardiographic abnormalities or only mild echocardiographic signs of volume overload. The findings of flail aortic valve leaflet, endocarditis, or echo markers of moderate to severe volume overload or myocardial failure indicate a poor prognosis for life and performance. One should obtain an exercising ECG in performance horses with significant aortic regurgitation to determine if the horse remains safe to ride. One also should consider a Holter ECG as a potential test in older, valuable animals because of the statistical relationship between AR, ventricular extrasystoles, and sudden death. Follow-up clinical and echocardiographic examinations are indicated for the horse with moderate to severe AR. One should anticipate the development of secondary MR, atrial fibrillation, ventricular arrhythmias, CHF, or sudden death in horses with severe volume overload.

Figure 8-37 Echocardiograms recorded from horses with aortic regurgitation (see also Figure 8-20, *C*). **A,** Aortic valve prolapse *(arrow)* in a 12-year-old Standardbred gelding. **B,** A well-circumscribed, echodense “bead” on the noncoronary cusp of the aortic valve in a 2-year-old Standardbred colt may represent a congenital or acquired lesion. This short-axis image plane is recorded across the base of the heart. **C,** Continuous wave Doppler recording from the same horse as in **A** demonstrates a turbulent diastolic signal that ends at the QRS complex *(arrow)*.



* References 3, 5, 18, 72, 103, 114, 116, 120, 128, 129, 131, 132, 317, 419, 538, 557, 558.

* References 81, 120, 128, 129, 131, 132, 142, 419, 425, 428, 558.

8.7.3 TRICUSPID REGURGITATION

TR may be the most frequently detected of flow disturbances and murmurs in the horse,[*] although virtually nothing is written about the etiopathogenesis of this condition. The anatomic basis of tricuspid valve incompetency can be any of the following lesions: idiopathic, degenerative thickening, prolapse, noninfective tricuspid valvulitis, bacterial endocarditis, pulmonary hypertension (including left-sided failure), ruptured

chordae tendineae, myocardial disease with secondary cardiomegaly, chronic tachyarrhythmia, and congenital malformation of the valve. TR is common in horses of racing age[437]; however, the anatomic correlation to this incompetency is uncertain. Degenerative, fibrotic thickening of the tricuspid valve in mature horses may lead to mild to moderate TR. Compared with the mitral condition, ruptured tricuspid valve chordae tendineae are uncommon[556] and better tolerated unless associated with endocarditis. Bacteria can infect the tricuspid valve, and in some cases infection occurs following an injudicious or septic jugular venipuncture or catheterization. Pulmonary hypertension, cardiomyopathy, and myocarditis can lead to secondary dilation of the tricuspid annulus or alteration of papillary muscle support, permitting valvular insufficiency. Tricuspid malformation does occur but seems more commonly associated with stenosis or atresia of the valve[*] (see the previous discussion in this chapter).

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A soft, grade 2 to 3 out of 6 systolic murmur of TR is most often an incidental finding detected during a routine examination. The murmur becomes more problematic when it is loud or is identified in a horse that is performing poorly. Many horses with TR race well; therefore the clinician first should exclude other likely reasons for poor performance before incriminating the tricuspid valve. Of course, if chronic hemodynamically significant TR has developed, then peak work effort suffers and even right-sided CHF may develop. The latter situation is not common unless the TR follows pulmonary hypertension or bacterial endocarditis or unless severe TR is associated with atrial fibrillation. Prominent jugular pulses (giant c-v waves) are typical of horses with TR associated with CHF.

Auscultation of the horse with TR typically reveals a grade 2 to 5 out of 6 holosystolic murmur with the point of maximal intensity over the right hemithorax at the tricuspid valve area (see Figure 8-8; Tables 8-2 and 8-3). The murmur can be holosystolic, decrescendo, or mid to late systolic. Although the timing and intensity at times may remind the examiner of a functional murmur, the location of greatest murmur intensity argues against that possibility. The murmur usually radiates dorsally and if loud to the extreme left cranioventral thorax. The intensity of the TR murmur in many cases does seem to correlate to the regurgitant volume and definitely depends on the pulmonary artery and RV systolic pressure. Thus a grade 4 or louder murmur is anticipated with moderate to severe TR or when TR is related to pulmonary hypertension. Atrial fibrillation or atrial premature beats are present in some horses with TR, particularly when the right atrium is dilated or the regurgitant jet is large.

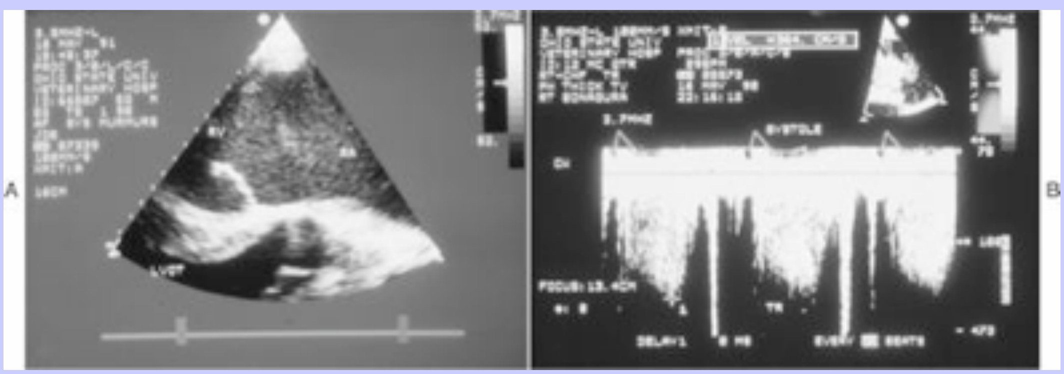
Echocardiography with Doppler studies are useful in assessing TR (Figure 8-38). One typically performs examinations of the tricuspid valve from the right side of the thorax, because the tricuspid valve and RV inlet are closer to the right thoracic wall; however, an extreme left cranial transducer location also can be successful for examining tricuspid flow. Because a number of potential reasons for tricuspid incompetency exist, one must direct careful attention to the valve and its support apparatus, the size of the pulmonary artery, and the left side of the heart. Usually one can diagnose or exclude valve thickening, prolapse, vegetations, chordal rupture, regurgitation following RV dilation, and pulmonary hypertension with careful imaging. However, in most cases one cannot detect a clear-cut lesion of the tricuspid valve leaflet. As with MR, moderate to severe TR leads to RA and RV volume overload; however, quantifying these chamber volumes is more difficult because of their complex geometry. Doppler studies can identify the tricuspid regurgitant jet. In trivial or silent TR, this jet is typically narrow at the origin and directed at the aorta. However, when the jet is wide at its origin or projects centrally or laterally into the right atrium, cardiomegaly is more likely to be present and one should examine the heart carefully. One can estimate the RV (and pulmonary artery) systolic pressure by faithfully recording the peak jet velocity. Estimation requires the examiner to align the continuous wave Doppler cursor to regurgitant flow. When the jet velocity is less than 2.5 m/sec, pulmonary hypertension is not present. Jets exceeding 3.2 to 3.4 m/sec indicate pulmonary hypertension (provided that ventricular outflow obstruction or VSD is not present).

The identification of pulmonary hypertension should prompt careful examination of the left side of the heart, because the main cardiac lesion may be centered over the mitral valve, aortic valve, or LV cavity.

The prognosis for horses with TR is generally favorable. Generally a soft murmur of TR in a trained athlete is disregarded as clinically insignificant; although defining such a disturbance as functional would be imprecise in the authors' opinion. The presence of TR is more likely to cause concern if right-sided cardiomegaly, pulmonary hypertension, or atrial fibrillation is evident. Abnormalities detected during echocardiography of the tricuspid valve leaflets may be instructive because the visualization of a vegetation or chordal rupture would indicate a poor prognosis for performance and a guarded prognosis for life. The width of the regurgitant jet at its origin, recent performance history, and results of exercise testing are useful in developing a prognosis for life and future work. Knowing whether exercise and the associated pulmonary hypertension worsen the degree of TR in horses over time would be interesting, and the increased use of exercise testing and postexercise echocardiography may provide answers to this question. When TR is judged to be moderate or severe, periodic reexaminations are indicated to follow the progression of the lesion and to detect cardiomegaly or cardiac arrhythmias if they develop.

* References 3, 72, 103, 114, 120, 139, 162, 205, 430, 438, 442, 556, 565. 415

Figure 8-38 Echocardiograms from horses with tricuspid regurgitation (see also Figure 8-23, *C*). **A,** Two-dimensional echocardiogram demonstrating probable valve thickening, significant right atrial distention, and spontaneous right atrial contrast in a horse with severe tricuspid regurgitation, atrial fibrillation, and congestive heart failure (1-cm calibrations at the left of the sector; *LVOT,* left ventricular outflow tract). **B,** Continuous wave Doppler recording of a high-velocity tricuspid regurgitation jet from a horse with tricuspid regurgitation caused by pulmonary artery obstruction caused by a mass lesion. The high velocity of regurgitation, greater than 3.6 m/sec, indicates that elevated right ventricular pressure is driving the regurgitant jet. In this case the high pressure is caused by supravalvular pulmonary stenosis caused by the tumor. Tricuspid regurgitation jets associated with normal right ventricular pressures are typically less than 2.6 m/sec.



* References 141, 146, 151–153, 155, 317, 554, 555.

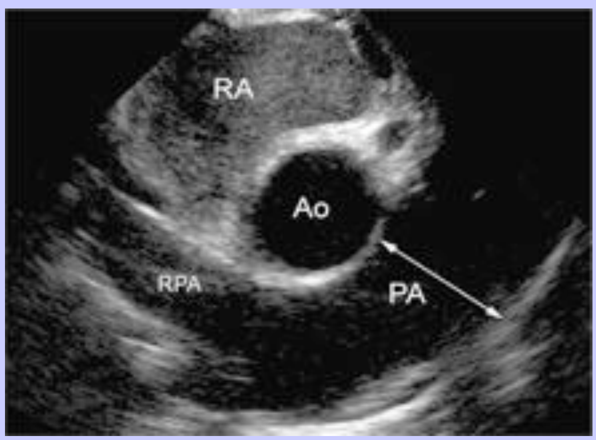
8.7.4 PULMONARY INSUFFICIENCY

One often can detect trivial and clinically silent physiologic pulmonic insufficiency by Doppler studies; however, this is a normal finding.[120,139,205,430] Clinically significant pulmonic insufficiency is uncommon and occurs most frequently with pulmonary hypertension associated with left-sided heart failure. Bacterial endocarditis and congenital abnormalities of the valve leaflets (bicuspid or quadricuspid) occur, but these are rare causes of pulmonary insufficiency.[125] A rare cause of this condition is rupture of the pulmonary valve.[127]

Murmurs of pulmonic regurgitation are usually undetectable unless the regurgitant volume is large or is driven by pulmonary hypertension; however, one can identify the flow disturbance using pulsed wave and color flow Doppler echocardiography. One may detect dilation of the pulmonary artery with echocardiography when pulmonary hypertension causes pulmonic insufficiency (Figure 8-39).

When severe pulmonic regurgitation does develop, it generally results from left-sided heart failure and is accompanied by clinical signs of biventricular failure, including increased respiratory rate and effort. Signs of right-sided CHF result from pulmonary hypertension, pulmonic regurgitation, RV volume overload, and TR. If 416
one detects a murmur of pulmonic regurgitation, the murmur is usually holodiastolic and decrescendo, with the 417
point of maximal intensity at the pulmonic valve area, radiating toward the right cardiac apex. The prognosis for life and performance is usually poor in these cases.

Figure 8-39 Right cranial echocardiogram with the aorta *(Ao)* in short axis demonstrating a dilated pulmonary artery *(PA)*. The cause was pulmonary hypertension following left-sided congestive heart failure. *RA,* Right atrium (with spontaneous contrast in cavity); *RPA,* right branch of the pulmonary artery.



8.7.5 BACTERIAL ENDOCARDITIS

Bacterial endocarditis is caused by bacterial invasion of the heart valves or endocardium. Endocarditis is not common in horses but occurs sporadically in most populations.[*] The infection may affect horses of any age, although the pathogenesis may differ in younger animals or in those that are immunosuppressed. In one report, the mean age of affected horses was 2.1 years.[442] Numerous bacteria have been associated with bacterial endocarditis. The offending microorganism likely depends on the environment, portal of entry (e.g., gastrointestinal tract, skin, lung, oral cavity, joint, surgical wound, or intravenous catheter), and the effects of prior antibiotic therapy that may select for resistant strains. *Streptococcus* sp., *Actinobacillus equuli,* and *Pasteurella* sp. have been isolated most frequently. *Rhodococcus equi, Candida parapsilosis, Erysipelothrix rhusiopathiae,* meningococci, *Staphylococcus aureus,* and other organisms including aspergillus also have been isolated.

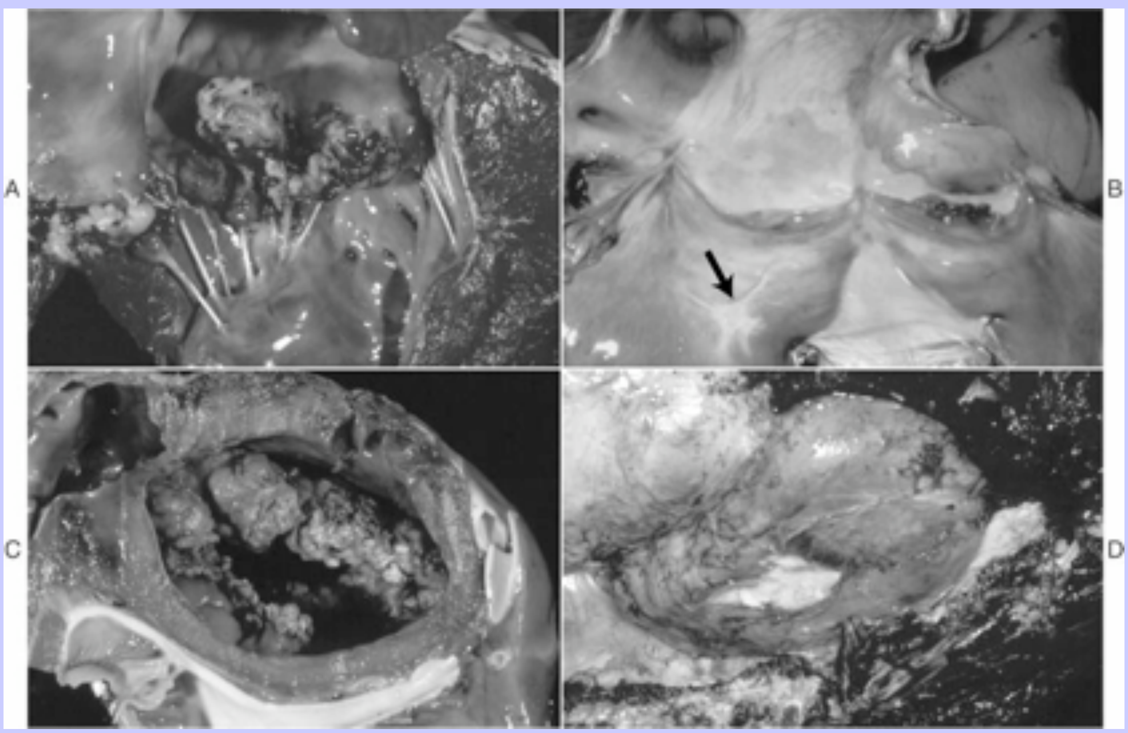
The pathogenesis involves bacterial invasion from the bloodstream and colonization of the heart valve or endocardial surfaces. Bacteremia is a prerequisite for development of this condition. Direct invasion of a previously normal valve by virulent bacteria, or in the context of overwhelming sepsis, represents the most likely pathogenic mechanism involved in foals with bacterial endocarditis. Disruption of the endocardial surface by jet lesions associated with congenital intracardiac shunts also may predispose horses to endocarditis, although this mechanism is not well established in horses. Preexisting valvular heart disease with endocardial changes or high-velocity jets may represent risk factors for bacterial colonization in older horses, but again this is not established. The most common sites of bacterial endocarditis are the aortic and mitral valves, although endocarditis lesions have been reported on all cardiac valves. Mural endocarditis lesions also have been reported but occur much less frequently. The combination of bacterial injury, exposure of valve collagen, thrombosis, and host leukocyte response contributes to the development of a vegetation (Figure 8-40), which consists microscopically of bacteria, platelets, fibrin, leukocytes, and varying degrees of granulation or fibrosis. Bacteria may not be evident at necropsy, especially if antimicrobial therapy has sterilized the vegetation.

The pathophysiology of endocarditis in horses is probably similar to that in other species. A host response, as well as the primary cardiac lesions, contributes to the morbidity of this disease. Cardiac manifestations include valvular injury leading to regurgitation, chordal rupture, or rarely to stenosis; secondary cardiomegaly; myocarditis from extension of the infection or through coronary embolization; myocardial infarction if emboli are shed to the coronary arteries; arrhythmias from cardiomegaly or myocarditis; and myocardial depression from bacteremia. Recurrent or chronic bacteremia, and hence fever, is characteristic of endocarditis. Metastatic infection, distant thrombosis and infarction, and immune-mediated host responses can occur. Distant infection or immune complex disease can lead to multisystemic clinical signs, including arthritis, osteomyelitis, vasculitis, or nephritis. Right-sided thrombi can lead to pulmonary thrombi, and or abscessation of the lungs (see Figure 8-40).[125,559]

Clinical features of endocarditis vary.[442] Affected horses usually have a history of intermittent fever, weight loss, depression, anorexia, lethargy, and often intermittent lameness (Figure 8-41). A predisposing condition or a concurrent infection may be evident, including jugular vein thrombophlebitis, strangles, septic joint, or an abscess. Most horses have no history of previous illness and no evidence of concurrent infection. The physical examination often reveals fever, and some horses may be tachypneic. The fever is often intermittent. One detects murmurs of mitral or aortic valvular insufficiency most commonly; those of TR are less common. One must differentiate systolic murmurs caused by valve destruction from the physiologic flow murmurs that are so often audible in febrile horses (see Table 8-2). One also may detect murmurs of valvular stenosis with bacterial endocarditis, but these occur infrequently.[125] Some horses with bacterial endocarditis have no auscultable murmur initially. The quality or intensity of the murmur may change over a number of days. Atrial fibrillation, atrial or ventricular premature depolarizations, and ventricular tachycardia have been observed with bacterial endocarditis.[102,561] One may note swelling of the joints or tendon sheaths.

Laboratory studies obtained from horses with bacterial endocarditis may reveal anemia, hyperproteinemia, elevated fibrinogen, and leukocytosis with a mature neutrophilia.[442] One should perform multiple blood cultures for suspected bacterial endocarditis. The result of blood cultures may be negative, however, particularly after antimicrobial therapy. One is more likely to obtain a positive blood culture by drawing multiple samples at different times of the day during or after febrile episodes. Antibiotic removal system media also might be of value when culturing blood from horses that have received antibiotics recently. 417

Figure 8-40 Bacterial endocarditis: postmortem lesions. **A,** Severe mitral valve endocarditis in a yearling. **B,** Focal aortic valve endocarditis is evident in this view of the left ventricular outlet and the ascending aorta. The septal mitral leaflet is at the lower right, and a jet lesion *(arrow)* is visible in the left ventricular outflow tract. Above the mitral leaflet, in the center of the left coronary cusp, is a raised, irregular vegetation that caused aortic regurgitation. **C,** Tricuspid valve vegetation in a weanling. Although less common than mitral or aortic vegetations, right-sided endocarditis is a definite risk in horses, particularly in animals subjected to repeated jugular venous catheterization. **D,** A lung abscess in a horse following pulmonary valve endocarditis. The center of the abscess is incised and reveals caseous exudate. Systemic embolization and metastatic infection are recognized complications of valvular infections.



One should consider a diagnosis of bacterial endocarditis in any horse that has a fever of unknown origin and the aforementioned clinical signs. Endocarditis is most likely when one cannot isolate the cause of fever to another body system and one suspects concurrent cardiac disease. Positive blood cultures in the setting of compatible

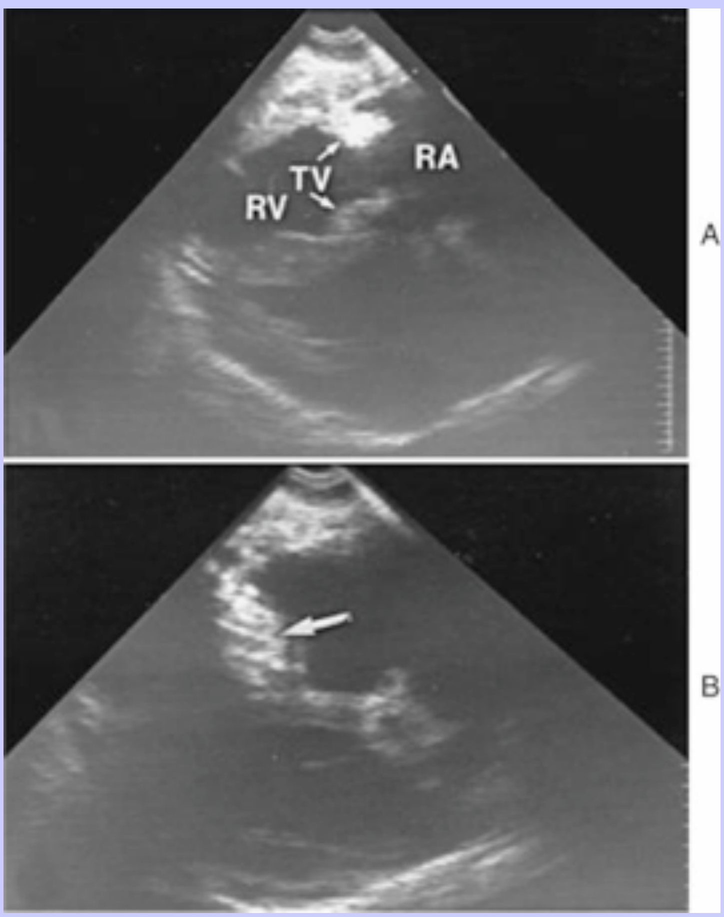
clinical findings or echocardiographic detection of vegetative lesions on the valve leaflets or endocardial surface confirm the diagnosis (Figure 8-42). Vegetative lesions usually appear on echocardiography as thickened, echogenic to hyperechoic masses with irregular or shaggy edges. The valve leaflet usually demonstrates diffuse thickening as well. The typical endocarditis lesion adheres to the endocardium (and therefore moves with the valve). When a fresh thrombus attaches to the vegetation, an oscillatory appearance may be evident with high frame rate, real-time imaging. With time the lesion may contract or develop a smoother contour. Echocardiography also may detect rupture of the chordae tendineae or avulsion of a valve leaflet, and this complication is common in mitral or tricuspid valve endocarditis. One can use pulsed wave and color flow Doppler echocardiography to confirm that the valve is incompetent or (rarely) stenotic. Valvular regurgitation often progresses because of continued damage to the valve leaflets associated with ongoing bacterial infection or following fibrosis associated with a bacteriologic cure.

Treatment of bacterial endocarditis should consist of high levels of bactericidal antibiotics, administered 418
parenterally and ideally based on culture and sensitivity patterns of blood culture isolates. Drugs that penetrate 419
fibrin well, particularly potassium penicillin (22,000 to 44,000 IU/kg every 6 hours), are reasonable initial choices because bacteria may be sequestered in fibrin and may be unavailable to leukocytes. Intravenous therapy is preferable in the initial stages of treatment. To extend the antimicrobial spectrum, one may administer gentamicin sulfate (3.3 mg/kg intravenously every 8 hours). Erythromycin (20 mg/kg every 6 hours intravenously or orally) or rifampin (5 to 10 mg/kg every 12 hours orally) may be useful in some cases. Initial therapy should be broad spectrum until the results of the blood culture are known or when a positive blood culture cannot be obtained. Therapy should extend to at least 4 weeks and as long as 8 weeks. The duration and type of long-term therapy depend on numerous factors, and one must weigh the bacterial isolate, clinical response, cost, and potential toxicosis of the antimicrobial therapy in these decisions.

Figure 8-41 Weight loss, loss of condition, and ventral edema in this weanling with endocarditis and right-sided congestive heart failure (see also Figure 8-40, *C*).

A poor expectation for survival is an appropriate initial prognosis in any case of established bacterial endocarditis. Even in the absence of significant valvular regurgitation, one may have difficulty in achieving a bacteriologic cure, or progressive valvular damage may occur as the vegetation heals and scars the valve. Although some horses have been treated successfully for bacterial endocarditis,[31,160,561] overall the likelihood of long-term survival is low with continued use of the horse as a performance animal or in breeding. The absence of an obvious echocardiographic lesion or signs of systemic inflammatory response creates a more favorable situation, provided that one can obtain a bacteriologic cure. Progressive cardiomegaly, CHF, rupture of the pulmonary artery, atrial fibrillation, or sudden death have been reported in horses affected with endocarditis. Accordingly, one should perform a periodic follow-up examination, including echocardiograms, in successfully treated horses.

Figure 8-42 Two-dimensional echocardiogram of tricuspid valve endocarditis *(arrows),* acquired rupture of the chordae tendineae, and flail tricuspid valve. The movement of the valve from diastole **(A)** to systole **(B)** is evident and is typical of a valvular involvement. The valve is highly echogenic and thick, which is typical of chronic infection. *RA,* Right atrium; *RV,* right ventricle; *TV,* tricuspid valve.



* References 3, 31, 72, 87, 102, 103, 114, 125, 128, 129, 132–134, 137, 138, 160, 161, 442, 523, 559–565.

8.8 Pericardial Disease

Pericardial diseases occur uncommonly and usually are associated with pericardial effusion and fibrinous pericarditis.[*] Pericarditis and pericardial effusion may be idiopathic, bacterial, viral, or traumatic or associated with cardiac or pericardial neoplasia. Mesothelioma and lymphosarcoma are the most common neoplasms affecting the pericardium in horses. Pericardial herniae also occur but are rare. Not all cases of pericarditis are septic, but one should consider the possibility of an infectious cause until proved otherwise. Sterile inflammatory and eosinophilic effusates have been recognized, and a recent outbreak of pericarditis centered in Kentucky has been attributed potentially to a caterpillar vector (Figure 8-43). The pathogenesis of noninfective pericarditis is unknown but might be immune mediated in some situations.[91]

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Figure 8-43 Pressure tracing demonstrating elevated right ventricular end-diastolic pressure in a horse with constrictive pericarditis and heart failure. A quick rise occurs from the nadir of pressure *(lower arrow)* to a plateau *(upper arrow),* which is typical of constrictive disease and ventricular filling that is limited to early diastole.

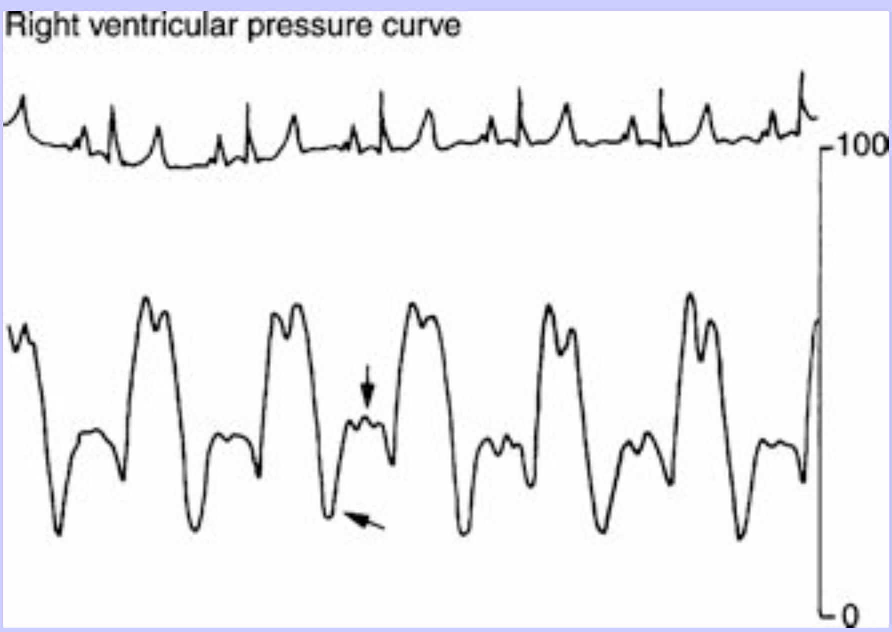
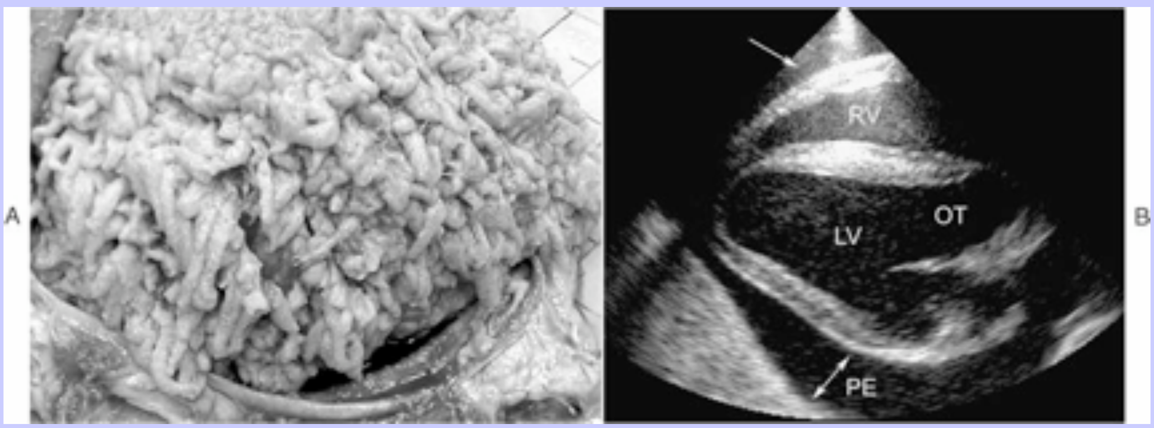


Figure 8-44 **A,** Considerably proliferative epicardial reaction in a horse with idiopathic, fibrinopurulent pericarditis. The heart is covered by a layer of organizing fibrin and inflammatory debris creating a shaggy appearance typical of inflammatory pericarditis. **B,** Two-dimensional long-axis echocardiogram from another horse demonstrating a moderate pericardial effusion. The effusion *(PE)* appears more prominent behind the left ventricle *(LV)* but is also evident *(arrow)* cranial to the right ventricle. *OT,* Left ventricular outflow tract.



The pathophysiology of pericardial disease in most cases is ascribed to cardiac compression (tamponade) or constriction of the heart with impairment of filling. Occasionally a mass lesion effect compresses the heart, venous drainage, or ventricular outflow. Other clinical signs may be referable to the underlying cause as with infection or neoplasia.

The clinical syndrome of pericardial effusion with tamponade is characterized by impairment of cardiac filling, increases in ventricular filling pressures (Figure 8-44), acutely by reductions in arterial blood pressure, and chronically by normal blood pressure with signs of right-sided CHF. The history usually includes systemic signs of illness such as fever, lethargy, depression, anorexia, tachypnea, ventral edema, colic, and weight loss. External thoracic trauma or penetration by a gastric foreign body may lead to bacterial inoculation of the pericardial space.[566,569,570] A recent history of an upper or lower respiratory tract infection is common. Physical examination abnormalities include various combinations of tachycardia, fever, pericardial friction rub, muffled heart sounds, tachypnea, pleural effusion, jugular and generalized venous distention, ventral edema, thready pulses, and ascites. Arterial blood pressure may be decreased in cardiac tamponade and pulsus paradoxus may be identifiable with careful measurement of arterial blood pressure. In chronic disease, blood pressure is normal because of fluid retention, elevated venous pressures, vasoconstriction, and tachycardia.

Laboratory studies contribute to the diagnosis. Clinical laboratory abnormalities are not specific, but the most 420
frequently detected abnormalities include anemia, hyperproteinemia, hyperfibrinogenemia, and a neutrophilic 421
leukocytosis. Other hematologic abnormalities may be related to inflammation, CHF, or organ hypoperfusion.

Thoracic radiographs usually reveal a globoid cardiac silhouette or pleural effusion and may reveal interstitial pulmonary infiltrates and enlarged pulmonary vessels. The ECG usually demonstrates decreased amplitude of the QRS complexes. If the effusion is large and the heart is swinging, one may observe electrical alternans. Pericarditis may elevate the ST segment in multiple leads, but this change may occur simply as a result of tachycardia. Sinus tachycardia is typical, although one may detect ventricular or atrial premature complexes.

The echocardiographic examination is diagnostic, demonstrating an anechoic or hypoechoic fluid space between the pericardium and epicardial surface of the heart (see Figure 8-44, *B*). Fibrin tags are frequently visible on the parietal and visceral pericardial surfaces. Diastolic collapse of the right ventricle or systolic collapse of the right atrium is diagnostic of cardiac tamponade. Inflammatory processes eventually may lead to adhesion between the parietal and visceral pericardial layers, causing constrictive pericarditis with minimal or no obvious effusion. One can detect associated pleural effusion easily by ultrasound; the finding is common. One also can exclude extracardiac mass lesions, which can mimic pericardial disease, by echocardiography.[94] Echocardiographic diagnosis of constrictive disease without effusion is more challenging, but typically reveals thickened pericardium, atrial dilation, systemic venous dilation, exuberant movement of the ventricular septum, and exaggerated inspiratory filling of the heart as documented by pulsed wave Doppler studies. In some cases, one requires right-sided heart catheterization to establish the diagnosis. Typical findings are increased central venous pressure, elevated RV diastolic pressure, and possibly a diastolic dip and plateau appearance to the RV waveform.[83]

Cytologic evaluation of the pericardial effusion is essential to distinguish septic, aseptic, or neoplastic pericardial effusion. One can obtain fluid in the course of needle pericardiocentesis or during the placement of an indwelling tube for pericardial lavage and drainage. One should perform bacterial culture and sensitivity tests of the aspirated fluid to guide antimicrobial therapy in cases of septic pericarditis. *Streptococcus* spp. have been reported most frequently with pericarditis, but *Actinobacillus equuli, Pseudomonas aeruginosa, Pasteurella* sp., and mycoplasma[572] have been isolated; and equine influenza, viral arteritis, and caterpillar vectors have been associated with fibrinous pericarditis (as well as abortion).

The treatment of pericardial diseases varies, depending on the cause and clinical situation. Pericardiocentesis or catheter drainage is appropriate in all cases of infective pericardial effusion or pericardial effusion with cardiac tamponade. Because the development of cardiac tamponade depends not only on the volume of pericardial fluid but also on the rate at which it accumulates, the clinician's urgency should be guided by blood pressure, clinical signs, magnitude of pleural effusion, and echocardiographic evidence of cardiac tamponade. Tamponade is an indication for immediate drainage of the pericardial sac. One can use echocardiography safely to localize a site for pericardiocentesis and choose an appropriate length of needle, catheter, or drainage tube. One should perform pericardiocentesis after locally anesthetizing the intercostal muscles and pleura and should perform electrocardiographic monitoring continuously during the procedure to monitor for cardiac puncture or in case ventricular arrhythmias develop. One usually performs pericardiocentesis within the left fifth intercostal space, above the level of the lateral thoracic vein, although one can perform the procedure at the right hemithorax. Drainage is achieved using a large-bore catheter, teat cannula, or chest tube; the latter is recommended for repeated drainage and lavage of the pericardial sac and is most successful for aggressive management of septic or idiopathic fibrinous pericarditis. After insertion of an indwelling catheter, pericardial lavage and direct instillation of antimicrobials, combined with systemic antimicrobials, have been shown to be effective in treating septic pericarditis.[510] Pericardial lavage should continue for several days, until accumulation of pericardial fluid declines (<1 L over 12 hours), clinical signs have improved, and the cytologic character of the fluid becomes less inflammatory. If the cytologic analysis and culture are negative for bacteria, one may use antiinflammatory doses of dexamethasone to treat idiopathic, nonseptic, effusive pericarditis.[84,91] Exudative pericarditis may not respond in all cases to conservative treatment or even to drainage. Surgery is a rarely used option for treating pericardial

disease but would be most appropriate for constrictive or constrictive-effusive pericarditis.[83] Presumably, introduction of minimally invasive thoracoscopic surgical techniques to manage pericardial diseases in horses might prove useful.

The prognosis for survival and maintenance of performance in horses affected by pericardial disease is guarded, because the condition may become chronic, but recent reports indicate good results have been obtained.[91] The potential of eventual constrictive or fibrotic pericardial disease is greatest with infective or inflammatory pericardial conditions so that early success may be tempered by later complications of the disease.[84] The best prognosis of horses with fibrinous pericarditis is when treatment includes repeated drainage and lavage. The clinician carefully should follow horses treated by catheter drainage and should reevaluated them by echocardiography. The prognosis for cardiac or pericardial neoplasia is poor.

* References 3, 77–81, 83–91, 94, 114, 510, 511, 566–572.

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8.9 Myocardial Disease

Myocardial diseases are probably underrecognized in clinical practice, and published reports regarding these disorders are few.[*] The potential certainly exists for myocardial injury related to drugs, toxins (ionophore antibiotics, poisonous plants), ischemia, hypoxia, infective agents, parasite migration, heavy metals, trauma, relentless tachyarrhythmia, metabolic disease, or nutritional imbalance. Myocardial injury also can occur from extension of a preexisting infection (pericarditis, pericardial abscess, or endocarditis). Infiltrative cardiomyopathies can occur following neoplasia (melanoma, lipoma, lymphosarcoma, hemangiosarcoma, mesothelioma) or the rare amyloidosis (Figure 8-45; see Table 8-2). Idiopathic, dilated cardiomyopathy also has been recognized in the horse. A potentially reversible cardiomyopathy may accompany relentless ventricular or supraventricular tachycardia. One can assess myocardial function in affected horses only a number of days following conversion to sinus rhythm.

8.9.1 CLINICAL FEATURES

The general manifestations of myocardial disease, regardless of the underlying injury, can be attributed to the following *pathophysiologic processes:*

1. Reduced myocardial contractility and ventricular ejection fraction
2. Diastolic dysfunction with impaired ventricular filling
3. Mitral or tricuspid valve incompetency caused by cardiac dilation or papillary muscle dysfunction
4. The development of arrhythmias

The overall cardiac disability in horses affected by myocardial disease varies greatly. Some horses have no detectable clinical signs, whereas others demonstrate exercise intolerance, life-threatening arrhythmias, low-output CHF, or sudden death.

Persistent ventricular premature depolarizations or ventricular tachycardia are observable in horses with primary myocardial disease. Atrial premature depolarizations, atrial tachycardia, or atrial fibrillation are more often primary electric disturbances, although these arrhythmias also can develop in horses with cardiomyopathies. Although diagnosing myocardial disease in the setting of any cardiac rhythm disturbance is tempting, one should appreciated that many rhythm abnormalities are functional, without a gross anatomic substrate. This point is

especially germane to horses suffering from electrolyte or other metabolic imbalances, high sympathetic tone, sepsis or toxemia, hypoxemia, or ischemia.

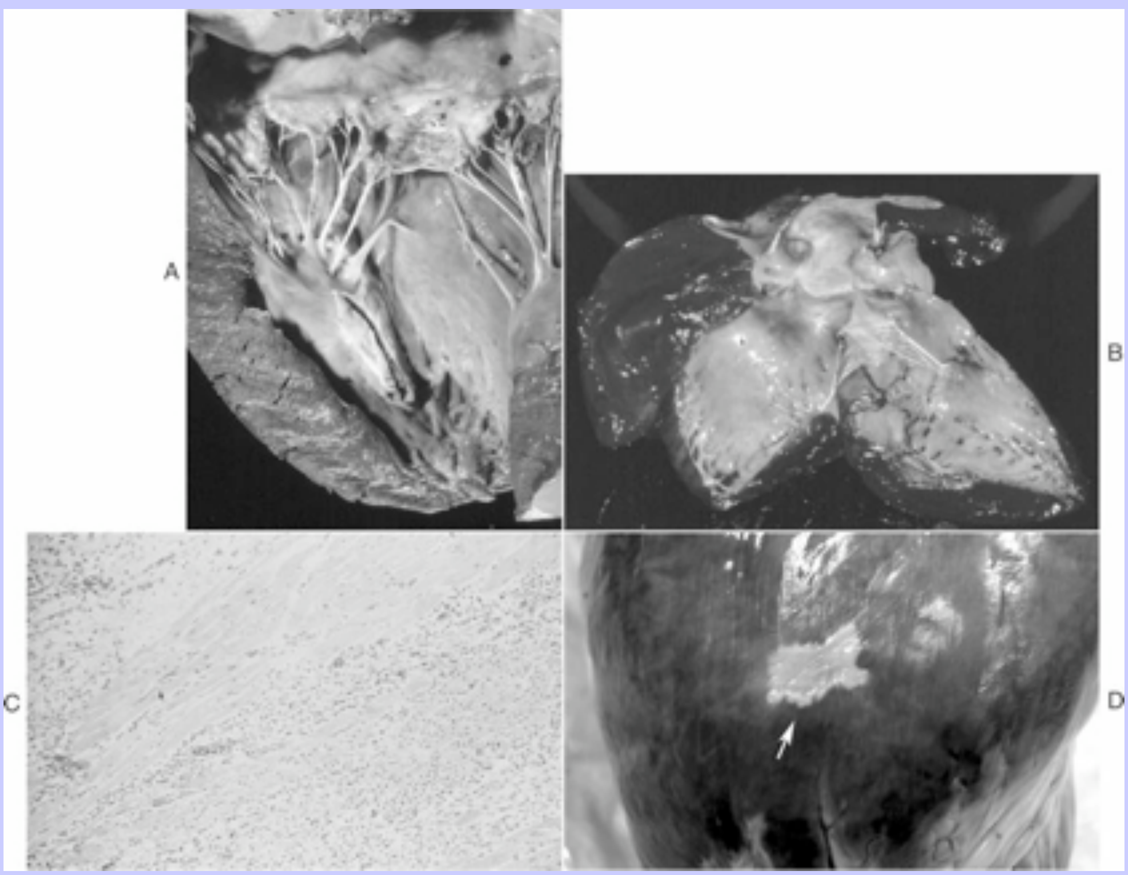
The onset of clinical signs may lag behind the initial myocardial insult, especially in cases of myocarditis or chronic myocardial injury. For example, a horse that apparently has recovered from an illness may develop problems once rigorous training begins. The trainer may complain that the horse is unable to achieve faster speeds or may stop or suddenly slow during hard training. The affected horse may take a long time to cool out after a workout. In more severe cases, significant exercise intolerance, weakness, ataxia, or even collapse may occur. One may detect respiratory distress, pulmonary edema, cyanotic mucous membranes, prolonged capillary refill time, and a rapid, thready pulse after exercise. In case of severe myocardial injury, one may observe signs such as fever, persistent tachycardia, arrhythmia, murmur, pulmonary or ventral edema, or respiratory distress. Sudden death may occur without premonitory signs.

Results of the *clinical examination* in horses with myocardial disease are inconsistent. Resting physical examination findings can be normal, or signs of heart disease may be evident. These can include persistent tachycardia, tachypnea, frequent premature beats, sustained arrhythmias, systolic murmurs of atrioventricular valvular insufficiency, or CHF. A postexercise examination often detects an abnormally rapid heart rate, which remains persistently high after exercise is discontinued. The ECG may demonstrate atrial or ventricular arrhythmias, and an exercise ECG typically records an inappropriately high heart rate for the level of work undertaken. Resting echocardiography usually reveals a low normal or unambiguously reduced ventricular shortening fraction. Postexercise echocardiography may demonstrate a paradoxical reduction of LV shortening fraction or regional dysfunction characterized by LV wall motion abnormalities. One may observe significant increases in LV or LA spontaneous contrast with poor myocardial function, although this is not a specific finding (Figure 8-46). Abnormal areas of myocardial echogenicity have been observed, but myocardial tissue characterization by echocardiography is not well established in horses and depends on technical factors.

Clinical laboratory tests may be useful for identifying myocardial damage but may not necessarily distinguish myocarditis from myocardial cell injury induced by a toxin or by ischemia. Elevated plasma or serum myocardial fractions of creatine kinase or of lactate dehydrogenase suggest myocardial injury.[272] A more specific marker of myocardial is elevation of serum troponin I.[584,585] Although normal values or mild elevations do not exclude cardiomyopathy or myocardial infiltration, elevated values point to cardiac muscle damage.

Diagnosis of myocardial disease requires clinical suspicion and integration of findings from clinical and laboratory examinations. Because of the extreme variability of findings, one can make the presumptive diagnosis of myocardial disease only after reviewing the history, physical examination, echocardiogram, ECG, and clinical laboratory tests. Definitive diagnosis of myocarditis requires transvenous endomyocardial biopsy, but this test is impractical and may not identify piecemeal inflammation, degeneration, infiltration, or necrosis. 422 423

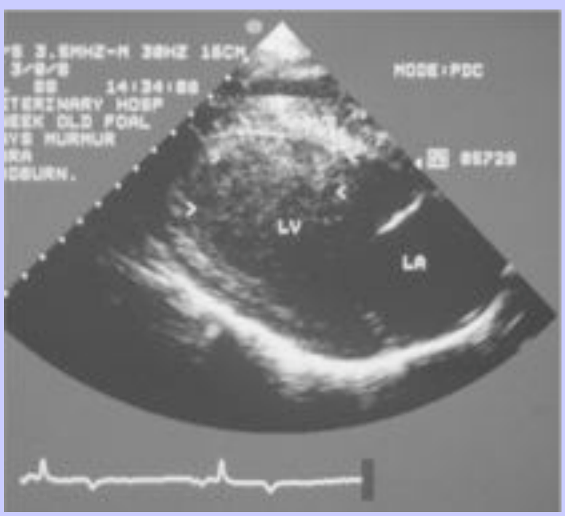
Figure 8-45 Myocardial diseases. **A,** An opened left ventricle revealing an *(incised)* large, oval area of subendocardial and myocardial fibrosis in a mare. No causative agent was found. **B,** Significant left ventricular dilation and subendocardial fibrosis in a horse with idiopathic dilated cardiomyopathy. The white discoloration of the left ventricle and left atrium may result from chronic distention or represent fibrosis following another injury. The opened right ventricle *(to the left)* is dilated but is not discolored. **C,** Myocardial lymphosarcoma. A substantial myocardial infiltration is evident in this photomicrograph. **D,** Close up of a left ventricular, subepicardial myocardial infarct *(arrow)* of undetermined cause.



Treatment of horses affected by myocardial disease is primarily supportive. Prognosis depends on the cause and severity of myocardial injury and the hemodynamic consequences of myocardial disease. All horses should be rested, preferably in a stall, until myocardial function, ECG, cardiac isoenzymes, and serum troponin return to

normal or at least remain stable for several weeks. The horse should rest a minimum of 1 month (and usually more) before returning to work. The clinician administers antiarrhythmic therapy when indicated for potentially life-threatening arrhythmias (see the following discussion in this chapter). Treatment of frequent premature supraventricular or ventricular complexes with an antiarrhythmic agent can be effective; however, the arrhythmia may return as soon as the antiarrhythmic drug is discontinued. If practical, the administration of an ACE 423
inhibitor such as enalapril or ramipril is recommended to reduce myocardial remodeling and unload the ventricle. 424
When CHF has developed, digoxin and furosemide are prescribed as described in the section on CHF. One should undertake digitalization with caution in horses with ventricular extrasystoles, because treatment may aggravate the arrhythmia. Digoxin is not indicated in monensin toxicosis (see the following discussion). If one believes noninfective myocarditis to be the cause of the arrhythmia or clinical signs, corticosteroid therapy may be indicated, although its value is unsubstantiated. When the principal manifestation of myocardial disease is electric (arrhythmias with otherwise normal myocardial function), the prognosis is fair to good for resolution of the arrhythmias. Horses with decreased myocardial function by echocardiography or those with CHF must be given a guarded prognosis for life and a poor prognosis for future performance. One should note, however, that some horses with acute onset of CHF have recovered completely and returned to their prior performance level. Such horses most likely suffered from acute myocarditis that resolved spontaneously or following antiinflammatory therapy. Other horses may achieve a less spectacular recovery but still serve successfully as breeding animals.

Figure 8-46 Spontaneous contrast in the apex of the left ventricle of a foal. The echodensities swirled in real time. One can compare the left ventricular density with the density within the left atrium. Bradycardia, low cardiac output, and rouleaux formation are thought to contribute to this finding (see also Figure 8-38, *A*).



* References 3, 28, 29, 72, 92, 99–100, 103, 106, 107, 112, 114, 527, 573–583.

8.9.2 MYOCARDITIS

Myocardial inflammation, or myocarditis, is difficult to diagnose. The diagnosis often is entertained in horses with cardiac arrhythmias or abnormal myocardial function, particularly when cardiac signs occur after another illness and are associated with elevated cardiac isoenzymes and serum troponin I concentrations. Often the prior disorder is a viral, influenza-type condition or an infection caused by *Streptococcus* spp. The logical conclusion is that immune-mediated myocarditis is operative in these cases, but definitive cause-and-effect proof is lacking because of the difficulty of obtaining myocardial biopsies. Myocarditis also may follow parasitic migration, pericarditis, or bacterial endocarditis. Hematogenous spread to the heart may be another mechanism for myocarditis. Severe necrotizing myocarditis can lead to arrhythmias or to signs of myocardial dysfunction as discussed previously. The signs, prognosis, and treatment of myocarditis are similar to that described in the preceding section.

8.9.3 TOXIC INJURY OF THE MYOCARDIUM

A number of chemicals and plant toxins are potentially injurious to the myocardium. Ionophore antibiotics are among the most notorious causes of myocardial necrosis in horses.[106–111] Toxic myocardial injury also can occur following ingestion of glycoside-containing Japanese yew plants or cut oleander,[586] or from eating feed contaminated by *Epicauta* species (blister beetles) that contain the toxic element cantharidin.[576,578,587] Myocardial necrosis also occurs following endotoxemia, particularly with clostridial infection, salmonellosis, and torsion of the large colon. Rattlesnake venom has been associated with cardiac injury and arrhythmias.[588]

Horses are uniquely sensitive to ionophore antibiotics, which are used as a coccidiostat in poultry production and as a growth promoter in steers. Monensin toxicosis (>2 to 3 mg/kg) has occurred most frequently, but salinomycin (0.6 mg/kg or higher) and lasalocid (21.5 mg/kg or higher) also have caused myocardial injury and death in horses. Ionophores react with polar cations to form lipid-soluble complexes, leading to cation transport across myocardial cell membranes. Various ionophores demonstrate particular affinities for different cations, though lasalocid may complex with a variety of ions. Exposure of horses to the ionophore antibiotics usually stems from accidental contamination of equine feedstuffs at the mill or through accidental delivery of poultry or cattle feeds to horses. In most outbreaks the culprit was a recently acquired ration.

Clinical signs of horses with ionophore toxicity vary with the specific type, quantity, and concentration of ionophore ingested and the preexisting health and body condition of the exposed horses. A wide range of clinical signs has been observed by one of the authors (Reef) in exposed horses ranging from none to clinical signs involving almost all body systems. Weakness, lethargy, depression, anorexia, ataxia, colic, diarrhea, profuse sweating, and recumbency have been observed. The cardiac findings are similar to those previously described for 424
myocardial diseases. If sudden death occurs, it is usually within 12 to 36 hours of ingestion of the contaminated 425
feed. Polyuria and hematuria also have been reported in ponies. A diagnosis of ionophore toxicity is based on the detection of the ionophore in the feed or stomach contents of exposed horses. One may observe various clinicopathologic abnormalities with monensin toxicity, including decreased serum calcium, potassium, magnesium, and phosphorus; and increases in serum urea, creatinine, unconjugated bilirubin, aspartate aminotransferase, creatine phosphokinase, and lactate dehydrogenase. Isoenzyme patterns of creatine phosphokinase and lactate dehydrogenase have indicated cardiac, skeletal, and red blood cell damage. Elevated packed cell volume and total solids have been associated with dehydration. Echocardiographic evaluation of affected horses has revealed significant decreases in shortening fraction with segmental wall motion abnormalities that range from mild to severe. Horses that exhibit decreased shortening fraction and dyskinesis

shortly after exposure to monensin generally do not survive. Horses with mild decreases in shortening fraction survive and may be useful breeding animals, but most do not return to previous performance levels. Horses with normal echocardiograms not only survive but also typically return to work at their previous level. Postmortem findings range from no visible lesions in horses with normal echocardiograms to severe myocardial necrosis and fibrosis in those with decreased shortening fraction or ventricular dyskinesis. A single dose may lead to death from cardiac arrhythmias before the development of myocardial necrosis. Postmortem lesions have been described elsewhere but include myocardial pallor and signs of CHF.

Treatment for affected horses is largely symptomatic (see the previous discussion), unless recent exposure is known. Vitamin E has been suggested to have a protective effect in other species and may be beneficial in affected horses. Digoxin and probably calcium channel blockers are contraindicated in acutely affected horses. If ingestion of the contaminated feedstuff is recent, treatment with activated charcoal or mineral oil is indicated to reduce absorption of the ionophore. Intravenous fluid and electrolyte replacement therapy may be indicated, as well as antiarrhythmic drugs, for any life-threatening arrhythmias. Stall rest in a quiet environment for up to 8 weeks after exposure is most important, because echocardiograms recorded after trivial exercise or excitement can reveal residual disease characterized by significant decreases in fractional shortening and myocardial dyskinesis.

8.9.4 DILATED CARDIOMYOPATHY

Idiopathic dilated cardiomyopathy is a disorder of the myocardium characterized by global reduction of LV systolic function that cannot be explained by valvular, vascular, coronary, or congenital heart disease. The inciting cause of dilated cardiomyopathy generally is undetermined, although myocarditis or prior toxic injury often is suspected. Relentless junctional or ventricular tachycardia also can lead to a dilated cardiomyopathic state. The clinical signs are similar to those described for myocardial diseases. Echocardiography is diagnostic, revealing cardiomegaly with biatrial and biventricular dilation and depressed shortening fraction (see Figure 8-20, *A*). Symptomatic therapy with digoxin and diuretics may stabilize CHF temporarily and lead to transient improvement, but most horses deteriorate in the 3 to 12 months after diagnosis and are destroyed humanely. Neither the history nor the postmortem examination reveals the cause, and generally only diffuse or multifocal myocardial degeneration, necrosis, and fibrosis are observed.

A form of dilated cardiomyopathy also can be caused by vitamin E and selenium deficiency and occurs primarily in fast-growing foals from mares with a marginal or deficient selenium status raised in selenium-deficient areas. Affected foals are usually younger than 6 months of age and have an acute onset of weakness, recumbency, respiratory distress, pulmonary edema, tachycardia, murmurs, and arrhythmias. The prognosis for foals affected with the myocardial manifestations of white muscle disease are poor, and most die within 24 to 48 hours after the onset of clinical signs. Laboratory abnormalities in affected foals include significant elevations of creatine kinase (including the cardiac isoenzyme or MB fraction in foals with myocardial involvement), aspartate aminotransferase, and lactate dehydrogenase, as well as hyperkalemia, hyponatremia, and hypochloremia. Myoglobinuria may occur. Echocardiography demonstrates the severity of myocardial involvement. Whole blood selenium, red blood cell glutathione peroxidase, and vitamin E levels may be helpful in the diagnosis; however, tissue samples provide a more accurate indication of selenium stores. Treatment with vitamin E and selenium might be successful, but typically the myocardial necrosis is extensive and incompatible with life. Postmortem findings reveal pale streaking of the myocardium with intramuscular edema, myodegeneration, myocardial necrosis, and fibrosis or calcification. Prevention of white muscle disease is important in selenium-deficient areas. Supplementation of pregnant mares should occur during gestation based on individual blood and tissue selenium levels and should continue during lactation, because more selenium is passed to the foal through the milk than across the placenta.

8.10 Vascular Diseases

Diseases of the arteries and veins can be congenital or acquired. Acquired disorders include a variety of conditions, varying in cause and ranging from subclinical to devastating.[*] Thrombophlebitis is probably the most common vascular problem encountered in clinical practice. Catheter embolization occurs periodically and has been amenable to surgical and percutaneous catheter retrieval.[610,611] Venous aneurysms,[591] vascular and lymphatic malformations, and angiomatous lesions are rare.[535] Lesions related to arteriosclerosis and arterial thrombosis are thought to be caused by parasite migration in the gastrointestinal (particularly the mesenteric) arteries[612] and at the terminal aortic quadrifurcation. Rupture of the aorta is usually a life-threatening event or leads to severe sequelae. Rupture of an aortic sinus aneurysm into the ventricular septum or right-side heart chambers; aortic rupture into the pericardium in older breeding stallions; and acquired aortic-pulmonary fistula have been reported.[34,57,196,434,609] Aortic inflammation of unknown cause has been observed.[436,444] Rupture of the pulmonary artery is a potential result of long-standing pulmonary hypertension and left heart failure. Arteriovenous communications occur rarely and may develop after vascular rupture or following growth of vascular tumors. Pulmonary vascular disease and associated hypertension may place a load on the right ventricle, a condition called cor pulmonale. However, no compelling evidence at this time incriminates cor pulmonale as an important or common cause of heart disease in horses. Idiopathic pulmonary hypertension has been reported as a cause of atrial fibrillation in horses; however, left-side heart dysfunction was not excluded completely in the reported cases.[61] Tumors of the great arteries and veins are rare.[535] Mycotic disease within the guttural pouch is a well recognized cause of arteritis and rupture of the internal carotid artery (see Chapter 7). 425 426

The echocardiographic observation of intravascular, spontaneous contrast within blood vessels and cardiac chambers has been suggested to indicate cardiovascular or rheologic disease.[413,613] This observation is common during ultrasound examinations and contrast may be observed in a variety of vessels and in the heart. Pronounced spontaneous echo contrast is especially likely when low flow rates are caused by bradycardia, arrhythmia, or myocardial failure or when actual obstruction to blood flow in vessels occurs. Contrast is often prominent in valvular endocarditis. Lesions, thrombosis, or even venipuncture of the jugular vein can lead to spontaneous echo contrast in systemic veins and the right side of the heart, becoming more obvious if CO suddenly increases. The identification of spontaneous contrast also depends on the operator with respect to transducer, gain, and gray-scale contrast settings (see Figure 8-46). The clinical significance of isolated intravascular contrast cannot be stated with certainty. Prominent contrast is observable with some cardiovascular diseases and has been suggested to indicate abnormal platelet function. However, in the majority of horses, spontaneous contrast probably represents a normal phenomenon. Currently, no compelling evidence indicates that, in the absence of other vascular or cardiac lesions, appearance of spontaneous contrast is abnormal.

One often can suspect serious vascular disease from the clinical examination, although it may not be obvious until a catastrophic vessel rupture or fatal hemorrhage occur. In general, significant *arterial* disease reduces perfusion to the affected vascular bed, impairing tissue metabolism and function. If severe or complete, ischemia may lead to tissue necrosis (infarction) and even perforation of a hollow organ. A bruit—auscultatory evidence of vascular narrowing or rupture—may occur over the affected artery, or hemorrhage may be obvious if a superficial vessel has ruptured. Ultrasound and Doppler examinations can demonstrate serious arterial disease, provided an acoustic window for examination exists. Angiography or nuclear scintigraphy rarely is required to demonstrate arterial thrombosis or disruption. In major venous disease, the typical sign is swelling of soft tissues drained by the affected vessel. Palpable evidence of local inflammation and thrombosis may be found in superficial venous disease. Right-

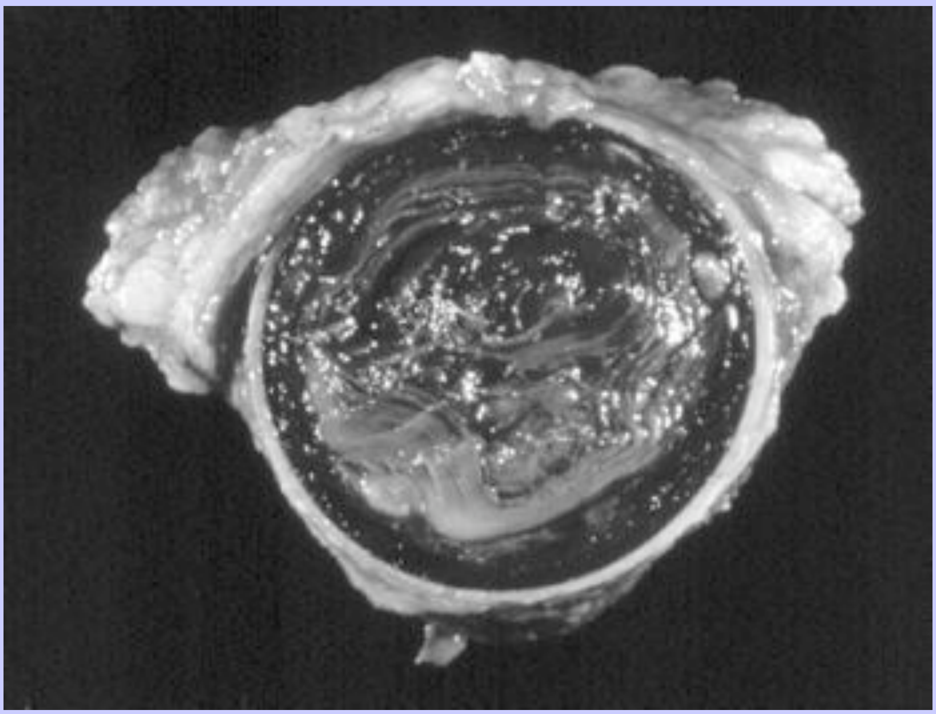
sided endocarditis, embolic pneumonia, and pulmonary embolism are potential consequences of systemic venous disease.

8.10.1

THROMBOPHLEBITIS

Intravenous catheterization or intravenous injection most commonly causes thrombophlebitis, the inflammation 426
and thrombosis of a vein (Figure 8-47),[349,600] which most often affects the jugular veins. Thrombophlebitis can 427
develop in the absence of preexisting vessel trauma following coagulation disorders. Clinical signs of phlebitis are straightforward, including swelling over the affected vein, and pain on palpation of the involved tissues. Thrombotic occlusion results in distention of the veins and possibly subcutaneous edema proximal or cephalad to the affected area. Significant swelling, heat, and pain on palpation, combined with fever, hyperfibrinogenemia, or a neutrophilic leukocytosis, indicate infection of the thrombus.

Figure 8-47 Thrombosis of a jugular vein following iatrogenic thrombophlebitis.



The diagnosis and assessment of thrombophlebitis is based on the detection of the previously described clinical signs and examination by duplex Doppler ultrasonography. Imaging of the affected vein reveals filling of the lumen with an anechoic, hypoechoic, hyperechoic (if gas filled), or mixed echogenic material that partially or completely occludes the vessel (Figure 8-48). Ultrasonographic evaluation also may reveal thickening of the vessel wall, perivascular swelling, tracts extending from the vein to the subcutaneous tissues or skin, or subcutaneous abscesses. Cavitation in the center of a thrombus suggests infection and represents a target for aspiration and bacterial culture. Stagnant or turbulent flow may be manifested by increased spontaneous venous contrast. One may evaluate the flow across the affected zone by pulsed wave or color-coded Doppler studies or with contrast venography using agitated saline as a contrast agent. One also should assess venous drainage

proximal and distal to the area of thrombosis because collateral circulation still may enter the vein distally. One should perform a careful evaluation of the communicating large veins and of the heart in horses with infective thrombophlebitis. Bacterial endocarditis may develop following embolization of infective thrombus or bacteremia.

Figure 8-48 Vascular imaging: jugular veins. **A,** Ultrasound image of normal jugular veins. The echolucent lumina of the right and left veins are indicated. The image is just off transverse. **B,** Image of a mixed, echogenic thrombus *(arrows)* in the right jugular vein. Doppler studies could be used to evaluate the flow across this area.

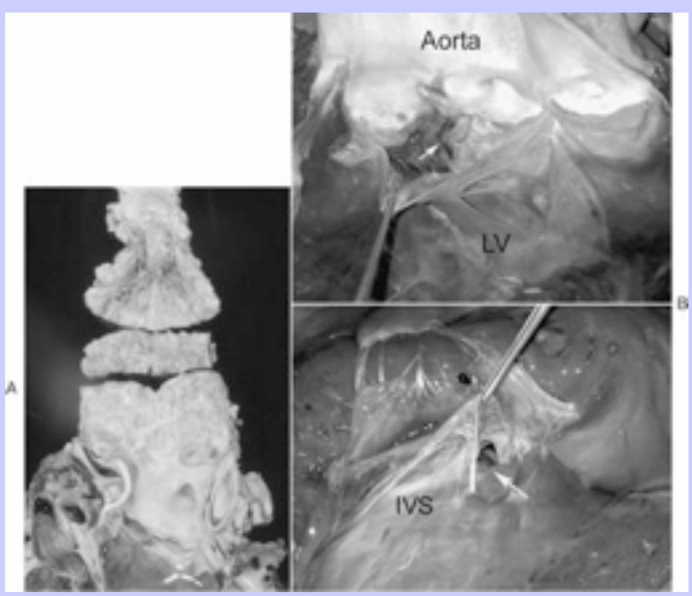
The clinician should anticipate infections in multiple organisms in cases of infective thrombophlebitis and should administer high doses of broad-spectrum antimicrobials until culture and sensitivity results of the aspirate are available. One should consider metronidazole (15 mg/kg every 6 hours) if one suspects anaerobic infection and may add flunixin meglumine (1 mg/kg every 12 hours) to reduce inflammation. Local therapy including hot compresses, topical ichthammol, or dimethyl sulfoxide may be helpful in treating thrombophlebitis. If antimicrobial therapy is unsuccessful, surgical resection of the affected vein may be indicated. Recanalization of an affected vein often occurs and can be documented by Doppler or contrast ultrasound studies demonstrating blood flow between the vessel wall and the thrombus. Occasionally, one may detect loculation within the ends of the thrombus. Such loculation may result in persistent fibrous webs within the vein that restrict venous return. Venous stricture also may occur following long-standing thrombophlebitis. Complete fibrous occlusion of the jugular vein (see Figure 8-47) may impede venous return from the head such that if adequate collateral circulation does not develop, impaired venous drainage of the head and neck may limit performance.

* References 3, 34, 52, 55–57, 61, 63, 65, 96, 99–101, 103, 114, 118, 127, 142, 159, 185, 195, 196–203, 274, 315, 319, 347–349, 398, 412, 425, 432, 434, 477, 519, 522, 535, 589–609.

8.10.2 AORTIC-ILIAC THROMBOSIS

Aortic-iliac thrombosis is an uncommon but potentially serious disorder. The gross appearance, histologic features, and clinical findings of this vasoocclusive disorder have been reviewed extensively, and the interested reader is directed to these and other related reports.[*] Arteriosclerotic and atherosclerotic aortic lesions also have been observed at other sites as well (see Figure 8-49). Suggested causes of arterial disorders such as these include *Strongylus vulgaris* infection, systemic infections, embolization, and vasculitis. No convincing evidence indicates that *Strongylus* infection consistently causes aortic-iliac thrombosis, and Azzie[199,201] has refuted this contention. The terminal aorta and the iliac arteries, including their proximal branches, are the focus of 427
428 involvement. Vessels become partially to completely occluded by multifocal ingrowths of fibrous tissue, laminated thrombi, or fibrous plaques.[197] Histologic lesions include organized, fibrous connective tissue, hemosiderin-laden macrophages, irregular vascular channels, and disruption of the intima and internal elastic lamina. The lesions are generally devoid of fat.

Figure 8-49 Aortic disease. **A,** Severe atheromatous change with secondary *Streptococcus* infection of the proximal aorta. The intimal and subintimal change begins just distal to the coronary ostia. Representative sections of the ascending aorta are shown. *Strongylus vulgaris* was not found. The most distal segment was necrotic and severely narrowed flow across the region. **B,** Dissecting lesion communicating the root of the aorta *(top panel, arrow)* to the right side of the heart across the intraventricular septum *(IVS)*. The distorted tricuspid valve is retracted to demonstrate the lesion on the right *(arrow)*.



Although aortic-iliac thrombosis is uncommon, it can be associated with severe performance problems, including reproductive failure.[601] The typical features include exercise-associated, typically unilateral hindlimb lameness, ataxia, or collapse.[197,199,201,202] Aortic-iliac thrombosis also has been diagnosed as a cause of breeding failure in stallions.[199,201,202] Physical examination of an affected horse at rest may reveal weak metatarsal arterial pulses or delayed saphenous refill in the affected limb. The temperature of the limb in the resting animal is usually normal, unless complete arterial occlusion has occurred, in which case the limb is cold and painful and may become edematous. Exercise in affected animals results in an exercise-associated gait abnormality (lameness, ataxia, or weakness) with a decreased or absent metatarsal and digital pulse and delayed or absent saphenous refill. Claudication may cause the horse to become uncomfortable and reluctant to bear weight on the affected limb. Significant hyperpnea, other signs of distress, and profuse, generalized sweating are often present with trembling of the affected limb. A rectal examination may reveal fremitus, a weak or absent pulse, or aneurysmal dilation of the affected artery or arteries. These abnormalities may be more evident after exercise and may help to confirm the diagnosis. 428

429
Ultrasonographic evaluation of the terminal aorta and iliac arteries (Figure 8-50) with a high-frequency (5- or 10-MHz) rectal transducer[198,200,348] or nuclear techniques[616] can confirm diagnosis. Doppler studies may indicate abnormal blood flow in the femoral arteries.[617] Essential abnormalities include a hypoechoic to echogenic mass protruding into the arterial lumen. One can estimate the degree of obstruction based on the percentage of the artery occluded. Many cases are long-standing, and hyperechoic areas, even tissues sufficiently echodense to cast acoustic shadows, may be imaged within the aortic or iliac thrombus. These findings suggest mature scar tissue and calcification.

The prognosis for this disorder is guarded. No controlled studies have evaluated therapy for this condition, though prior experience, including a recent case survey,[618] suggests no treatment consistently improves outcome. In cases of acute thrombosis associated with degenerative or inflammatory disease, surgical or catheter-based thrombectomy has potential benefit. Various medical treatments have been reported, including intravenously administered sodium gluconate, larvicidal dewormings, phenylbutazone, low-molecular-weight dextran, and a controlled exercise program. Early diagnosis is essential if therapy is to be beneficial. Treatment aims at improving collateral circulation and preventing additional thrombus formation.

* References 118, 197–202, 347, 348, 594, 601, 614–616.

8.10.3 PARASITIC INFECTION

The migrating larval forms, particularly L_4 of *S. vulgaris,* are known causes of arterial disease, particularly involving the aorta and the cranial mesenteric artery and its branches (Figure 8-51). Lesions have been described as far forward as the bulbous aorta and the aortic sinuses in infected horses. Rounded fibrous plaques and mural thrombi have been reported in the thoracic and cranial abdominal aorta in 9.4% of horses examined immediately after death by Cranley and McCullagh.[99] These investigators reported a statistical association between the occurrence of proximal aortic *S. vulgaris* lesions and the presence of focal ischemic lesions in the myocardium. They hypothesized that these lesions resulted from microembolism from parasitic lesions. With the advent of ivermectin and other new anthelmintics, and aggressive deworming programs currently recommended by practicing veterinarians, heavy *S. vulgaris* larval migration damage occurs less frequently. However, one must consider migration of *S. vulgaris* L_4 larvae in horses with poor deworming histories, high potential of exposure, high fecal egg counts, and palpable abnormalities on rectal examination or on finding fremitus or aneurysmal dilation of the aorta, particularly in the region of the cranial mesenteric artery or iliac system. An

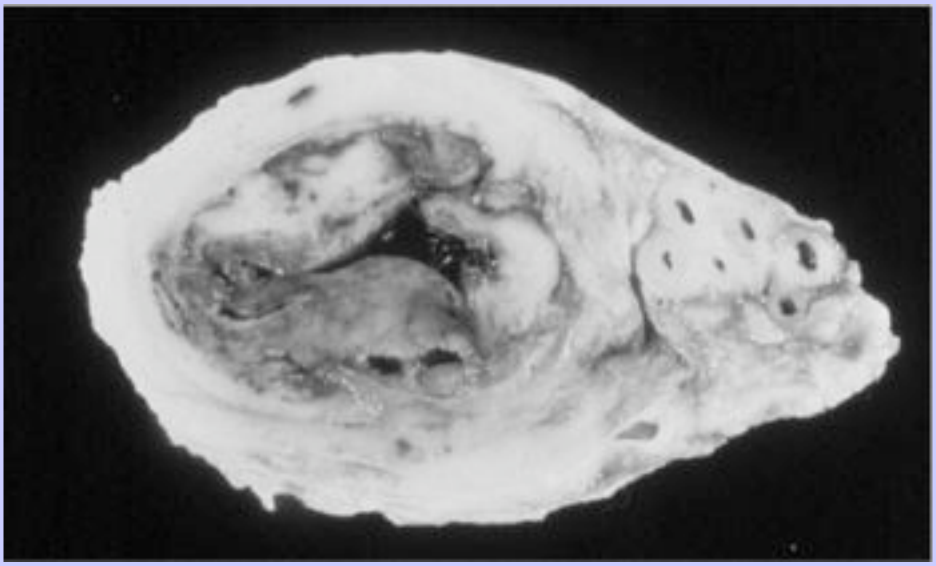
ultrasonographic evaluation of the cranial mesenteric artery is possible and can be used to confirm the diagnosis of thrombosis in these vessels (see Figure 8-51).[598,618] Treatment should consist of larvicidal deworming, combined with a rigorous individual and environmental parasite control program.

Figure 8-50 An ultrasound image of a distal aortic thrombosis obtained by transrectal probe. The thrombus, with hyperechoic areas and probable calcification as well as patent areas of the lumen with enhanced intravascular contrast effect, is evident.

8.10.4 ARTERIOVENOUS FISTULAE

Arteriovenous fistulae occur uncommonly and most often are detected with large vascular tumors (hemangiomata, hemangiosarcomata) or as a rare congenital defect or posttraumatic sequela.[597] Classic features include a continuous thrill and murmur over the affected area, localized edema, slowing of the heart rate after occlusion of the shunt, and signs of increased CO if the shunt flow is great. One can make a diagnosis with Doppler ultrasound, including pulsed wave and color flow Doppler to demonstrate continuous and pulsatile flow from the artery through the associated communication into the distended veins. The clinician should perform a 429
complete cardiovascular examination because a large arteriovenous communication could lead to cardiac failure. 430
Treatment depends on the underlying cause. No treatment may be indicated with small, uncomplicated arteriovenous communications. Large vascular neoplasms are usually inoperable at the time when they are diagnosed or are complicated by the possibility of metastasis.

Figure 8-51 Verminous arteritis and thrombosis in the cranial mesenteric artery caused by *Strongylus vulgaris*.



8.10.5

ARTERIAL RUPTURE

Rupture of the aorta or its branches or of the pulmonary artery are reported sporadically. Rupture of the aortic root has been reported commonly in older horses, usually older breeding stallions, but also may occur in older mares or in younger horses.[57] Rupture typically involves the right aortic sinus (of Valsalva). The aortic root can rupture into the right atrium, right ventricle, intraventricular septum, or pericardial space.[57,142,434,441] Dystrophic changes in the media of the aorta have been reported, and the hypertension associated with breeding has been implicated as a cause of aortic root rupture. Aneurysms of the right sinus of Valsalva also have been reported and are related to congenital defects in the media of the aorta near the right coronary sinus. Rupture of the middle uterine artery can lead to fatal peripartum hemorrhage.[195] Ruptures of the extrapericardial aorta or other blood vessels leading to fatal hemorrhage or a systemic to pulmonary shunt have been reported.[52,56–59,61,63–67]

Rupture of an artery is often a catastrophic event, and most diagnoses are made at the necropsy floor. Antemortem diagnosis of an aortic sinus of Valsalva aneurysm is possible by echocardiography. Aside from breeding, owners should not use horses with diagnosed aortic aneurysms or arterial-cardiac fistulae for athletic endeavor, because rupture of the aneurysm could occur at any time. When aortic root rupture or rupture of a sinus of Valsalva aneurysm communicates with the right atrium or right ventricle, right-sided heart failure can occur. The aortic-cardiac fistula causes a continuous heart murmur. Cardiac arrhythmias (usually ventricular tachycardia) also may develop, especially if dissection progresses into the intraventricular septum.[441] The prognosis for life in these horses is generally grave depending on the site and size of the communication. Some horses survive with supportive treatment for CHF.

Chronic pulmonary hypertension and pulmonary artery dilation most often cause pulmonary artery rupture. One detects rupture most commonly along with severe chronic MR, but rupture may occur in any horse with severe

pulmonary hypertension, regardless of the cause. One should consider the potential for pulmonary artery rupture whenever echocardiography detects a large, dilated main pulmonary artery or left and right pulmonary artery. Owners should consider affected horses unsafe for riding or driving and should not use them, except for breeding, because of the possibility of sudden death (see Figures 8-39 and 8-49). Focal medial calcification of the pulmonary artery is a common finding at necropsy.[432]

8.11 Cardiac Arrhythmias

Cardiac arrhythmias are disturbances in heart rate, rhythm, or conduction and can be classified based on atrial and ventricular rate, anatomic origin of the impulse, method of impulse formation, and conduction sequence. A variety of cardiac arrhythmias have been recognized.[493,619] Of principal concern to the clinician are the hemodynamic consequences of arrhythmias (reduced pressure, flow, and perfusion) and the potential for further electric instability (myocardial fibrillation, sudden death).

The electrophysiologic basis of cardiac arrhythmias will not be discussed, and the interested reader is referred elsewhere for descriptions of abnormal automaticity, reentry, reflection, entrainment, and other cellular mechanisms of arrhythmogenesis. Cardiac arrhythmias are classified based on the anatomic origin of the manifest ECG mechanism (Box 8-9). The reader should be aware that some arrhythmias, especially those originating in the atrioventricular junction, may mimic atrial rhythm disturbances or high ventricular rhythms. For the purpose of discussion, the authors have elected to distinguish *atrial* from *junctional;* however, one also may apply the term *supraventricular* to these rhythm disturbances. Box 8-10 reviews the clinical evaluation of the horse with an arrhythmia.

8.11.1 SINUS RHYTHMS

A number of physiologic sinus rhythms are recognized and can be explained by the effect of autonomic nervous system traffic on the sinoatrial node (Figure 8-52). Normal horses at rest demonstrate vagal-mediated sinus bradycardia, sinus arrhythmia, or sinus block or arrest; yet fear or a sudden stimulus may provoke sympathetically driven sinus tachycardia. Exercise leads to pronounced sinus tachycardia with heart rates often exceeding 200 beats/min. As a general rule, atrioventricular conduction tends to follow sinus activity. During sinus tachycardia, the P-R interval shortens, whereas during periods of progressive sinoatrial slowing, the P-R interval prolongs. Usually second-degree atrioventricular block, type I (Wenckebach), follows a progressive prolongation of the P-R interval. Sinus rate and atrioventricular conduction usually change in parallel; however, some horses appear to control blood pressure during high sinus tachycardia by blocking impulses in the atrioventricular node. Second-degree atrioventricular block is more likely in a standing horse, in a horse that suddenly has stopped submaximal exercise, or after a brief surge of sinus tachycardia in an anxious animal. 430 431

8.11.1.1 BOX 8-9 CARDIAC RHYTHMS OF THE HORSE

8.11.1.1.1 Sinus Mechanisms*

Normal sinus rhythm

Sinus arrhythmia

Sinoatrial block or arrest

Sinus bradycardia

Sinus tachycardia

8.11.1.1.2
Atrial Rhythm Disturbances

Atrial escape complexes[†]

Atrial premature complexes

Atrial tachycardia: nonsustained and sustained

Atrial flutter

Atrial fibrillation

Reentrant supraventricular tachycardia

8.11.1.1.3
Junctional Rhythm Disturbances

Junctional escape complexes[†]

Junctional escape rhythm[†] (idionodal rhythm)

Junctional premature complexes

Junctional (“nodal”) tachycardia

Reentrant supraventricular tachycardia

8.11.1.1.4
Ventricular Rhythm Disturbances

Ventricular escape complexes[†]

Ventricular escape rhythm[†] (idioventricular rhythm)

Ventricular premature complexes

Accelerated ventricular rhythm (idioventricular tachy-cardia)

Ventricular tachycardia

Ventricular flutter

Ventricular fibrillation

8.11.1.1.5
Conduction Disturbances

Sinoatrial block[*]

Atrial standstill (hyperkalemia)

Atrioventricular blocks: first degree,* second degree,* third degree (complete)

Ventricular preexcitation

* Generally physiologic.

† Escape complexes develop following another rhythm disturbance.

One must monitor sinus rate and rhythm carefully in the critically ill or anesthetized horse because heart rate is a major determinant of CO and arterial blood pressure. Sedative drugs and anesthetics can cause sinus bradycardia. Anesthetic drugs or hypoxia can depress sinus node function, as can traction on an abdominal viscus. Concurrent depression of the sinus node with stimulation of latent pacemakers in the coronary sinus or atrioventricular junction can lead to ectopic rhythms in the anesthetized horse. Conversely, an increasing sinus rate may indicate 431
inadequate depth of anesthesia, pain, or hypotension. On identifying sinus tachycardia, one must seek out and 432
treat the cause. One should consider and manage the possibilities of pain, hypotension, and sepsis when appropriate.

8.11.1.2

BOX 8-10 EVALUATION OF THE HORSE WITH

General medical history, past illnesses, past and current medications

Work history and exercise tolerance

General physical examination

Physical examination: evidence of congestive heart failure

- Subcutaneous edema
- Jugular venous distention and pulsations
- Pulmonary edema
- Pleural and peritoneal effusion
- Weight loss, poor condition

Auscultation of the heart

- Assessment of heart sounds (rhythm, third heart sound)
- Assessment of heart murmurs

Resting electrocardiogram

- Heart rate and cardiac rhythm
- Analysis of P-QRS-T waveforms

Postexercise electrocardiogram

Exercise (or treadmill) electrocardiogram

Clinical laboratory studies

- Complete blood count (rule out anemia, infection)
- Blood culture (in cases of suspected endocarditis)
- Serum electrolytes (particularly potassium and possibly magnesium)
- Serum biochemical tests (especially renal function)
- Skeletal muscle enzymes (cardiac isoenzymes)
- Serum troponin concentration
- Red blood cell potassium
- Fractional excretion of potassium

Echocardiogram/Doppler echocardiogram

- Examine for cardiomegaly or reduced myocardial function
- Identify predisposing cardiac lesions and abnormal flow

Serum/plasma concentrations of cardioactive drugs

- Serum digoxin concentration
- Plasma quinidine concentration

Response to medications

Modified from Bonagura JD: Clinical evaluation and management of heart disease, *Equine Vet Educ* 2:31-37, 1990.

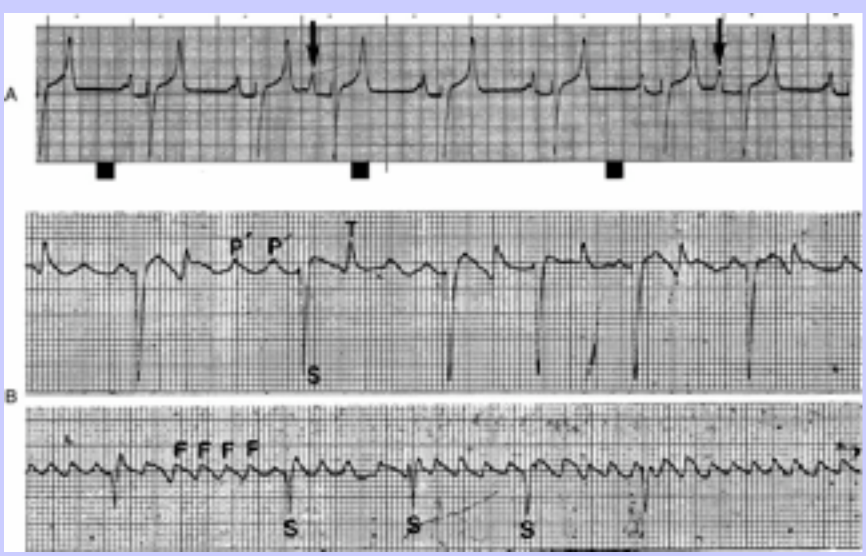
Figure 8-52 Sinus rhythms. **A,** Sinus bradycardia *(top)* and tachycardia *(bottom)*. Base-apex lead recorded at 25 mm/sec. **B,** Sinus arrhythmia with second-degree atrioventricular block *(arrows)*. An ambulatory electrocardiogram is shown; transthoracic leads recorded at 25 mm/sec.

Sinus bradycardia is generally a benign rhythm in standing horses; however, when occurring during sedation or anesthesia, sinus bradycardia or arrest may cause CO to decrease and result in significant hypotension. Treatment of symptomatic sinus bradycardia can include the infusion of catecholamines (dobutamine, dopamine, epinephrine) or the administration of anticholinergic drugs (atropine, glycopyrrolate) (see Table 8-6). One can infuse dopamine or dobutamine to increase heart rate, contractility, and arterial blood pressure; however, reflex atrioventricular block may develop in some horses.[187,191,212,257] Excessive administration of catecholamines causes sinus tachycardia and ectopic beats. Anticholinergic drugs may not be effective in the setting of anesthetic-induced depression of sinoatrial function, particularly if vagal efferent traffic is low. Gastrointestinal complications including ileus and colic may develop after anticholinergic drug therapy; thus one should not choose it for trivial rate problems.

8.11.2 ATRIAL ARRHYTHMIAS

Rhythms originating in the atria are common. These disturbances often develop as functional disorders with no overt structural cardiac lesion. Autonomic imbalance (including high sympathetic activity), hypokalemia, β-agonists, infections, anemia, and colic can cause atrial premature complexes. Of course, atrial rhythm disturbances are common with structural lesions of the valves, myocardium, or pericardium. Myocarditis after viral or bacterial infection may precipitate atrial arrhythmias in some horses. Atrial premature complexes can precipitate sustained atrial arrhythmias such as atrial tachycardia, atrial flutter, and atrial fibrillation. The large size of the equine atria and the frequent presence of microscopic atrial fibrotic lesions predispose the horse to these sustained arrhythmias. The high resting vagal tone present in most horses serves to shorten the duration of 432
the action potential of atrial myocytes and also facilitates development of sustained atrial tachyarrhythmias, 433
which probably depend on reentry.

Figure 8-53 Electrocardiograms with atrial rhythm disturbances. **A,** Premature atrial complexes *(arrows)*. The premature P waves of different morphology, the slightly prolonged P-R interval indicating atrioventricular nodal refractoriness, and the normal-appearing QRS complex indicating a supraventricular origin are notable. A slight conduction aberrancy exists in the second premature QRS complex (see also Figure 8-15). **B,** Sustained atrial tachyarrhythmias. The top picture shows atrial tachycardia with rapid, regular P waves *(P')* and a variable ventricular rate response to conducted P waves. The base-apex lead recorded at 25 mm/sec. The lower trace shows atrial flutter *(F)* with variable ventricular response *(S)*. Lead 3 recorded at 25 mm/sec.



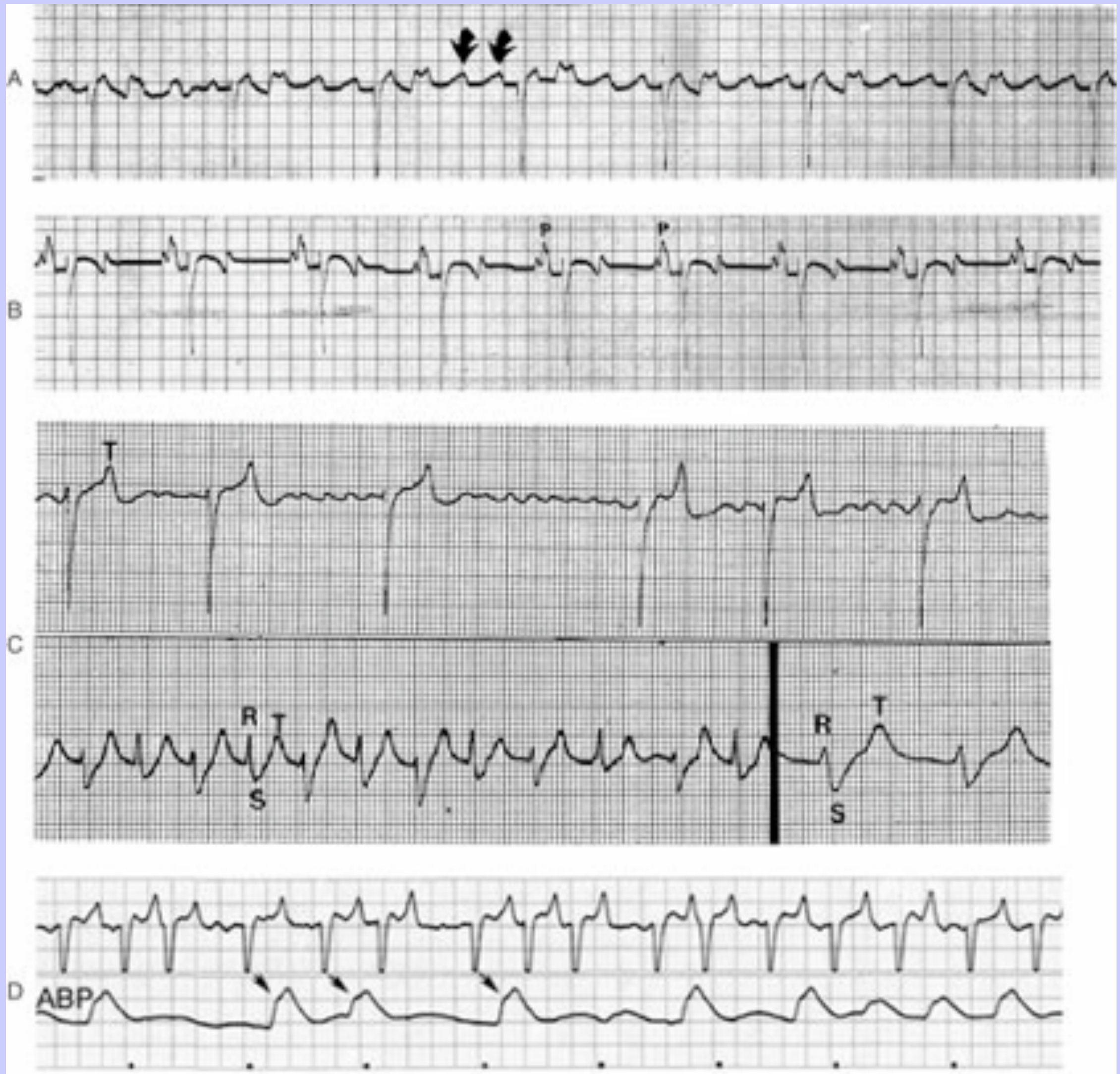
Atrial arrhythmias are the most common abnormal rhythms detected in horses (Figures 8-53 and 8-54).[*] Atrial premature complexes are the least complex of these rhythm disturbances and may be clinically insignificant or associated with exercise intolerance or other signs of cardiac disease. In contrast, atrial tachycardia, atrial flutter, and atrial fibrillation are likely to be clinically significant. The overall importance of atrial premature complexes is often difficult to ascertain. Routine ECG rhythm strips recorded from more than 950 horses and interpreted by one of the authors (Bonagura) indicated that atrial arrhythmias, overall, were present in less than 3% of the horses studied. However, when one of us (Reef) examined clinically normal horses by Holter monitor, atrial premature complexes were present in 28% of the horses. Thus the incidence of atrial arrhythmias appears to depend not only on the population examined but also on the methods used. Clearly, one should assess these rhythm disturbances in light of other clinical findings.

* References 2, 33, 39, 42, 46, 55, 102, 124, 130, 168, 169, 171, 173, 179, 256, 314, 391, 418, 420, 474, 487, 494, 501, 503, 505, 561, 620–632.

8.11.3 ATRIAL PREMATURE COMPLEXES

The clinician can detect atrial premature complexes by auscultation and document them by ECG. Auscultation usually reveals a regular sinus rhythm that is interrupted by an obviously premature beat (see Table 8-1). Premature atrial contractions may be interpolated fully (the regular sinus rhythm continues after the premature 433
atrial contraction) or a pause may occur, if the sinus node is reset. The ECG is characterized by a premature 435
(usually narrow) QRS complex, which is preceded by an abnormal, premature P wave that is often buried within the preceding T wave. Often the P-R interval is longer than normal (physiologic first-degree atrioventricular block), and in other cases the ectopic P wave is nonconducted (physiologic second-degree atrioventricular block) (see Figure 8-53). One must take care not to overdiagnose atrial premature complexes. Sinus arrhythmia and sinus bradycardia often lead to variations in the P-P intervals, and often a “wandering atrial pacemaker” gradually alters the P wave morphology. Exercise or excitement abolishes such physiologic rhythms.

Figure 8-54 Atrial arrhythmias. **A** and **B,** Successful conversion of atrial fibrillation using a combination of digoxin and quinidine (see text for details). **A,** Incomplete conversion of atrial fibrillation to atrial tachycardia with rapid, regular atrial activity *(arrows)*. **B,** Conversion to normal sinus rhythm during combination therapy. **C,** Quinidine toxicosis can be manifested as abnormalities on the electrocardiogram. In this horse, atrial fibrillation (*top*; base-apex lead recorded at 25 mm/sec) was not converted. The lower recording shows persistent atrial fibrillation with a rapid atrioventricular conduction response, electrical alternans, and widening of the QRS complex (lead 2 recorded at 25 mm/sec; right panel, at 50 mm/sec). The rapid rate response is related to the vagolytic effect of quinidine. The electrical alternans is common with regular supraventricular tachycardias with rapid ventricular response rates; this finding indicates varying conduction into the ventricle. The widened QRS is a sign of quinidine toxicosis. An idiosyncratic reaction leading to polymorphous ventricular tachycardia also has been recognized (see Figure 8-56). **D,** Hemodynamic consequences of increasing ventricular response rate in atrial fibrillation. The short QRS-QRS intervals do not generate effective arterial pulsations recorded in the arterial blood pressure *(ABP)* tracing, which is related to inadequate ventricular filling time during short cycles. Pulse deficits are detectable by physical examination.



As a general rule, atrial premature complexes are more likely to be clinically significant in the following circumstances: if they (1) are frequent at rest, (2) are associated with nonsustained or sustained runs of atrial tachycardia, (3) are related to poor performance (while other causes are excluded), (4) precipitate paroxysmal atrial flutter or fibrillation, or (5) develop along with other signs of cardiac disease. Documentation of atrial arrhythmias *during* exercise may be critical for determining if paroxysmal atrial tachycardia or fibrillation is likely to be the cause of poor performance.[354] One must use clinical judgment, however, because supraventricular premature complexes are most likely to occur in the immediate postexercise period, probably associated with autonomic imbalance. If these postexercise arrhythmias are not associated with clinical signs and are not detected during exercise, they are unlikely to be clinically significant. Cardiac rhythm monitoring during exercise may be necessary to be certain. In summary, routine electrocardiography, clinical chemistry, hematology, echocardiography, exercising electrocardiography, postexercise electrocardiography, and continuous 24-hour electrocardiographic monitoring may play a role in the complete evaluation of a horse suspected of having intermittent cardiac arrhythmias.

One generally does not treat isolated atrial premature complexes. The hemodynamic consequences of these rhythm disturbances is minor, unless sustained abnormal activity develops. Consideration of antiarrhythmic therapy might be appropriate if one documents frequent atrial extrasystoles as precipitating atrial fibrillation. In such cases, one may contemplate using quinidine or procainamide (though both therapies are impractical for chronic use) or digoxin. Although digoxin is easiest to administer long term, the effectiveness of such treatment has not been established except in the setting of CHF. Maintenance of normal serum potassium may be important in suppressing atrial ectopic activity. In cases of suspected immune-mediated myocarditis, one might consider antiinflammatory doses of dexamethasone as empirical therapy.

8.11.4 ATRIAL TACHYCARDIA

Atrial tachycardia, a sustained, ectopic, abnormal atrial tachyarrhythmia, occurs infrequently. Atrial tachycardia may be sustained or nonsustained (paroxysmal) and is precipitated by an atrial premature complex. The atrial rate is rapid and regular; however, because many of the ectopic P waves are blocked physiologically in the atrioventricular node, an irregular ventricular rate response often results. Atrial rates of 120 to 300 beats/min are typical in horses with sustained atrial tachycardia. At lower atrial rates, 2:1 atrioventricular conduction may yield a regular, relentless heart rate. At the higher atrial rates, the rhythm may be indistinguishable from atrial flutter. Differentiation of atrial tachycardia from flutter is not critical because both arrhythmias carry the same clinical significance and are treated identically. One recognizes sustained atrial tachycardia most often during treatment of horses with quinidine sulfate. Before conversion of atrial fibrillation to sinus rhythm, one may observe atrial tachycardia; thus this rhythm indicates a partial therapeutic effect of quinidine on the atrial myocardium. When this atrial tachycardia occurs as an isolated finding, one should suspect structural or underlying myocardial disease and should consider the horse predisposed to development or recurrence of atrial fibrillation. Treatment is the same as that described for atrial fibrillation.

8.11.5 ATRIAL FLUTTER

Atrial flutter is even less common than atrial tachycardia and represents a form of atrial circuit movement or macro-reentry. The clinical circumstances and assessment of this rhythm disturbance are identical to those of atrial tachycardia. The ECG in atrial flutter is characterized by a rapid abnormal but regular atrial activity usually manifested as a saw-toothed ECG baseline. The atrial frequency often exceeds 300 beats/min. The RR intervals are usually irregular because of variable atrioventricular conduction, and fewer QRS and T complexes than flutter waves are present (see Figure 8-53). The ventricular rate depends on the refractory period of the atrioventricular node and on the strength of the atrial stimulus. Treatment of atrial flutter is similar to that for horses with atrial fibrillation.

8.11.6 ATRIAL FIBRILLATION

Atrial fibrillation is the most common atrial arrhythmia associated with poor performance and exercise intolerance.[*] CO at rest is normal in most horses with atrial fibrillation[474]; yet maximal CO during exercise is 435
limited because the atrial contribution to filling is most important at higher heart rates. Undoubtedly, the already 436
high LA pressure present in heavily exercising horses[481,482] is increased further by the loss of active atrial transport function. As expected, exercise intolerance is most common in high-performance horses (racehorses, advanced combined training horses, polo ponies, and some Grand Prix jumpers) and less common in show hunters and in pleasure, dressage, and endurance horses. Exercise-induced pulmonary hemorrhage, respiratory

distress, CHF, ataxia or collapse, and myopathy have been reported with atrial fibrillation; conversely, the arrhythmia often is detected as an incidental finding.[314]

Atrial fibrillation may be paroxysmal or sustained. Paroxysmal atrial fibrillation often is associated with a single episode of poor performance, with the horse often decelerating suddenly during a race. The arrhythmia usually disappears spontaneously within 24 to 48 hours. Paroxysmal atrial fibrillation may be associated with transient potassium depletion, particularly in horses treated with furosemide or bicarbonate solutions, and most often is unrelated to other clinical or echocardiographic abnormalities of cardiac disease. Sustained atrial fibrillation may be less common than paroxysmal atrial fibrillation but is easier to diagnose because the arrhythmia is sustained. A point of emphasis is that many horses with sustained atrial fibrillation have no other evidence of significant underlying cardiac disease. Box 8-11 summarizes those factors that interact in the prognosis of a horse with atrial fibrillation. Of these, CHF represents the overall worst prognostic indicator. Atrial fibrillation is most common in adult horses and has been reported infrequently in foals, weanlings, yearlings, or ponies. Several reports indicate a higher incidence in Standardbreds, Draft Horses, and Warmblood Horses compared with the general hospital population.

Atrial fibrillation also occurs commonly in horses with advanced mitral or tricuspid insufficiency or CHF. In these horses, treatment should aim at controlling CHF and resting heart rate with furosemide, an ACE inhibitor, and digoxin as opposed to quinidine conversion back to sinus rhythm.

Atrial fibrillation is characterized on auscultation by an irregular heart rhythm, variable intensity heart sounds, and an absent S_4 (see Table 8-1). Arterial pulses vary in intensity, and pulse deficits may be present. The ECG is characterized by an absence of P waves; instead, fibrillation or f waves occur in the baseline. These f waves may be coarse (large) or fine (small), and the number of atrial impulses per minute cannot be counted but usually exceeds 500. The QRS-T complexes are normal in morphology and duration, but ventricular rate response is irregular, although one may observe periodicity infrequently.[169] As for all atrial tachyarrhythmias the ultimate ventricular rate response depends on the refractory period of the atrioventricular node and the strength of the atrial stimuli. In the otherwise healthy horse in atrial fibrillation, vagal tone is high and sympathetic tone is low when standing; consequently, the ventricular rate is close to normal or slightly increased (30 to 40 beats/min). If sympathetic activity increases for any reason or if vagal activity is blocked (as occurs with quinidine sulfate therapy), the ventricular rate response increases as the atrioventricular nodal refractory period shortens. This explains the clinician's simple but useful dependence on measuring pretreatment, resting heart rate in horses with atrial fibrillation.[314,501] Because the horse with structural heart disease is more likely to require sympathetic support to maintain CO and arterial blood pressure, persistent resting tachycardia, higher than 60 beats/min, is associated with a poorer prognosis.

Laboratory studies in horses with atrial fibrillation are usually normal. Infrequently, a horse is found to have low serum potassium, fractional excretion of potassium, or red blood cell potassium. Chest x-rays are usually normal unless pulmonary disease is concurrent, and this study is not a high-yield procedure in horses without compatible signs of lung disease or structural heart disease. The echocardiogram is usually normal, unless valvular or ventricular myocardial disease is concurrent. Commonly an otherwise normal horse demonstrates a slightly reduced LV shortening fraction (usually 24% to 32%), which returns to normal once the horse has been converted to sinus rhythm.[418,632] This decrease in fractional shortening is probably multifactorial in origin but is related in part to decreased preload from loss of the atrial contribution to ventricular filling. Doppler studies are useful in evaluating horses with concurrent valvular disease.

One may give an excellent prognosis for conversion (>95% conversion rate) for horses with a heart rate of less than or equal to 60 beats/min, murmurs less than or equal to grade 3 out of 6, and atrial fibrillation of less than 4

months in duration.[501] Recurrences affect approximately 25% of these horses. Horses with longer duration of atrial fibrillation or significant other cardiac disease may be more difficult to convert to sinus rhythm (80% conversion) and have a higher recurrence rate (60% recurrence). Horses usually can return to training within 24 to 48 hours after conversion, once plasma quinidine concentrations are undetectable. One should evaluate carefully any horses with heart rates exceeding 60 beats/min for heart failure or structural disease and probably should not treat the horses with quinidine before a complete evaluation.

Conversion of atrial fibrillation to sinus rhythm using quinidine sulfate has been successful in many horses and using a variety of treatment plans (see Figure 8-54).[276,619,638] Box 8-11 summarizes general recommendations for therapy, including alternative treatments. Intravenously administered quinidine gluconate can be successful in conversion of horses with no other significant cardiac disease and atrial fibrillation of recent onset.[629] If this treatment is not successful or the suspected duration is longer than 1 month, one should attempt conversion with quinidine sulfate administered via nasogastric intubation at a dosage of 22 mg/kg every 2 hours until the horse converts or develops initial signs of toxicosis. One should consider toxicosis or a cumulative dose of 88 to 132 mg/kg the endpoints for therapy and if conversion has not occurred, one should obtain a plasma sample for determination of quinidine plasma concentration and should discontinue therapy temporarily for at least 6 hours. Therapeutic plasma quinidine concentrations for conversion from atrial fibrillation to sinus rhythm are 2 to 5 μg/ml. One should base subsequent quinidine sulfate administration on the most recent plasma concentration. The mean quinidine elimination half-life after an oral 10-g dose is 6.65 hours.[257,639] Thus after the loading regimen every 2 hours, one can prolong treatment intervals to every 6 hours (approximately every half-life) and can continue these treatment intervals until the horse shows toxic signs or converts to sinus rhythm, or the owner elects to discontinue treatment. If conversion to sinus rhythm has not occurred after 24 hours of therapy, one may add digoxin at 0.0055 to 0.011 mg/kg orally twice a day to the treatment regimen for 24 to 48 hours. As in other species, a digoxin-quinidine interaction effectively can double the serum concentration of digoxin.[640] Thus combination therapy beyond 24 hours should continue only with drug level monitoring of the serum digoxin and consideration of using the lower end of the digoxin dosage range. Even horses that do not convert on the standard administration regimen every 2 hours may convert after the initiation of an every-6-hour interval or the combined use of quinidine and digoxin. The value of using such a treatment plan every 6 hours is reaching of steady-state levels, having sufficient time to attain myocardial concentrations, and lower frequency of quinidine toxicity compared with the every-2-hour regimen.

8.11.6.1 BOX 8-11 MANAGEMENT OF ATRIAL FIBRILLATION

8.11.6.1.1 General Examination of the Horse

(see Box 8-4)

8.11.6.1.2 Identification of Negative Prognostic Factors

Congestive heart failure (CHF)

Consistently elevated resting heart rate

Dilated cardiomyopathy

Mitral, tricuspid, or aortic valve regurgitation with cardiomegaly

Long history of atrial fibrillation

Prior bouts of atrial fibrillation

Unresponsive to therapeutic doses of quinidine

8.11.6.1.3 Horse Without Heart Failure

8.11.6.1.3.1 Recommended method: initial oral dosing at 2-hour intervals

- Initiate therapy with quinidine sulfate via nasogastric tube, 22 mg/kg body mass every 2 hours to a cumulative dose of 88 to 132 mg/kg, or until conversion or toxicosis occurs.
- When the aforementioned treatment fails to achieve conversion to normal sinus rhythm, continue quinidine therapy at a dose of 22 mg/kg every 6 hours for an additional 2 to 4 days, monitoring plasma quinidine concentration.
- Administer digoxin concurrently during quinidine therapy in the following situations: (1) if the vagolytic effect of the drug causes significant acceleration in ventricular rate response, (2) if resting heart rate exceeds 90 to 100 beats/min, (3) if the horse exhibits low resting vagal tone (e.g., a nervous animal), or (4) beginning on the second day of quinidine therapy if conversion has not yet occurred.[*]
- An oral maintenance dose range of digoxin for control of ventricular rate response in atrial fibrillation is 1.1 to 1.5 mg/100 kg body mass every 121 hours[†]; alternatively, administer an intravenous maintenance dose of digoxin at 0.22 to 0.375 mg/100 kg body mass every 12 hours.[†]
- Monitor plasma levels of quinidine if possible; therapeutic levels associated with conversion to normal/sinus rhythm are 2 to 5 µg/ml.
- Monitor plasma levels of digoxin to avoid digoxin toxicity.

8.11.6.1.3.2 Alternative therapy: IV dosing

- Administer quinidine gluconate 1 mg/kg intravenously every 5 to 10 minutes to a maximum of 10 mg/kg cumulative dose.
- This treatment is used most frequently for conversion of atrial fibrillation of recent onset (<7 days), during anesthesia, or when nasogastric delivery is difficult or impossible.

8.11.6.1.3.3 Toxicosis

- Monitor the electrocardiogram for response and toxicosis (increasing ventricular rate response, increasing QRS duration, or proarrhythmic effect causing ventricular tachycardia).
- If toxicosis develops (see text), discontinue therapy until all signs resolve, and initiate oral therapy using the every-6-hour method as described earlier.
- Consider a 24-hour Holter electrocardiogram on the day after conversion to evaluate for recurrent atrial arrhythmias.

8.11.6.1.3.4

Rest the horse

For 1 to 2 weeks if possible (in racing horses, training can begin 48 hours after conversion).

8.11.6.1.3.5

Reevaluate the horse

For atrial arrhythmias, before starting training and during training.

8.11.6.1.4

Horse with Heart Failure

- Low probability for return to serious work; however, successful therapy may permit breeding or keeping the animal as a pet.
- Establish diuresis with furosemide (1 mg/kg intravenously or intramuscularly every 12 hours) as needed to control edema. Continue maintenance furosemide therapy at 0.5 to 1 mg/kg per os every 12 to 24 hours; titrate the daily dose to effect.
- Digitalize the horse to control heart rate and treat heart failure.

  Use an intravenous maintenance dose for treatment of CHF at 0.22 mg/100 kg body mass every 12 hours[†] to initiate therapy until clinical signs of CHF have been controlled; alternatively, begin therapy using the oral maintenance dose below.

  Administer an oral maintenance dose of 1.1 to 1.75 mg/100 kg body mass every 12 hours[†] chronically.
- Prescribe an angiotensin-converting enzyme inhibitor such as enalapril (see text).
- Monitor the serum digoxin concentration (therapeutic trough levels are 1 to 2 ng/ml).
- Monitor serum electrolytes, blood urea nitrogen, and serum creatinine.
- Monitor the resting heart rate, clinical signs, and electrocardiogram.

* The clinician should consider the potential for quinidine-digoxin drug interaction elevating the serum digoxin level (see text).

† When a dosage range is given, generally begin at the lower end of the dose.

Conversion of horses with atrial fibrillation generally results in a return to their previous performance level.[314,501] Horses with repeated episodes of atrial fibrillation often are converted numerous times with quinidine sulfate treatment. Most horses that experience a recurrence of atrial fibrillation do so within 1 year of initial conversion, but periods as long as 6 years have occurred between episodes of atrial fibrillation in some horses. If the duration of sinus rhythm becomes shorter, repeated treatments may no longer be practical, and a career change may be indicated. Some horses eventually become refractory to quinidine sulfate, probably because of the progressive atrial fibrosis or underlying myocardial disease.

One should perform careful clinical and continuous electrocardiographic monitoring on horses with atrial fibrillation during conversion to sinus rhythm and should monitor and compare the QRS duration with the pretreatment QRS duration before administering each additional treatment. Prolongation of the QRS duration to greater than 25% of the pretreatment value is an indication of quinidine toxicity and should prompt discontinuation of therapy. The simplest ECG change is an acceleration of atrioventricular nodal conduction related to the vagolytic effect of quinidine. Rapid supraventricular tachycardias with ventricular rate responses of 300 beats/min have been observed in several horses receiving quinidine sulfate. These horses have been treated intravenously with digoxin at 0.002 mg/kg to slow the ventricular response rate, replacement fluids to improve perfusion, sodium bicarbonate at 1 mEq/kg to bind any free plasma quinidine, and if needed, a phenylephrine drip to restore blood pressure if hypotension is critical. Ventricular arrhythmias (torsades de pointes, multiform ventricular tachycardia, and ventricular complexes) also have been detected with quinidine toxicosis (see Figure 8-54). Intravenously administered sodium bicarbonate also is indicated in these horses to bind free circulating quinidine, whereas intravenously administered magnesium sulfate (up to 25 g in a 450- to 500-kg horse) is the treatment of choice for quinidine-induced ventricular arrhythmias. One also may use lidocaine hydrochloride if needed, starting with an intravenous bolus of 0.5 to 1.5 mg/kg given slowly intravenously.

Clinical markers of quinidine toxicosis include ataxia, colic, and nasal edema causing upper respiratory tract stridor. Most toxic reactions to quinidine sulfate are associated with higher plasma concentrations of the drug (>5 μg/ml) and can be avoided with careful clinical and electrocardiographic monitoring. Many of the adverse effects of quinidine administration are idiosyncratic reactions. These adverse effects should prompt discontinuation of therapy or altering of treatment intervals. Depression and paraphimosis occur in most horses treated with quinidine but disappear with the discontinuation of the drug. Diarrhea often develops with the administration of higher doses of quinidine, but this sign also disappears with discontinuation of the drug. Convulsions, hypotension caused by vasodilation, CHF, laminitis, urticaria, and sudden death also have been reported 438
associated with quinidine sulfate administration, although these fortunately are rare. Tachycardia exceeding 100 439
beats/min is an adverse idiosyncratic reaction that one should avoid if at all possible. Particularly nervous horses or those prone to the vagolytic effects of quinidine may benefit from pretreatment with digoxin at 0.011 mg/kg orally twice daily to blunt the ventricular rate response. When the ventricular rate response exceeds 100 beats/min in any horse undergoing quinidine sulfate conversion, one should consider digitalization, particularly if no other signs of quinidine toxicosis occur (indicating an excessive plasma concentration).

Future treatments of atrial fibrillation or flutter in horses might involve overdrive pacing of the atria or intracardiac mapping and radiofrequency ablation of tissue.[638] Electric cardioversion has been used in a limited number of horses to induce sinus rhythm,[641] but such therapy requires general anesthesia. These may represent future approaches to therapy but currently are considered experimental.

* References 2, 33, 39, 42, 46, 55, 102, 124, 130, 136, 168, 169, 171, 179, 256, 312–314, 353, 354, 391, 418, 420, 474, 487, 494, 501, 503, 505, 507, 561, 620, 621, 623–638.

8.11.7 JUNCTIONAL ARRHYTHMIAS

Cardiac arrhythmias that originate within the atrioventricular conducting tissues, the ventricular specialized conducting tissues, or myocardium are classified as junctional (atrioventricular nodal or bundle of His) or ventricular in origin. Unlike sinus or atrial arrhythmias, a conducted P wave does not precede these arrhythmias. Junctional and ventricular rhythms often lead to dissociation between the sinoatrial (P wave) activity and that of the ventricle (QRS-T), resulting in atrioventricular dissociation.[359] Atrioventricular dissociation in these cases

develops because the premature atrioventricular junctional or ventricular depolarization causes interference with the conduction of normal sinoatrial impulses.

Distinguishing between junctional and ventricular arrhythmias and determining the exact location of the abnormal impulse formation may be difficult. One sometimes can make the differentiation of junctional and ventricular rhythms by inspecting the QRS complex. Junctional impulses are more likely to result in a narrow, normal-appearing QRS complex with normal initial activation and electric axis, because they originate above the ventricular myocardium. Complexes of ventricular origin, by contrast, are conducted abnormally and more slowly, resulting in a widened QRS, an abnormal QRS orientation, and abnormal T waves. However, junctional tachycardias may be conducted aberrantly, resulting in a bizarre and wide QRS complex. When sustained, both types of rhythms cause atrioventricular dissociation with an independent atrial rhythm superimposed on the ectopic ventricular rhythm. The normal heart contains potential cardiac pacemakers within the atrioventricular and ventricular specialized tissues. These potential pacemakers may become manifest during periods of sinus bradycardia or atrioventricular block. Such complexes are termed *escape complexes* or, if the junctional or ventricular complexes are linked together, *escape rhythms*. Escape rhythms are characterized by slow ventricular rates, often in the realm of 15 to 25 beats/min. These normal pacemakers may be enhanced under certain conditions, including the administration of anesthesia or catecholamines. Preanesthetic drugs such as xylazine and detomidine and anesthetic drugs (halothane) suppress sinoatrial function, resulting in sinus bradycardia or periods of sinus arrest while enhancing the effects of catecholamines on latent junctional and ventricular pacemakers (Figure 8-55). Such accelerated idioventricular (or idionodal) rhythms generally have little clinical significance other than to prompt the reduction of anesthetic dosages or promote administration of vagolytic drugs to increase the sinoatrial rate. Specific antiarrhythmic drug suppression of escape rhythms is generally not necessary and is contraindicated because these rhythms serve as rescue mechanisms for the heart.

Junctional complexes that arise early relative to the normal cardiac cycle are designated as *premature* junctional complexes. These complexes may occur as single or repetitive events and can resemble ectopic rhythms that might originate in the atria. Repetitive ectopic complexes that occur in short bursts or runs are termed *nonsustained* or *paroxysmal tachycardias*. Sustained junctional tachycardias also may occur and can lead to CHF.

The clinical significance of an occasional junctional premature complex is difficult to ascertain. Persistent or repetitive junctional or ventricular rhythms indicate heart disease, systemic disease, or a drug-induced abnormality of cardiac rhythm. The best management choice is uncertain inasmuch as some junctional rhythms behave more like atrial tachyarrhythmias, whereas others cause atrioventricular dissociation and appear to act like ventricular ectopic impulses. Because the mechanism responsible for junctional tachycardias can be abnormal automaticity or reentry (circuit) movement using the atrioventricular node, empirical therapy usually is required to control sustained arrhythmias. If atrioventricular dissociation is obvious, then quinidine would seem a reasonable first choice. If the mechanism is uncertain but is clearly supraventricular, then intravenously administered digoxin, verapamil, or propranolol may silence the rhythm or slow the rate.

8.11.8 VENTRICULAR ARRHYTHMIAS

Ventricular arrhythmias are less common than atrial arrhythmias but are more likely to be associated with underlying cardiac disease or a multisystemic disorder.[*] One may observe ventricular arrhythmias with severe toxemia or sepsis or primary gastrointestinal disorders including proximal enteritis and large bowel disorders; with electrolyte (potassium, magnesium) or metabolic disorders, hypoxia, or ischemia; or associated with halothane anesthesia. In particular potassium, magnesium, and calcium activities can affect myocardial 439

electrophysiology.[650] Drugs, myocardial toxins such as the ionophores, viral or bacterial myocarditis, 441

endocarditis, or pericarditis may lead to significant ventricular arrhythmias. The approach to the horse with ventricular arrhythmias should emphasize ruling out the noncardiac causes, correcting them (if possible), and then following if necessary with a complete cardiovascular examination, including electrocardiography and echocardiography. One should consider evaluating cardiac isoenzymes to identify cardiac injury, although separating primary from secondary causes of cardiac isoenzyme elevation may be difficult (see Box 8-10). Ventricular ectopic rhythms are classified as indicated in Box 8-9.

Figure 8-55 Ventricular extrasystoles (see also Figure 8-15). **A,** The ectopic complex *(arrow)* is premature and abnormal in morphology. A compensatory pause follows the extrasystole because the next sinus P-wave is blocked in the atrioventricular node. The effect of the premature complex on arterial blood pressure in the lower tracing *(arrow)* is noticeable. **B,** Ventricular rhythms. The top tracing demonstrates an idioventricular tachycardia in a postoperative gastrointestinal surgery case. Sinus complexes *(arrow)* are followed by ventricular ectopic beats at varying rates (base-apex lead recorded at 25 mm/sec). The lower tracing demonstrates atrioventricular dissociation caused by a sustained high ventricular (or junctional) ectopic rhythm. The atria are discharged independently by the sinus node (*arrowheads*; lead 2 recorded at 25 mm/sec).

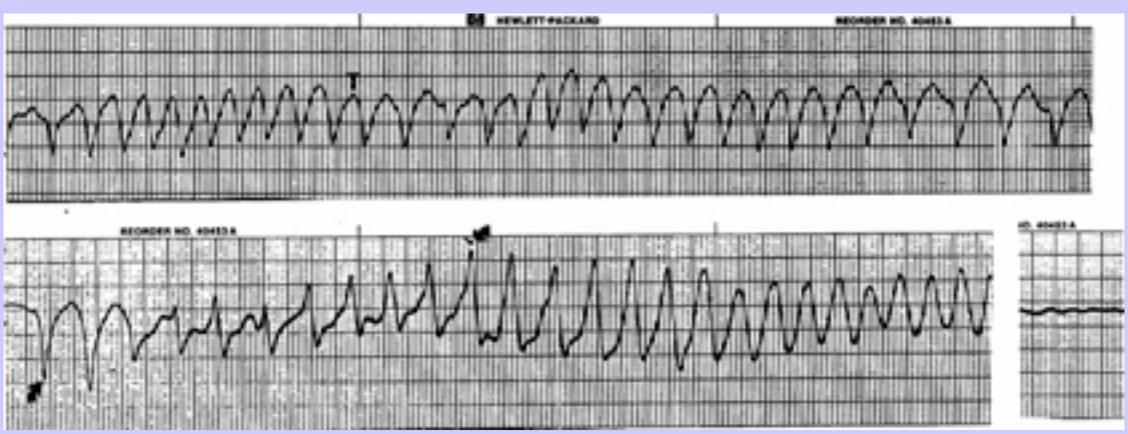
* References 177, 310, 359, 391, 412, 582, 584, 642–649.

8.11.9

VENTRICULAR PREMATURE COMPLEXES

Ventricular premature complexes are characterized by an ectopic, premature complex usually followed by a compensatory pause, because the next sinus impulse is blocked by the refractory atrioventricular conduction system. If the sinus rate is slow or the ventricular premature depolarization is coupled closely with the preceding normal sinus beat, the premature complex may be interpolated between two normal beats. Ventricular ectopic impulses are characterized by QRS and T waves that are wide and often bizarre in appearance (Figure 8-56). Of course, the premature QRS bears no relationship to any preceding P waves, though genesis of ventricular ectopics may depend on underlying cardiac cycle length. The morphology of the ventricular premature complex may be uniform or multiform. The relationship of a ventricular extrasystole to the preceding sinus QRS-T is expressed by the coupling interval between them. Often the coupling interval is fixed, although it may vary minimally[648] or considerably in cases of ventricular parasystole. A short coupling interval may place the ectopic QRS on the preceding T wave, a phenomenon called *R on T* and related experimentally to increased ventricular vulnerability for fibrillation. Ventricular premature complexes occurred in 14% of clinically normal horses during routine 24-hour continuous electrocardiographic monitoring.[310] The distribution pattern of ventricular ectopics may include haphazard distribution, bigeminy, couplets (pairs of ventricular premature complexes), or runs of ventricular ectopics. Runs of accelerated ventricular rhythm, usually developing at slow heart rates, are common in horses with ventricular ectopic activity. Infrequently, protected, totally independent ventricular rhythms typical of parasystole are recorded by Holter ECG. Runs of ventricular ectopics at a rapid rate—ventricular tachycardia—are more likely to be hemodynamically and electrically unstable, particularly when they develop at rates faster than 100 beats/min (see the following discussion).

Figure 8-56 Sustained, rapid ventricular tachycardia *(top)* of varying rate that develops into a polymorphic ventricular rhythm with varying morphology complexes (torsades de pointes). The horse died of cardiac arrest *(lower right panel)*. The arrows indicate QRS complexes of varying polarity (25 mm/sec).

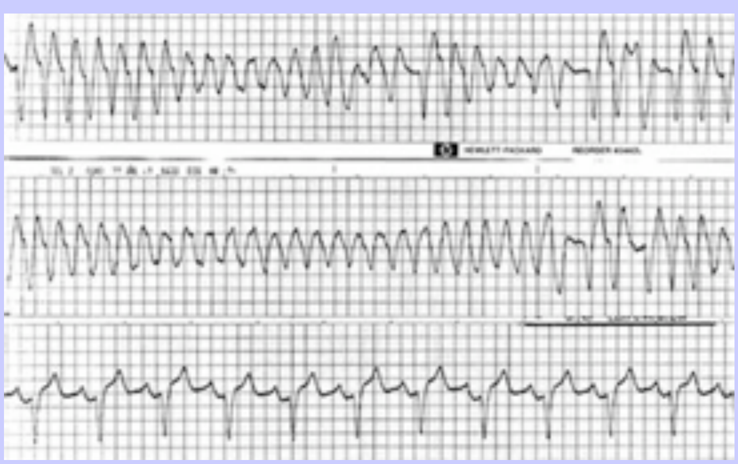


On identifying premature ventricular beats by auscultation, one should obtain an ECG and should undertake a workup similar to that outlined in Box 8-9. Occasional premature ventricular complexes, as with occasional supraventricular premature complexes, may be clinically insignificant and not require treatment. Rest is highly recommended for horses with frequent ventricular premature complexes. In the majority of horses with premature ventricular complexes, the arrhythmia seems to resolve spontaneously after 4 to 8 weeks of rest. The 441
horse then may be able to return successfully to its prior performance level. Antiarrhythmic therapy is usually 442
successful in abolishing ventricular premature complexes, but most complexes return when the antiarrhythmic agent is discontinued, unless the underlying problem has been resolved. Dexamethasone and other antiinflammatory drugs also may be helpful in abolishing ventricular premature complexes, but their use is controversial and they certainly should not be administered to horses with a recent or current infection.

8.11.10 VENTRICULAR TACHYCARDIA

Ventricular tachycardia is an ectopic ventricular rhythm characterized by a regular or irregular ventricular rate (Figure 8-57; see also Figure 8-56). Ventricular tachycardia is recognized clinically by the increased heart rate, often exceeding 100 beats/min, and the loud, sometimes booming heart sounds one may detect. Arterial pulse intensity is typically weak or variable. Multiform ventricular tachycardia is characterized by an irregular rhythm and usually is associated with more clinical signs of cardiovascular disease than uniform (unifocal) ventricular tachycardia. With multiform ventricular tachycardia, heart sounds are more likely to vary in intensity and arterial pulses are likely to be abnormal. One may detect jugular pulsations caused by atrioventricular dissociation. Syncope is associated with higher rates of ventricular tachycardia (180 beats/min or higher). Respiratory distress and pulmonary edema may develop from systolic and diastolic dysfunction. Sustained junctional or ventricular tachycardias (>120 beats/min) may lead to CHF.[582]

Figure 8-57 Polymorphous ventricular tachycardia following administration of quinidine sulfate for attempted conversion of atrial fibrillation. In this case normal sinus rhythm was established *(lower tracing)* following treatment with lidocaine, bicarbonate (to facilitate elimination of quinidine), and phenylephrine (administered to maintain arterial blood pressure).



Ventricular tachycardia may be life threatening if one detects R on T complexes; the arrhythmia is rapid (>180 to 200 beats/min); multiform ventricular tachycardia is detectable; or polymorphic tachycardia with torsades de pointes is present. The horse may require immediate treatment for cardiovascular collapse.[619] Intravenously administered lidocaine or quinidine usually is chosen for emergencies, although administration of either drug has been associated with side effects, including sudden death. Quinidine is a myocardial depressant and vagolytic drug. Lidocaine causes central nervous system excitation; thus one should weigh the risks and benefits of treatment carefully. However, recent studies of lidocaine indicate that boluses and infusions can be well tolerated in horses and causes minimal hemodynamic or electrophysiologic alterations at nontoxic doses.[547,548,651] In clinical practice one can use an initial bolus of 0.5 to 1.5 mg/kg and constant rate infusions of 25 to 50 mg/kg/min with careful observation for side effects. Quinidine gluconate at 1.1 to 2.2 mg/kg intravenously as a bolus and repeated up to 8.8 mg/kg is an alternative. Procainamide boluses of 2 mg/kg have electrophysiologic effects similar to quinidine but are less vagolytic and possibly have less gastrointestinal side effects. Magnesium sulfate 442
is an alternative antiarrhythmic that can be effective in normomagnesemic and hypomagnesemic horses. One 443
may administer magnesium sulfate intravenously in 2 g or larger boluses or as a rapid drip, up to a total dosage of 25 g per 450 to 500 kg. One may try other antiarrhythmics, including intravenously or orally administered procainamide[652] or propafenone,[653] in refractory ventricular arrhythmias (see Table 8-6).

A period of rest (4 to 8 weeks) followed by electrocardiographic and echocardiographic re-evaluation (including an exercising ECG) is indicated for follow-up of horses that have developed sustained ventricular tachycardia. A substantial number of these horses can be returned successfully to performance after treatment with antiarrhythmics or antiinflammatory drugs and rest.

8.11.11 CONDUCTION DISTURBANCES

Once formed, a cardiac electric impulse is conducted rapidly throughout the heart. The sequence of cardiac electric activation usually is dictated by the specialized conducting tissues in the atria, the atrioventricular node, the bundle of His, the bundle branches, and the Purkinje fiber system. This conduction system permits orderly activation of atrial and ventricular muscle and facilitates effective mechanical activity of the heart. A variety of conduction disorders are recognized, including sinoatrial nodal exit block, atrial standstill (usually caused by hyperkalemia), atrioventricular block, bundle branch block, and ill-defined ventricular conduction disturbances (Figures 8-58 and 8-59).[*]

Rarely, accelerated conduction occurs in the heart, which involves a pathway around the normally slow-conducting atrioventricular node and results in early excitation of the ventricles.[655] These syndromes are termed *preexcitation* and have various associated labels related to similar human disorders (e.g., Wolff-Parkinson-White). The preexcitation syndromes usually are characterized by short P-R intervals and widened QRS complexes. Some examples of these conduction disorders are described next.

8.11.11.1 Atrioventricular Conduction Block

Delays in atrioventricular conduction are the most common conduction disorders. These delays are classified as first-, second-, and third-degree (or complete). *First-degree atrioventricular block* occurs when the P-R (or PQ) interval exceeds a certain value (while the atrial impulse still transmits through the atrioventricular conduction system and activates the ventricle, causing a QRS). Some P waves are not conducted to the ventricles during *second-degree atrioventricular block,* which results in occasional P waves not followed by a QRS-T complex. *Third-degree* or *complete atrioventricular block* is characterized by an absence of atrial to

ventricular conduction. P waves are never followed by or related to QRS complexes, and to prevent ventricular asystole, a junctional or ventricular escape rhythm must develop below the level of the atrioventricular block.

First- and second-degree atrioventricular block are considered normal variations.[643] These rhythms most often are associated with high vagal tone and may occur with sinus bradycardia or sinus arrhythmia. One usually can abolish second-degree atrioventricular block with exercise, stress, or vagolytic drugs such as atropine or glycopyrrolate. Complete atrioventricular block indicates organic heart disease, severe drug toxicity, or rarely, high vagal activity. Generally speaking, third-degree atrioventricular block caused by vagal-efferent traffic is short-lived. Intraventricular conduction blocks, such as bundle branch block, are less common and more difficult to diagnose. Widening of the QRS complex and axis deviation are typical features. One also may find these abnormalities after atrial premature complexes, from overdosage with quinidine sulfate, or following supraventricular tachycardias with rapid ventricular response.

Sudden development of high-grade or complete atrioventricular block may require the administration of atropine or a catecholamine, or rapid transvenous insertion of a pacing wire into the right ventricle. Chronic third-degree atrioventricular block requires treatment with a pacemaker[433,656–659]; such a pacemaker has been placed successfully epicardially and transvenously in several horses and in miniature (Jerusalem) donkeys (Figure 8-60).

* References 161, 170, 176, 299, 392, 643, 654.

8.11.11.2 Preexcitation

Preexcitation, or accelerated atrioventricular conduction, has been reported sporadically,[655] but the clinical significance of this electrocardiographic abnormality has yet to be determined in this species. Ventricular preexcitation in human beings and in dogs often is caused by an anomalous conducting pathway around the atrioventricular node, which serves as a path for reentrant supraventricular tachycardias. These rhythm disturbances may cause hypotension and syncope. Whether preexcitation syndromes are important has yet to be determined. Nevertheless, ECG traces occasionally show evidence of ventricular preexcitation and are characterized by a P-QRS-T relationship but with an extremely short P-R interval, early excitation of the ventricle characterized by a slurring of the initial QRS complex (a delta wave), and an overall widening of the QRS complex.

8.11.11.3 Hyperkalemia

Hyperkalemia can cause significant depression of atrial, atrioventricular, and ventricular conduction and can 443
shorten ventricular repolarization. Serum potassium is most likely to be greatly elevated following oliguria, 444
rupture of the ureter, during shock, in foals with ruptured bladder and uroperitoneum, or after excessive intravenous potassium replacement. Experimentally, changes in the ECG are usually evident at potassium serum concentrations greater than 6 mEq/L, with severe changes evident when serum concentrations are between 8 and 10 mEq/L.[176,660–662] Broadening and flattening of the P wave are the most consistently observed change. Inversion or enlargement (tenting) of the T waves is also likely. One may note considerable widening of the QRS complex as near-lethal concentrations of potassium are approached. Ventricular asystole 444
or fibrillation can develop. The QT interval is not a reliable indicator of induced hyperkalemia, and other 446
electrolyte/acid-base alterations, including serum calcium and sodium, influence the effect of hyperkalemia on the heart.

Figure 8-58 Conduction disturbances. **A,** Second-degree atrioventricular block. Nonconducted P waves and varying P-R intervals are evident (base-apex lead recorded at 25 mm/sec). **B,** The tracings are from a horse with third-degree atrioventricular block. The upper strip demonstrates multiple blocked P waves. The lower tracing shows nonconducted P waves and ventricular escape complexes (base-apex lead recorded at 25 mm/sec). **C,** Permanent transvenous ventricular pacing in a miniature donkey with a complete atrioventricular block. A pacemaker spike *(arrow)* precedes each paced ventricular complex.

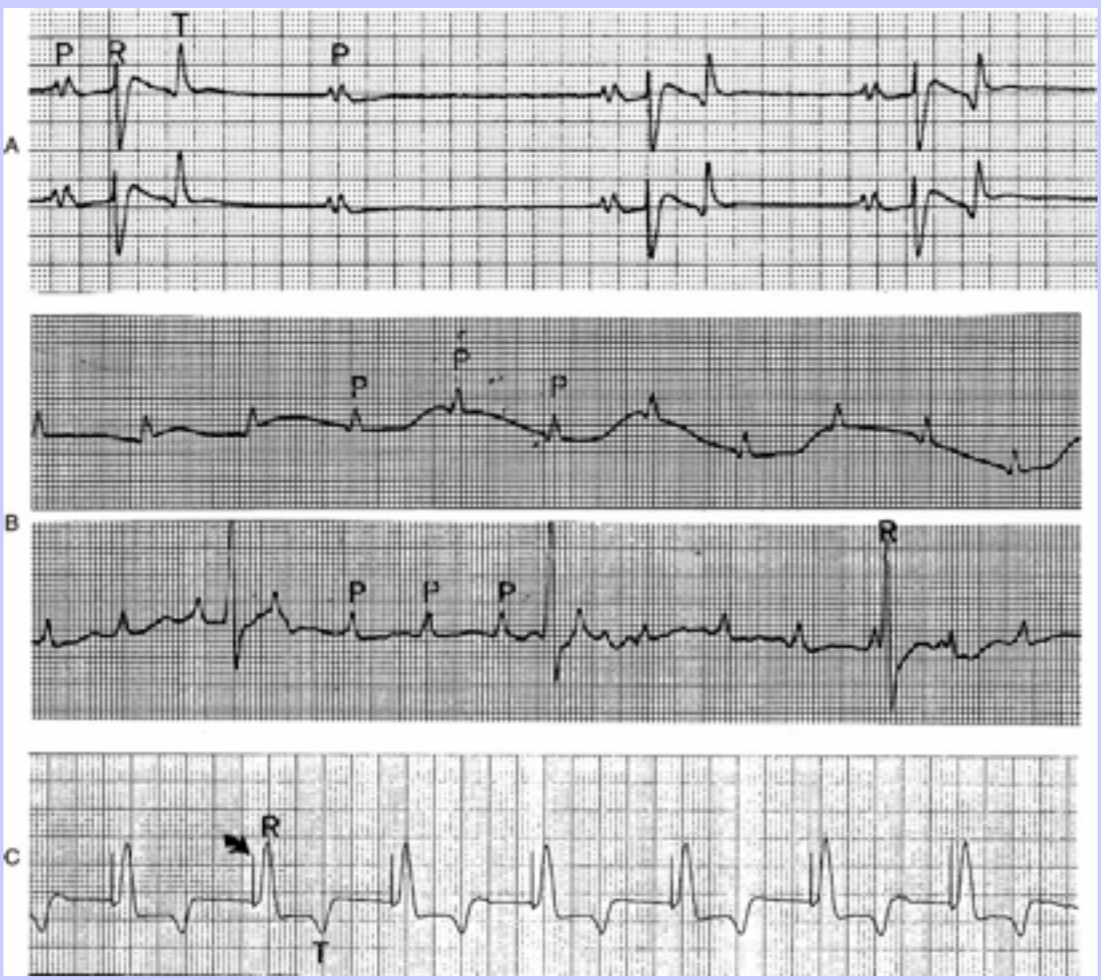
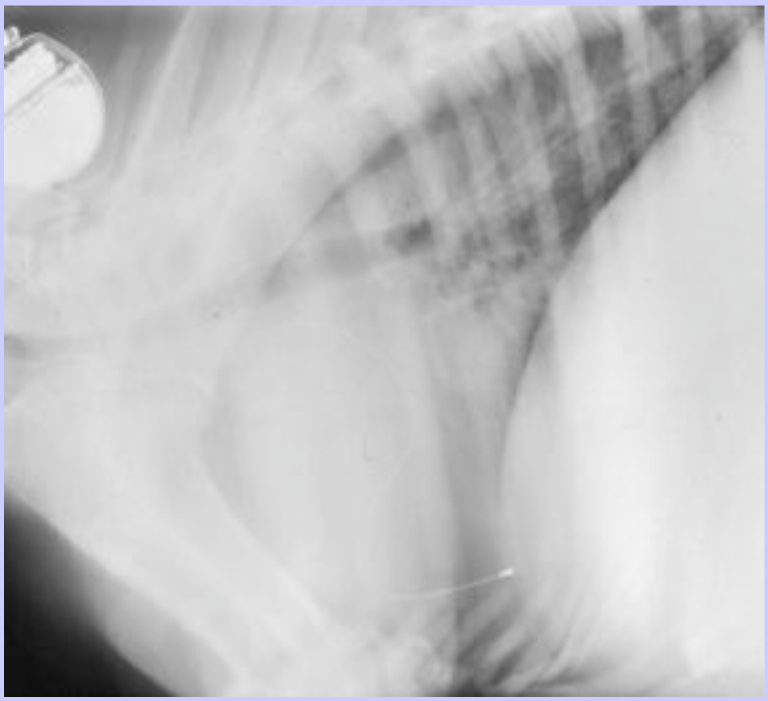


Figure 8-59 Conduction disturbances. **A,** Ventricular preexcitation. The short P-R interval *(arrow)* and small deflection in the P-R segment (delta wave) indicate an accessory pathway around the atrioventricular node with premature activation of a portion of the ventricle (delta wave and initial portion of the QRS). The large QRS and secondary T wave changes can be explained by the loss of normal cancellation of ventricular electrical forces. **B,** A sinus arrhythmia and intraventricular conduction disturbance. The sudden change and widening of the ventricular conduction pattern are notable, despite the consistent P-R interval and probably represent a bundle branch block, although this diagnosis is difficult to make in horses (base-apex lead recorded at 25 mm/sec). **C,** Hyperkalemia in a foal with a ruptured bladder. The top strip demonstrates atrial standstill (no P waves), significant widening of the QRS complexes, and large T waves with a shortened ST segment. The lower tracing shows the effects after initial medical therapy for hyperkalemia, which included treatment with saline and sodium bicarbonate. The normalization of the QRS complexes and the appearance of low-amplitude P waves *(arrows)* are notable. (Tracing courtesy of Ron Hilwig.)

Figure 8-60 A lateral radiograph of a permanent transvenous pacing system in a miniature donkey. The pacemaker is evident at the upper left of the radiograph. The thin transvenous pacing wire extends from the device through the jugular vein and vena cava and into the right ventricular apex.



Therapy for hyperkalemia includes correction of the underlying problem; infusion of saline; and administration of sodium bicarbonate (initial dose 0.25 to 0.5 mEq/kg) and possibly calcium chloride. Although controversial, one may consider administration of regular insulin along with dextrose infusion in cases of life-threatening hyperkalemia. Potassium follows glucose intracellularly and lowers the extracellular potassium concentration.

8.12

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9 CHAPTER 9 DISEASES OF THE MUSCULOSKELETAL SYSTEM

Jennifer M. MacLeay

The innate ability of the horse to learn and trust is a special attribute that allows human beings to take advantage of the extraordinary strength and agility of the horse. Horses have contributed to human life by assisting in agriculture, business, travel, and recreation. Although effective locomotion depends on the coordination ofmany body systems, movement ultimately depends on skeletal muscle. This chapter endeavors to review the many disorders that affect the muscular system of the horse.

9.1 Structure

Mammalian skeletal muscle consists of approximately 75% water, 18% to 22% protein, 1% carbohydrate, and1% mineral, with variable lipid content. Depending on the breed and type of horse, between 44% and 53% of the live weight of a mature 500-kg horse has been estimated to be muscle.[1] The myofiber, or individual muscle cell, is the fundamental building block of muscle, and each is a fusiform, multinucleated cell. Combined, myofibers constitute 75% to 90% of the volume of muscle. The myofibers are arranged in parallel along the length of the muscle such that the force of contraction is additive. The rest of the muscle tissue is made up of fibroblasts, capillaries, adipose cells, nerves, and connective tissue fibers. The composition of any muscle varies depending on the muscle surveyed and the overall fitness level, age, and breed of the horse. Muscles begin and end with tendons of various sizes, which attach the muscle to bone. Golgi tendon organs act as end organs of muscle sense andare found in the major tendinous origins and insertions. The nerve and blood vessels that supply an individual muscle typically enter near the midpoint of the muscle belly at a region called the neurovascular hilum. The nerve bundle splits off into individual nerves once it enters the muscle. A single nerve contacts several myofibers such that they contract in unison when stimulated (motor unit). A single nerve innervates each myofiber at a single point known as the motor end plate.

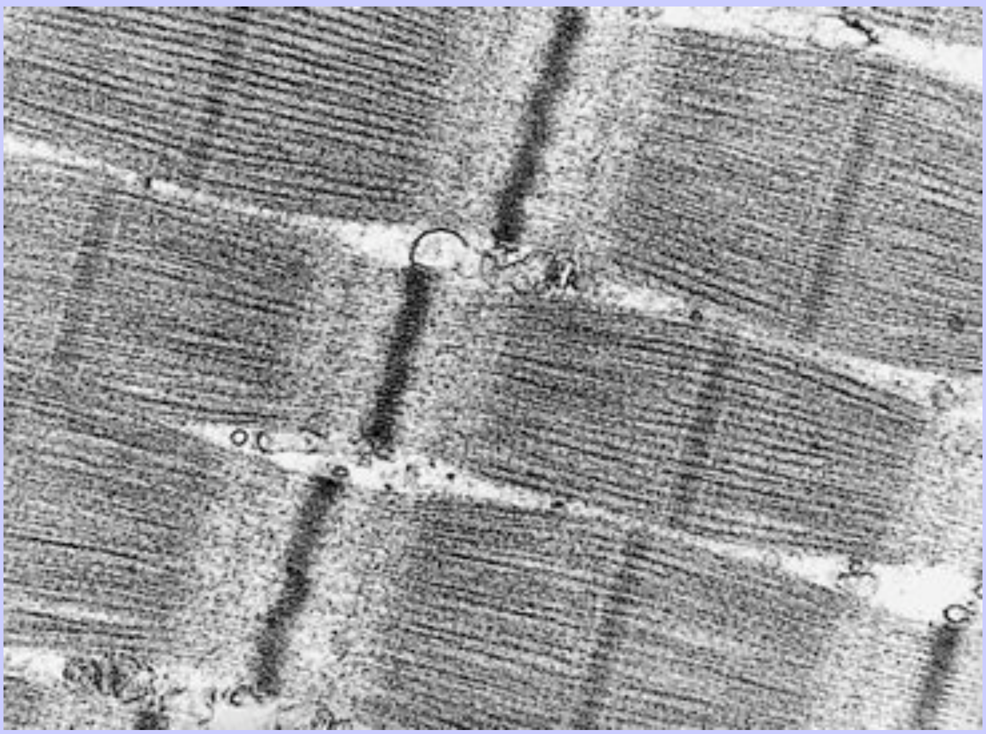
Each myofiber is bounded by a complex membrane called the sarcolemma, which invaginates into the muscle fiber at numerous points to form the T, or transverse, tubules. The T tubules terminate within each muscle cell in proximity to the sarcoplasmic reticulum of the cell, contacting the myofibrils between the A and I band twice within each sarcomere. The T tubule lies between two terminal cisterns of the sarcoplasmic reticulum. Together these three structures form the *triad*.

A basement membrane surrounds the sarcolemma and attaches closely to a layer of connective tissue called the endomysium. The endomysium is continuous with the perimysium, which surrounds groups or bundles of muscle fibers. The perimysium in turn is continuouswith the epimysium, which surrounds the entire muscle. Satellite cells, which consist of a simple cell membrane around a nucleus with a minimal amount of cytoplasm and few mitochondria, lie in shallow indentations on the myofiber surface between the sarcolemma and the basement membrane. Satellite cells give rise to new myofibers after rhabdomyolysis (dissolution of muscle fibers). The 461
nuclei of the satellite cells and mature myofibers are oriented to the long axis of the muscle fiber. The muscle fiber 462
nuclei lie at the periphery of the cells just within the sarcolemma.

Within each myofiber are parallel arrays of myofibrils, the basic contractile units of muscle. Myofibrils are composed of end-to-end stacks of rodlike structures or contractile units called sarcomeres. Each stack of sarcomeres forms a long filament, and arrays of filaments form the myofibrils. Sarcomeres are made up of the contractile proteins actin, myosin, tropomyosin, and troponin. Troponin is a complex of troponin T, troponin I, and troponin C, whereas myosin is made of heavy and light chains and a globular head that contains the myosin

adenosine triphosphatase (ATPase). Figure 9-1 shows an electron micrograph of several myofibrils. The alternating bands of light and dark are notable. The dark A band is composed of the thick myosin filaments and is transected by a darker line called the M line. Each myosin filament is surrounded by, and interdigitates with, six thin filaments composed of actin, tropomyosin, and troponin. Areas where the thin filaments are not overlapping with myosin form the lighter I band. The dark line running across the I band is called the Z line. A *sarcomere* is defined as the region between each Z line. At the Z lines, the protein α-actinin anchors actin. Because of the differing way these structures absorb light, the regular alternation of A bands and I bands in adjacent myofibrils produces the striated appearance characteristic of skeletal muscle fibers as viewed with light microscopy. Mitochondria fit into the small spaces around the myofibrils and produce the adenosine triphosphate (ATP) necessary for the contraction-relaxation cycle. The number, size, and shape of mitochondria vary depending onthe fitness of the individual and the muscle fiber type predominant within the muscle sampled.[2–4]

Figure 9-1 Electron micrograph of several muscle myofibrils. (×45,000.)



9.2 Muscle Contraction

To understand the pathophysiology of many muscle disorders, defining the sequential steps that lead to contraction is valuable. This process is known as excitation-contraction coupling.

9.2.1 EXCITATION

Excitation begins with the origination of a nerve impulse within the central nervous system. The impulse exits the spinal cord, travels along a motor neuron, and arrivesat the motor end plate of an individual myofiber. Motor end plates are located in deep primary clefts or folds of the sarcolemma. The nerve impulse arrives at the motor

end plate, releasing acetylcholine, which traverses the synaptic cleft and binds to nicotinic receptors. The binding of acetylcholine produces a conformational change in the nicotinic receptor, which results in the opening of ion channels, producing increased sodium and potassium conductance in the end plate membrane and causing depolarization and generation of an end plate action potential. The T tubule system propogates the action potential across the sarcolemma in all directions and carries it deep into the muscle fiber. Diffusion of acetylcholine from the receptors or hydrolysis by acetylcholine esterase causes depolarization to cease.

9.2.2 EXCITATION-CONTRACTION COUPLING

Coupling of excitation with contraction begins with the arrival of the action potential at the triad region, which causes opening of sodium channels embedded in the T tubule membrane and rapid influx of sodium into the cell. The rapid influx through the sodium channel results inthe change of polarity across the membrane that we associate with depolarization. As a result the inside of the sarcolemma becomes more positive relative to the outside of the membrane. Depolarization of the T tubule membrane triggers a voltage-gated calcium channel known as the dihydropyridine receptor, which also is located within the T tubule membrane. Activation of the dihydropyridine receptor triggers the closely related ryanodine receptorin the terminal cisternae of the sarcoplasmic reticulum, causing it to release calcium into the sarcoplasm.

9.2.3 CONTRACTION

Calcium released into the sarcoplasm binds to troponin C, resulting in a conformational change of the tropomyosin molecule and unmasking part of the actin filament responsible for binding to myosin: the G-actin monomer. With binding of myosin to the G-actin monomers, a 90-degree cross-bridge forms. As soon as the cross-bridge forms, myosin ATPase cleaves ATPto form adenosine diphosphate (ADP) and adenosine 462
monophosphate, resulting in another conformational change converting the 90-degree to a 45-degree angle. The 463
contractural force is believed to be generated bythe movement of the cross-bridge myosin head from a 90-degree to a 45-degree angle with a coincident sliding of the myofilaments relative to one another toward the center of the sarcomere. The hydrolytic products of ATP then detach from the myosin head. This enables the cycle to recommence, because the addition of new ATP to the myosin molecule results in the rapid dissociation of the actin and myosin filaments.[5,6] Sustained contraction occurs with the rapid repetition of this mechanicochemical cycle.[5]

Each sarcomere shortens in unison in successive ratchetlike movements comprised of multiple acts of relaxation and reengagement depending on the degree of shortening demanded by the nervous system. With input from the central nervous system to relax opposing muscles, the process of excitation-contraction coupling produces smooth coordinated movement.

9.2.4 RELAXATION

For the cell to relax and return to baseline polarity, the sarcoplasmic reticulum and the plasmalemma must reaccumulate calcium, thereby lowering the concentration of calcium in the sarcoplasm and the T tubule while sarcolemmal membranes begin to repolarize. Sarcoplasmic calcium concentrations decrease when calcium-magnesium ATPase pumps return calcium to the sarcoplasmic reticulum and sodium-potassium ATPase pumps move sodium extracellularly and potassium intracellularly. Energy-dependent reaccumulation of calcium by the sarcoplasmic reticulum results in the release of calcium from troponin C, restoration of the resting configuration

of the troponin-tropomyosin complexes, and ultimately disruption of the linkage between the myosin and actin filaments. Further details on muscle structure and contraction are available.[5–8]

An important note is that all of the processes necessary for relaxation are active, meaning they require energyin the form of ATP for them to occur. In the absence of ATP, actin and myosin remain engaged and the muscle remains in the shortened or contracted position known as rigor.

Several terms frequently are used when referringto the shortening of muscle in veterinary medicine. *Contraction* implies a shortening or tightening of the muscle that is initiated by the central nervous system. This electric activity is recorded readily using electromyography. A *contracture* is considered to be the contraction of a muscle because of abnormal activity within the muscle cell. A contracture originates within the muscle fiber itself and is therefore electrically silent on an electromyograph. Contractures occur in disorders characterized by muscle cramping such as polysaccharide storage myopathy or recurrent exertional rhabdomyolysis in which muscle shortening is typically electrically silent. The term *spasm* is more generic and may refer to a transient or sustained contraction or contracture. If a spasm is painful, the term *cramp* often is used. Fibrous tissue within a muscle may prevent complete stretching and shortening of a muscle, thereby limiting the range of motion of a limb or joint. Such occurs in fibrotic myopathy as described in a subsequent section.

9.2.5 SOURCE OF ENERGY FOR CONTRACTION

The hydrolysis of ATP provides the energy for contraction. When the phosphate bond of ATP is cleaved, the resulting by-products include ADP, inorganic phosphate, and energy. Numerous different processes within the muscle fiber can generate ATP and include glycogenolysis, glycolysis, Krebs cycle, oxidative phosphorylationby the electron transport chain, β-oxidation of free fatty acids, and purine nucleotide deamination. Energy also can be derived by cleavage of the phosphate bonds of creatine phosphate (or phosphocreatine) by the enzyme creatine kinase. (The frequently used term *creatine phosphokinase* is a misnomer.) Which pathways are most active depends on many factors, including the underlying muscle fiber composition, the number of motor units recruited, capillarization of the muscle, the underlying oxidative and glycolytic capacities of the muscle fibers, and the delivery of oxygen and other energy substrates to the muscle. In turn, the breed and age of the horse, the length or stage of exercise, the intensity of exercise, and the fitness level of the horse influence thesefactors.

The major energy sources for muscle include creatine phosphate, glucose, and free fatty acids. At the initiation of exercise, creatine phosphate serves as an immediate source of energy. However, these stores are small and serve only to provide a short-term energy source until glucose can be used to produce ATP. Glycolysis or the breakdown of glucose to carbon dioxide and water in the presence of oxygen liberates 38 moles of ATP per mole of glucose. Free glucose is present in small quantities within the sarcoplasm and can be transported into the cell from the blood. However, most of the glucose is stored in the muscle cell as the branched structure glycogen. Breakdown of glycogen, or glycogenolysis, results in the liberation of 37 moles of ATP per mole of glycogen. A large, extramuscular storage site of glycogen is the liver. With exercise, the liver begins to liberate free glucose into the blood. Muscle cells take up glucose by glucose transporters located in the cell membrane. Athletes that have exercised frequently over time develop a denser network of capillaries about each muscle cell to facilitate the delivery of oxygen and glucose to the cell and the removal of by-products. 463

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Glycolysis and glycogenolysis are rapid processesand produce adequate energy for aerobic exercise. When exercise intensity increases and oxygen demand cannot be met, glucose is metabolized anaerobically. Anaerobic glycolysis yields 4 moles of ATP per mole of glucose and produces lactic acid as a by-product. The accumulation of lactic acid within the cell lowers the pH of the sarcoplasm, inhibits the function of many intracellular enzymes, and contributes to fatigue.

After a period of exercise (this varies between individuals but in many is after about 15 to 20 minutes of aerobic exercise) the muscle begins to rely more on the β-oxidation of free fatty acids than on glycolysis for the production of ATP. Depending on the free fatty acid, β-oxidation may produce up to 146 moles of ATP per mole of fatty acid. Small stores of fat are located within the muscle. However, most of the free fatty acids are liberated from body fat stores or the liver and are taken up by the muscle cell during exercise. The longer the aerobic exercise, the more energy is derived from metabolism of free fatty acids. This metabolism appears also to be influenced by diet. The higher the intake of fat, the more muscle appears to rely on free fatty acids for energy. The optimal ratio of fat in the diet of the horse respecting type and duration of exercise is yet to be determined.

The onset of fatigue during aerobic exercise is different from that during anaerobic exercise. During aerobic exercise, glycogen depletion, hyperthermia, and electrolyte depletion appear to be major factors. Whereas during anaerobic exercise, lactic acidosis and depletion of creatine phosphate and ATP appear to be important factors initiating fatigue. Accumulation of lactic acid withinthe muscle cell leads to decreased intracellular pH and inhibition of the enzymes important in glycolysis. A low intracellular pH also inhibits excitation-contraction coupling. In aerobic and anaerobic forms of exercise, eventual onset of myalgia and decreased motivation appear to be additional factors in fatigue.

When most forms of energy within the muscle are exhausted after extensive submaximal exercise or vigorous anaerobic exercise, the last source of energy withinthe cell is the formation of ATP from two molecules ofADP in a process known as the myokinase reaction. A by-product of this reaction is ammonia. Rising ammonia levels in the circulation are well correlated with the depletion of ATP within muscle. Depleted fibers may develop a painful, electrically silent contracture that is similar to rigor, and severely affected fibers may die. The death of muscle cells related to exercise is known as exertional rhabdomyolysis. Rhabdomyolysis often is assessed by the measurement of enzymes such as creatine kinase or lactate dehydrogenase that are released into theblood when muscle cells are severely stressed (the cell membranes become leaky) or lyse.

9.3 Fiber Type

9.3.1 DIFFERENTIATION

Under light microscopy using simple staining techniques, skeletal muscle appears to be a homogeneous tissue. However, muscle is composed of many fibers with differing characteristics. Myofibers may be grouped into two categories: type 1 and type 2. However, this classification is broad, and a spectrum of fibers has characteristics of both categories. The differences between fibers arise from variability in the proteins that comprise the contractile elements. Several different isoforms have been identified for the myosin heavy and light chains, tropomyosin, troponin-I, troponin-T and troponin-C. The greatest variability is in the isoform of the myosin heavy chain. Additionally, the calcium pumping capacity of the sarcoplasmic reticulum may vary from cell to cell.

All types of fibers are found in most skeletal muscles. Type 1 fibers are high in oxidative capacity and therefore rely on glucose, free fatty acids, and a high amount of oxygen to supply energy for contraction. Their sarcoplasmic reticula have a moderately slow reuptake of calcium capacity. Therefore, type 1 fibers are best suited to moderate contraction speeds and sustained contractions over long periods of time. As one would expect, these fibers predominate in muscles involved in posture. Type 2 fibers have a fast myosin ATPase rate and a rapid capacity to reuptake calcium into the sarcoplasmic reticulum. They are larger diameter fibers compared with type 1 fibersand have a high glycolytic capacity, making them suited to powerful, faster contractions. These fibers predominate in the larger skeletal muscles of the body involved in locomotion and rapid fine motor movements such as around the eye. Type 2 fibers that have activities closer to type 1 fibers are called type 2A,

whereas those that exhibit vast and high anaerobic capacity are characterized as type 2B. Because of the high oxidative capacity oftype 1 fibers, these muscles often appear to have a deeper red color than fibers high in type 2A and 2B fibers.

Histologically, differentiation of muscle fiber types relies on the measurement of myofibrillar ATPase activity (the enzyme responsible for the breakdown of ATP at the actin-myosin cross-bridges). Staining for this enzyme distinguishes two distinct fiber types at pH 9.4. The slow-twitch type 1 fibers have a low activity at this pH and so appear lighter in color than the fast-twitch type 2 fibers. The type 2 fibers can be divided further by preincubation at a more acidic pH into types 2A, 2B, and even 2C (the pH values required for this differentiation vary with the laboratory but are around 4.5 and 4.3). Work is currently in progress at various centers to develop and refine antibody techniques for the identification of fiber types. These techniques may clarify some of the issues concerning fiber types in the horse, may overcomemany of the practical difficulties of myosin ATPase staining, and may be adaptable to automatic tissue analysis systems. 464 465

The metabolic properties of the myofibers also can be used to differentiate the fiber types. Most commonly they are divided into those with a low oxidative ability, that is, having a high concentration of the sarcoplasmic enzyme glycogen phosphorylase, or a high oxidative ability with high concentrations of the mitochondrial enzymes such as succinic dehydrogenase.[9–11]

9.3.2 INNERVATION

Two types of motor nerve fiber are found. Each motor neuron may innervate a few or several hundred myofibers. Each myofiber, however, is only innervated by a branch of one motor neuron. The type of motor neuron determines the physiologic and biochemical nature and thus the type of myofiber. The large phasic motor nerve fibers innervate the type 2 myofibers, whereas the small tonic fibers innervate the type 1 fibers.

Sensory axon endings contact the muscle spindles, which are a bundle of specialized muscle fibers that run parallel to nonsensory muscle fibers. Muscle spindle cells are important in telling the nervous system about the tone and length of the muscle. They are important in maintaining posture. Discharges are relayed directly to the ipsilateral spinal motor neurons that innervate the muscle in which the spindle is located, resulting in a reflex arc. Two types of sensory neurons innervate each spindle:the primary endings are from the large-diameter group 1A afferent axons, whereas the less elaborate secondary endings are from the smaller group 2 afferent axons. The spindle cell fibers also receive motor neuron innervation from small γ-motor neurons, which helps keep the spindle taut when the muscle contracts, which in turn enables the sensory endings to respond to a wide range of muscle lengths.

TABLE 9-1 Fiber Type Characteristics

	TYPE 1	TYPE 2* A	TYPE 2* B
PHYSIOLOGIC CHARACTERISTICS			
Speed of contraction/twitch	Slow	Fast	Fast
Fatigability	Low	Intermediate	Rapid
Maximum tension developed	Low	High	High
HISTOCHEMICAL PROPERTIES			
Myofibrillar ATPase stain pH 9.4	Light	Dark	Dark
Preincubation at a pH of			
≈4.5	Dark	Light	Medium
≈4.3	Dark	Light	Light
Oxidative capacity	High	Intermediate-high	Low
Enzymes for glucose breakdown	Intermediate	High	High
Enzymes for free fatty acid breakdown	High	Intermediate	Low
No. of capillaries	High	Intermediate	Low
Glycogen content	Low	High	High

* Type 2C fibers have been described following acid preincubation with staining intensities between the light type 2A and medium type 2B fibers.

Muscle spindle cells are different from the muscle Golgi tendon organ. A sensory nerve ending wraps around each Golgi tendon organ. These organs are found at the ends of the muscle fibers at the musculotendinous junction and are formed from a common tendon to which a number of muscle fibers are attached. The sensory nerve innervating the Golgi tendon organ responds to tension generated by the contraction of any of these muscle fibers and the discharge is relayed to the ipsilateral α-motor neurons, where it is inhibitory. This forms another reflex arc responding to muscle tension and helps ensure that muscles of flexion and extension do not contract simultaneously.

9.3.3 CHARACTERISTICS

Type 1 fibers tend to depend largely on aerobic metabolism of glucose and fatty acids for their energy. They are capable of prolonged activity but slower contraction responses. The type 2 fibers derive energy mainly from anaerobic glycolysis with glycogen as the main substrate. These fibers become fatigued more rapidly but are capable of rapid contraction and therefore are found in the highest proportions in muscle groups that move limbs rapidly. Table 9-1 gives a summary of the characteristics of the different fiber types.

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9.3.4 FIBER RECRUITMENT

Under neural control an orderly selection of fibers occurs with increasing demands. When just maintaining posture or walking, the horse uses only the nerves supplying the slow-twitch type 1 and possibly a few of the intermediate type fast-twitch high-oxidative type 2A fibers. As the speed or intensity of work increases, more and more fibers are recruited. Thus at a medium trot, approximately 50% of the muscle cells within a muscle belly are contracting, whereas at the gallop, most or all fibers are involved. The fibers are recruited in a set order: type 1, type 2A, and then type 2B. The gradation in response of a muscle is known as the recruitment of motor units. A motor unit consists of a motor nerve and the cells it innervates. The smoothness of a muscular contraction occurs because of the asynchronous contraction of the various motor units.

9.3.5 DISTRIBUTION

The proportions of fiber types present within a muscle vary according to the muscle, the breed, and the age of the horse, as well as the horse itself.[10–18] In some muscles (in particular, the middle gluteal muscle, which is sampled most commonly) the distribution also depends on the sampling site or depth, because the distribution of fiber types is nonhomogeneous.[10,17,19,20] The suggestion has been made, however, that within a specific muscle of an individual, the variation in fiber types is small if samples are taken from the same site or an identical contralateral site under controlled conditions.[17,21]

The type 2C fibers are found in large numbers in young animals but are rare in the mature horse, in which they usually are referred to as transitional fibers. These fibers have been suggested to be a stage in the development of new fibers from satellite cells or fibers in direct transition from one fiber type to another.[19,22]

9.3.6 EFFECT OF TRAINING

The suggestion has been made that the relative proportions of type 1 and type 2 fibers are under genetic control and under normal training conditions cannot be altered significantly.[22,23] Changes in the relative proportions of type 2A and 2B fibers have been reported, however, although some controversy has arisen regarding this interconversion.[7,24,25] In general, the suggestion is that training results in (1) an increase in the ability of a fiber to use oxygen by increasing the number of mitochondria within the sarcoplasm, (2) a decrease in the use of muscle glycogen and blood glucose with a greater reliance onfat oxidation to supply ATP, and (3) a decrease in the amount of lactate produced per given intensity of exercise.

Variable results have been reported on the effects of exercise on fiber size and capillarization. The extent and nature of the changes appear to depend on the duration, intensity, and type of exercise involved, as well as theage of the animal. Further information on the effect of training on muscle is available.[7,19,23–30]

9.3.7 RELATIONSHIP TO PERFORMANCE

A relationship has been suggested to exist between performance and the proportion of type 1 and type 2 fibers and their subtypes.[22,24,31,32] Performance, however, depends on many factors, just one of which may be the genetic endowment of fiber type distribution, coupled with the beneficial effects of appropriate training. Given

the heterogeneity of muscle, basing any performance characterization solely on the relative proportions of the fibers is therefore likely to be misleading.[22,24]

9.4 Muscle Development and Growth

Mammalian skeletal muscle develops from the embryonic myotomes. Some myoblasts remain as single cells, retain their mitotic ability, and form regenerative satellite cells. Fusion of the remaining mononuclear myoblasts to form multinucleated myotubules is believed to be the waythat muscle fibers develop in the fetus. Smaller secondary myotubules then form on the surface of these large primary myotubules. Initially, these myotubules share a common basement membrane but soon become separate. The suggestion has been made that the primary myotubules develop slow-twitch (type 1) fiber characteristics, whereas the secondary myotubules develop fast-twitch (type 2) characteristics. Genetic, hormonal, and nutritional factors may influence the number of these primary and secondary myotubules.[33–36] Near to the time of birth and for a period afterward, a proportion of the secondary fibers that lie closest to the primary fibers may become modified to form type I fibers,[37] although by this stage, innervation of the fibers with their specific nerve types already should have occurred. Therefore the suggestion has been made that an "unplugging" from one neural type to another can occur during development in response to motor stimulation. However, much confusion still exists regarding fiber differentiation.[33,38]

Tertiary and later generations of myotubules form between the first- and second-degree myotubules. Formation is accompanied by concurrent maturation of the established myotubules to myofibers, including migration of the nuclei to a peripheral position and synthesis of contractile proteins. Established fibers then increase in diameter and length. This process is associated with the fusion of mononuclear satellite cells with the fibers. Postnatal increases in fiber diameter are associated with the addition of myofilaments and an increase in the number of myofibrils (perhaps derived from the satellite cells). Increases in length occur by the formation of extra sarcomeres together 466
with a slight increase in mean sarcomere length. In the horse, training has been suggested to cause an increase in 467
the absolute number of fibers,[26,29] but this is disputed because the number of cells in most muscles is believed to be established earlyin life.[7,24] Other work has supported the idea that the increase in muscle volume, associated with growth and training, does not depend on an increase in the number of fibers but on the hypertrophy of each muscle fiber.[39]

9.5 Pathologic Changes

9.5.1 GENERAL RESPONSE OF MUSCLE TO INJURY

Muscle responds to injury or damage in a limited number of ways. A growing body of evidence indicates that all myopathic conditions share a common final pathway of muscle fiber degeneration,[40] although the number and type of fibers affected and the degree of damage varies. The final common pathway involves failure to sequester calcium ions from the sarcoplasm. Prolonged calcium accumulation within the sarcoplasm leads to activation of cellular enzymes and prolonged activation of myofilaments. This process is visible histologically as hypercontraction. Consequently, mitochondria take up excess calcium in an attempt to prevent cellular damage. Accumulation of calcium within the mitochondria leads to failure of energy production (ATP) within the cell and eventually failure of all intracellular energy-dependent mechanisms, which leads to failure of membrane pumps and ultimately cellular swelling and lysis.[40,41]

The exact nature of these calcium-activated degenerative pathways is still unknown. A recent suggestion is that a key step may be calcium-induced membrane phospholipid hydrolysis via the activation of phospholipase enzymes, resulting in the production of tissue-damaging metabolites. Other processes, however, involving nonenzymatic lipid peroxidation also may be activated. During exercise, the flow of oxygen increases. At the same time, an overall depletion of ATP sources occurs. The resultant metabolic stress to the cells leads in turn to a greatly increased rate of oxygen free radical production that may exceed the scavenger and antioxidant defense systems of the cell, leading to a loss of cell viability and damage. This process could initiate skeletal muscle damage caused especially by exhaustive exercise. Possibly the increase in free radical activity can lead to a failure of calcium homeostasis and consequent muscular damage, or alternatively, calcium overloading may lead to an activation of free radical–mediated processes.[42] Multiple initiators likely may lead to this final common pathway depending on the type of exercise, fitness level, presence or absence of underlying genetic defects in muscle metabolism, and nutritional status of the horse. Free radical–induced skeletal muscle damage may be especially important if reperfusion follows a period of ischemia.[43]

Because muscle responds to stressors similarly, recognizing that muscle disorders only rarely can be diagnosed on the basis of muscle histopathology alone is important. A thorough history including genetic, exercise, nutrition, patient signalment, and clinical description of the muscular disorder is mandatory. This information—along with histologic evidence of the distribution, character, and age of the lesions; the presence or absence of certain cell types and parasites; and lesions in other organs—in most cases provides the practitioner with enough evidence to make a diagnosis.

Histochemical staining of frozen muscle sections also can aid the detection of glycolytic enzyme deficiencies and indicate the storage of various atypical metabolic compounds.[44–47] For example, phosphorylase deficiency has been described in human beings (McArdle's disease) and Charolais cattle.[48,49] The nature of any cellular infiltrate also may be of interest, for example, in trichinosis, in which an eosinophilic and neutrophilic infiltration of any necrotic sarcoplasm may be present. In most cases of segmental necrosis only a limited secondary involvement of the interstitial tissues or blood vessels occurs. With vascular injury and regional necrosis (following arterial or venous occlusion, hemorrhage, or trauma), the necrosis of the muscle fibers is only part of a more extensive lesion involving the interstitial tissue and blood vessels.

Other, more general pathologic changes that can be found in muscle include changes in fiber volume, number, shape, malformation, degeneration or proliferation of organelles, and disruption of the basic architecture of the fiber. Aggregates of inflammatory cells, reactive changes in vessel walls, occlusion of blood vessels, and increased amounts of fibrous tissue are other possible pathologic findings. In some conditions, such as tetanus or botulism, however, no significant changes have been identified in the muscles themselves. Further information on the types of changes that occur is available.[37,44,50]

Various histologic stains have been developed to highlight the different morphologic changes and inclusions that occur.[11,44,51,52] The most important of these is the periodic acid–Schiff stain that highlights glycogenstores within a cell and membrane structures containing mucopolysaccharide, glycoproteins, mucoproteins, glycolipids, or phospholipids. Large aggregates of abnormal polysaccharide occur in Quarter Horses with polysaccharide storage myopathy and Draft-breed horses with equine polysaccharide storage myopathy. Histochemical staining for fiber type enables the pathologic changes to be recognized as affecting all fibers or those of one type only. In cases of reinnervation, large groups of a single fiber type are visible as opposed to the more interspersed pattern typically observed.

These procedures can be combined with a morphometric examination of the muscle[4] to provide further 467
information. Immunocytochemical studies have been used in the investigation of neuromuscular disorders in 468
human beings and to diagnose autoimmune streptococcal-associated myositis in the horse.[44] Specialized staining techniques help to localize specific enzymes and intracellular and extracellular muscle components such as the various collagen and myosin types, complement, and fibronectin. Electron microscopic studies enable one to investigate the ultrastructural reactions of muscle fibers. To date, these advanced techniques have been applied sparingly in the horse; however, their use is a developing field in human and veterinary medicine.[34]

9.5.2 ATROPHY AND HYPERTROPHY

Atrophy may be defined as a decrease in muscle fiber diameter or cross-sectional area. Atrophy can occur in a variety of circumstances, including denervation, disuse, and cachexia, and in many muscle diseases, following circulatory disturbances, and extensive myolysis. Within 2 or 3 weeks following peripheral denervation, up to two thirds of the originally supplied muscle mass may be lost, although this may not always be obvious clinically because of the continued presence of superficial and intramuscular fat deposits. The changes seen histologically reflect the affected nerve and the muscle fiber type it supplied. In disuse, atrophy resulting from tenotomy—for example, a preferential atrophy of type I fibers—is apparent, often with hypertrophy of type II fibers. In contrast, with cachexia and malnutrition, generally the type II fibersare affected, especially in the essentially postural muscles. Not all muscles show the same degree of atrophy, even within one disease process. In cachexia, the back and thigh muscles tend to be the first affected, and the loss of muscle is usually symmetric, with a concurrent loss of fat deposits. A localized asymmetric atrophy is associated with paralysis, immobilization, and denervation.[37]

As indicated previously, hypertrophy may occur through training. A compensatory hypertrophy also may occur in the fibers surrounding an area where fibers have been lost or have decreased greatly in size; for example, with chronic denervation atrophies and in advanced cachectic atrophy. Often, large fibers are visible histologically in such conditions, with evidence of incomplete longitudinal division.[37]

9.5.3 REPAIR AFTER DENERVATION AND INJURY

9.5.3.1 Following Denervation

Reinnervation can occur in two ways. Damage to a nerve without severing the nerve is called axonotmesis. The axon is damaged but the Schwann cell and endoneural fibrous sheaths remain. Axons from the proximal partof the damaged nerve may reestablish connections with empty residual Schwann cell sheaths in the distal portion of the original nerve. Initially, atrophy of scattered muscle fibers is visible, but after reinnervation, if this occurs within a certain time, a proportion of the muscle fibers are restored to their normal size and functional capability. Even if the affected muscle is not restored fully, other surrounding unaffected muscles may be able to compensate so that overall function is maintained. A slight gait abnormality may remain, however.

The second type of reinnervation tends to occur when some nerve fascicles are severed completely or when the lesion is located a great distance from the muscle; for example, when lesions have involved the motor neuron cell bodies and nerve roots. In these circumstances, collateral reinnervation from adjacent, unaffected axons can occur. As the nerve type characterizes the muscle type, this results in regrouping of muscle fibers so

that with successive episodes of denervation and reinnervation, clusters of fibers of the same histochemical type are found rather than the normal checkerboard pattern. This effect is referred to as *fiber type grouping*.

9.5.3.2 Following Injury

Regeneration where a break in the continuity of the muscle fiber has occurred differs considerably from that involving primarily neurologic damage. In such cases repair involves multiplication of nuclei and formation of new internal structures and organelles followed by fusion and alignment into a new multinuclear myofiber. Regeneration seems to take place only if parts of the affected fiber remain intact. If this is so, recovery can be quick. Regeneration can occur at the healthy end of the severed fiber (continuous or budding regeneration) or by fusion of mononuclear myoblasts to form a myotube, which develops in a similar way to fetal fibers (discontinuous or embryonic regeneration). The origin of myoblasts is disputed. Some workers believe they are derived from undamaged sarcoplasmic nuclei, others think that they come from satellite cells.[52] If the scaffolding, basement membrane, and supporting tissues remain intact, and the initiating disease process subsides, new fibers tend to orient in a way similar to the original fibers. This type of regeneration usually occurs with segmental necrosis, in which necrosis of the whole diameter of the fiber has occurred, often involving several sarcomeres, but the basement membrane has been preserved. Fibroblastic and vascular reactions are minimal in this type of regeneration. Therefore full function of the muscle is restored without residual dysfunction.

Massive trauma, hemorrhage, infection, or infarction can result in damage to the basement membrane and other supportive structures, resulting in complete disorientation of the regenerating fibers with significant proliferation of fibroblasts and vessels. In such cases, after regeneration significant deformation of the muscle may occur with potential disruption of its normal function. 468

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9.6 Diagnosis of Skeletal Muscle Disorders

The clinical signs of muscle disorders are few and nonspecific. In acute muscle injury, clinical signs may include “knots” or regions of varying size of hypercontracted fibers within the muscle or areas of edema with or without heat within the muscle, pain on palpation, fatigue with light exercise or reluctance to move, atrophy, occasionally weakness, and discolored urine (myoglobinuria). In cases of infectious myositis such as clostridial infections, one may palpate gas accumulation evidenced by crepitus.

In cases of chronic muscle disease such as fibrotic myopathy, one may palpate firm areas of fibrosis or calcification with limited pain and heat. Gait abnormalities in cases of chronic muscle disease most commonly are associated with denervation atrophy and fibrotic myopathy. The practitioner may perform ancillary diagnostic testing to characterize the significance of the disorder further and to aid in diagnosis. These tests can include histologic and histochemical examinations of the muscle, thermography, electromyography (EMG), and most commonly in equine medicine, plasma-serum biochemical investigations for creatine kinase (CK) and aspartate aminotransferase (AST).

9.6.1 MUSCLE BIOPSY

The practitioner can obtain muscle biopsy samples by surgical excision under general or local anesthesia[53] or more commonly by percutaneous needle biopsy from the standing horse.[9,13,54] Because of the variation in fiber composition with sample site in certain muscles, the position and depth of sampling are important. Good specimen preparation for frozen sections is vital to prevent artifacts such as ice crystals, sarcomere shortening,

and fiber kinking.[9,44,55] For laboratories that prefer examination of frozen sections, specimens typically are shipped chilled so that the laboratory may freeze the sample themselves to minimize artifacts. Other laboratories prefer formalin-fixed specimens. The practitioner should discuss sample procurement, preparation, and shipment procedures with the laboratory before biopsy.

Muscle biopsy allows the morphologic, biochemical, and physiologic properties of the myofibers to be examined with the animal still alive and with minimal complications, especially if obtained by percutaneous needle biopsy, for which chemical sedation rarely is required. Typical sites used for percutaneous biopsy in cases of generalized muscle disease include the semimembranosus, the biceps femoris, and most commonly the middle gluteal muscle. In horses in which a specific muscle is abnormal, the two methods described next can be adapted readily to that particular muscle.

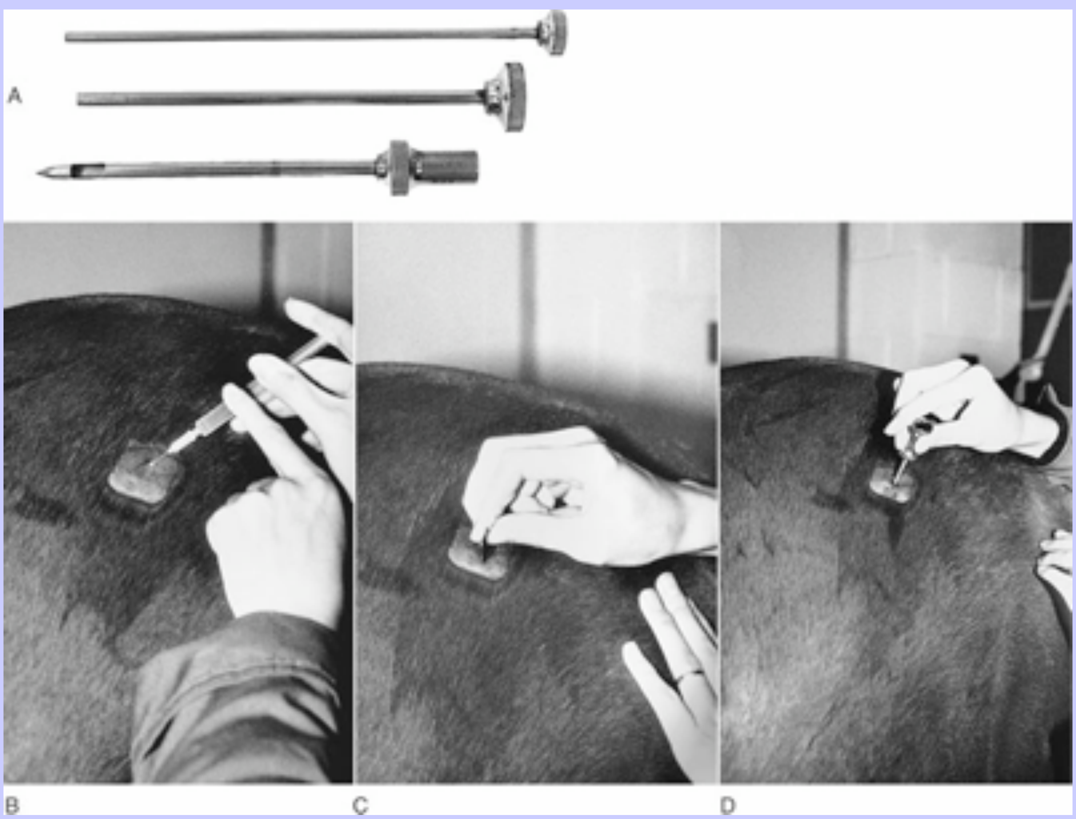
Figure 9-2 gives details on the procedure for obtaining a muscle biopsy from the middle gluteal muscle. The practitioner draws a line from the tuber coxae to the base of the tail. Approximately midway along this lineis the highest point of the middle gluteal muscle. At this point the practitioner closely shaves, washes, and cleans a small area of skin. The practitioner then injects local anesthetic subcutaneously along the proposed line of incision and into the connective tissue or fascia overlying the muscle but does not inject anesthetic into the muscle itself, which may induce artifact into the biopsy. The muscle belly is innervated poorly for pain, and therefore reaction to the insertion of the muscle biopsy needle is minimal. Horses react because with insertion of the needle the muscle spontaneously contracts. The practitioner then makes an incision through the skin and fascia and inserts a 4- to 6-mm biopsy needle to a predetermined depth. The practitioner catches and removes several small pieces of muscle with the needle and leaves the incision unsutured or uses a single skin suture to minimize bleeding and for client reassurance. Biopsies smaller than 4 mm typically do not provide enough muscle tissue for an adequate diagnosis, and for most horses6 mm is preferrable. This procedure rarely has complications; however, the horse should be current on tetanus prophylaxis as a general precaution. A scar from the incision rarely occurs.

When practitioners do not have muscle biopsy needles, they may take excisional biopsies from the semimembranosus muscle. The biopsy site is slightly lateral to and below the anus. At this location, if a scar occurs following the procedure, the tail will hide it. The practitioner surgically prepares the site and makes an inverted L lidocaine block of the skin and subcutaneous tissues. The practitioner then obtains a piece of muscle measuring approximately 1 × 1 × 2 cm and sutures closed the subcutaneous layer and skin. Buried skin sutures that do not need to be removed appear to be preferable because they are less likely to cause skin irritation and therefore are less likely to be rubbed out by the patient. Should the horse open the biopsy site, the wound typically heals well by second intention with general wound care.

9.6.2 THERMOGRAPHY

An infrared thermographic scanner converts the radiated thermal energy of the skin to electric signals that canbe amplified and displayed on a video screen. By using isotherm colors of known temperature, the practitioner can obtain two-dimensional, graphic, and quantitative information regarding the precise temperature of the skin surface. The practitioner can use this technique noninvasively as a means of detecting changes in skin temperature resulting primarily from changes in peripheral blood flow. Inflammation, atrophy, neoplasia, and neurologic lesions, particularly of the autonomic supply to the skin, can alter local blood flow. 469

Figure 9-2 **A**, Percutaneous muscle biopsy needle, often referred to as a Bergstrom biopsy or Stille-Eschmann cannula, available with 4-mm, 5-mm, and 6-mm internal diameters (6 mm is preferred). **B**, One closely shaves, cleans, and prepares a small area (approximately 2.5 cm) of skin over the gluteal muscle (two thirds of the distance on a line from the tail head to the tuber coxae) in a sterile manner and injects 1.5 ml of local anesthetic (lidocaine 2% with epinephrine) subcutaneously along the proposed line of incision and into the connective tissue or fascia overlying the muscle. One does not inject anesthetic into the muscle tissue.**C**, One makes an incision through the skin and fascia using a No. 23 scalpel blade. **D**, One inserts the biopsy needle into the muscle to a predetermined depth (indicated by an indelible mark on the shaft of the biopsy needle). Using the Tru-Cut principle, one can catch and remove with the needle a small piece of muscle (up to 100 mg). The incision is left unsutured.



Abnormalities have been found in exercise-exacerbated focal thoracolumbar gait abnormalities, as well as disuse atrophy.[56] The technique has been particularly usefulin documenting hindlimb muscle strain as a cause of lameness in horses.

9.6.3 ELECTROMYOGRAPHY

Needle EMG is the study of the electric activity of muscles. The practitioner places a recording needle electrode into the muscle, and the electric activity is amplified, recorded on an oscilloscope, and projected audibly into a loudspeaker. The electric status of muscle membranes depends on the integrity of the wholemotor unit. Thus an EMG evaluates the function of the ventral motor horn cell, its axon, axon terminals, and neuromuscular junctions, as well as the muscle fibers it innervates.

Investigations usually are carried out in two phases. First, the practitioner assesses the electric potentials 470
associated with physical disruption of muscle membranes resulting from insertion of the needle in the muscle. 471
These insertional potentials, if relayed through a loudspeaker, tend to sound like short bursts of loud static. They vary among muscles, probably because of differences in the size and number of motor units present.

The second phase involves assessment of electric potentials when the needle electrode is at rest in the muscle. Normally, electric silence occurs with cessation of needle movement, unless the needle is located in proximity to a nerve branch or the end plate zone when miniature end plate potentials are recorded continuously; these sound like a low-intensity static. In cases of denervation, insertional activity may persist after needle movement has stopped because of increased excitability of the muscle fiber membranes. In relaxed, diseased muscles, different types of abnormal electric activity have been recognized. Positive, sharp waves are slow monophasic waves, rapid in onset, with a slow decay to the baseline, which occur repeatedly with variable amplitude (100 μV to 20 mV). Their cause is uncertain, and they often occur with denervation and may represent a nonpropagated depolarization region in the muscle fibers near to the tip of the electrode. When occurring in trains, these positive sharp waves sound like a waning *brrrr*.

Fibrillation potentials are electric signals generated by a single muscle fiber. Long, random volleys of mono- or biphasic (occasionally triphasic) potentials of short duration (0.5 to 5.0 ms), with amplitudes of usually less than 200 μV, commonly occur in denervation (depending on the stage). Constant and repetitive fibrillation potentials, which sound like rain on a tin roof or the sizzling of frying eggs, can be found especially in the early stages of denervation. Fibrillation potentials also can be seen in myopathic disorders in which segmental muscle necrosis may have caused, for example, the separation of a muscle fiber and its nerve supply.

Myotonic discharges—high-frequency (up to 1000 Hz) repetitive discharges with a waxing and waning of the potentials seen and heard with a characteristic, musical, dive-bomber, or more precisely, revving motorcycle–like sound—are found in the myotonias (myotonia congenita, myotonia dystrophica, and hyperkalemic periodic paralysis) if the practitioner moves the exploring EMG electrode or percusses the muscle externally. Bizarre, high-frequency discharges (often referred to as pseudomyotonia) may produce a dive-bomber–like sound. No true waxing and waning occurs, although the amplitude and frequency of the potentials may change abruptly to mimic a revving motorcycle–like sound. The discharges are often in couplets or triplets and are likely to terminate abruptly. These, or similar discharges, occur in long-standing denervations, ventral horn cell disease, polymyositis, and certain myopathies. Unlike the myotonic discharges, pseudomyotonic discharges may be abolished by curare and therefore are believed to originate presynaptically.

Fasciculations, often seen in association with visible muscle twitching, are caused by the spontaneous contraction of some or all of the constituent fibers of a motor unit. They occur primarily with any cause of muscular weakness (myasthenia), local or generalized, especially in neurogenic disorders, and in tetanus and certain debilitating and metabolic disorders. In addition, one can assess the electric activity induced by electric stimulation of nerves or associated with voluntary or induced muscle contraction.

Further information on EMG is available in the literature.[57–60]

9.6.4 SCINTIGRAPHY

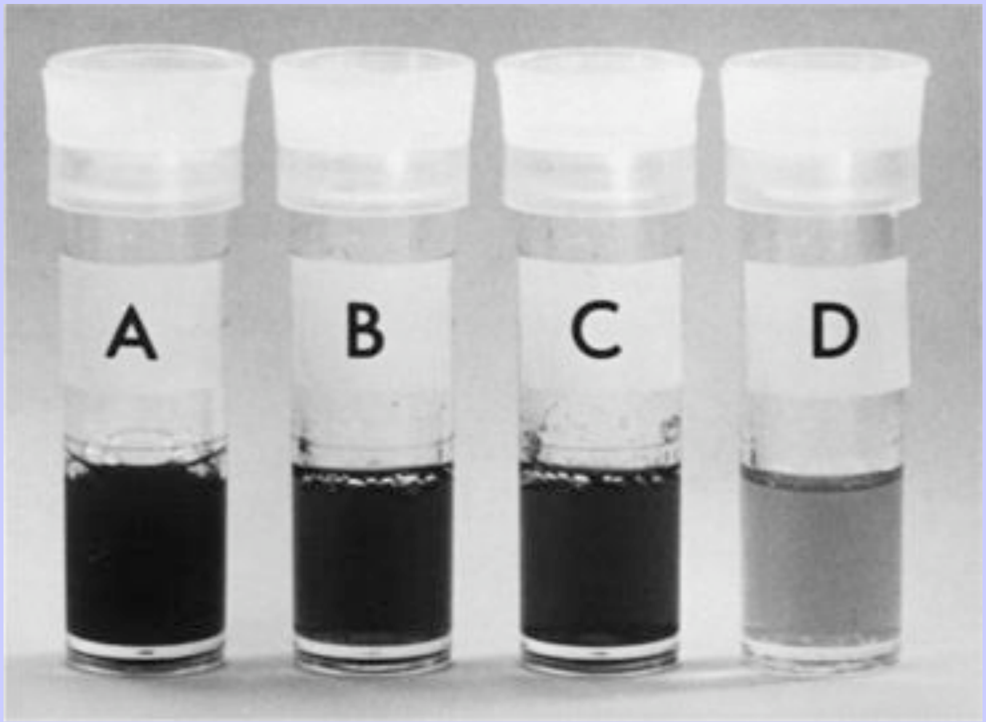
Bone-seeking radiopharmaceuticals have been used to detect and localize skeletal muscle involvement, especially in poor performance cases, but are likely to be of limited routine value in the field. The accumulation in damaged muscle may be related to the deposition of calcium in damaged fibers, binding by tissue hormones or enzyme receptors, tagging to denatured proteins, or altered capillary permeability. Uptake of labeled phosphates appears to occur only when muscle damage is ongoing and does not occur in areas of repair. Three main types of muscle uptake were identified in one study of horses with skeletal muscle damage: a diffuse, severe, and generalized uptake; bilateral symmetric uptake involving muscle groups that perform synergistic functions; and asymmetric radioisotope uptake in one or more muscle groups on one side of the animal.[61] The reasons for the various patterns have not been elucidated fully.

9.6.5 URINALYSIS: MYOGLOBINURIA

Myoglobin (molecular weight 16,500) is essential for the transport of oxygen into and within muscle cells. Most mammalian muscles contain about 1 mg myoglobin per gram of fresh tissue, and the suggestion has been made that acute destruction of at least 200 g of muscle must occur before serum myoglobin levels rise sufficiently for detection in the urine.[62] Serum myoglobin has a short half-life, and therefore measuring serum myoglobin is of limited diagnostic value. In human beings, myoglobinuria occurs in a variety of conditions, including myocardial infarction, crush and burn injuries, malignant hyperthermia, idiopathic and exertional rhabdomyolysis, and certain genetic metabolic abnormalities.[50,51,62–64] In the horse, myoglobinuria has been seen, for example, in equine exercise-induced rhabdomyolysis,[65,66] white muscle disease in foals,[67] and postanesthetic myositis.[68]

Pigmenturia or dark urine may be caused by increased myoglobin, hemoglobin, or whole blood in the urine. Low 471
levels of myoglobin, hemoglobin, or whole blood may occur in the urine and can be detected by urine dipstick or 472
laboratory test before visible changes in the color of urine. Therefore considerable amounts of myoglobin, hemoglobin, or whole blood must be present for visible pigmenturia to occur. Many causes of hemolysis in the horse can result in hemoglobinuria, including acorn poisoning[69] and hepatic and renal disease.[70]

Figure 9-3 Four urine samples. *A*, With 3 mg/ml hemoglobin. *B*, With 3 mg/ml myoglobin. *C*, Normal stored for 1 month at 18° *C*. *D*, Control (fresh urine).



Unfortunately, stored urine, concentrated urine, and urine containing myoglobin, hemoglobin, or other porphyrins can appear similar in color[69,71,72] (Figure 9-3). Therefore one cannot distinguish myoglobinuria by color alone and must use other methods. The orthotoluidine-impregnated strips commonly used by veterinary surgeons (urine dipstick tests such as BM-Test-8, Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut) are insensitive to the presence of myoglobin and hemoglobin.[66,73] The differential salting out of hemoglobin with ammonium sulfate has been shown to give false resultsin human beings[62,63,71,74] and the horse but is used commonly in many laboratories.[73] Visual inspection of the serum or plasma may indicate the cause of the pigmenturia. The low affinity of myoglobin for haptoglobin means that myoglobin is excreted at plasma concentrations around 0.2 g/L, whereas hemoglobin only appears in the urine at plasma concentrations greater than 1.0 g/L. At this concentration a pink discoloration of the plasma occurs, indicating that hemoglobin is present.[53,62,63]

Spectroscopy has been used to differentiate between the oxyforms of myoglobin and hemoglobin but does not identify oxymyoglobin when oxyhemoglobin isalso present. In fresh urine, oxymyoglobin is converted rapidly to its met-form, and in older urine samples both oxyforms will have been converted spontaneously to their met-forms. These oxyforms can be reduced chemically, but this can result in a denaturation of the proteins. In human beings, because methemoglobin and metmyoglobin have almost identical absorption spectra, spectrophotometry has been stated not to be able to distinguish one from the other if both are present in the sample.[74] The same has

been shown for the horse. Ultrafiltration also has been shown to be unreliable in the horse, perhaps because of the higher viscosity of equine urine compared with human urine.[73]

On electrophoretic separation on cellulose acetate, myoglobin migrates as a β_2-globulin and hemoglobinas an α_2-globulin. This method can distinguish the two proteins in urine containing high concentrations(>125 μg/ml) of either protein, provided no other proteins, apart from albumin, are present. Immunoassays are the most sensitive and specific tests and can be used for detecting small amounts of myoglobin in blood and urine.[73,75,76] Sequelae of acute tubular necrosis and acute renal failure have been associated with myoglobinuria in human beings[77] and horses.[53,78] Renal disease associated with myoglobinemia in the horse seems to be associated most with horses that are highly stressed (causing decreased renal blood flow), dehydrated, and have lactic acidosis associated with rhabdomyolysis. Therefore horses suffering from “capture” myopathy (e.g., cast in a stall or trailer accident and struggling for a prolonged period of time) and endurance horses are most at risk.

9.6.6 PLASMA AND SERUM ENZYME ACTIVITIES

The enzymes found to be most useful in evaluating the equine muscular system are CK, AST, and lactate dehydrogenase (LDH). A change in the plasma activity ofany enzyme can occur for a variety of reasons, including alteration in the permeability of the enclosing cell membrane, cell necrosis, impaired removal or clearance of the enzyme, and increased or impaired synthesis. Decreases in plasma enzyme activities are not usually clinically significant. Often no one specific organ of elimination is responsible, although most elimination occurs via the liver, kidneys, and lungs. Therefore under most circumstances the elimination rate of an enzyme from the plasma remains fairly constant, and the rate of influx to the plasma is the crucial factor.

Increases most commonly occur because of a defect in the integrity of the membrane containing the enzyme.[79,80] The defect results from complete disruption of the cell or a transient change in the permeability of the membrane without cell death. A complete explanation forthe pattern of release of intracellular constituents from diseased muscles cannot be given here. Most of the enzymes that are detectable in increased concentrations in the blood
with the various muscle disorders are the major soluble (sarcoplasmic) enzymes, although one also can find the 472
mitochondrial form of AST with severe injury. Intensification of cell function, as would occur during exercise or 473
as a reaction to cell damage, resultsin increased substrate use, which in turn may result in greater membrane permeability. Whether the increase in substrate permeation of the boundary surfaces themselves is sufficient to allow incidental escape of cell enzymes or whether a reduction in the energy potential is needed before cells leak is not clear. Therefore assuming a particular degree of cell death or damage from moderately elevated serum enzyme activity is inappropriate.

The suggestion has been made that considerable enzyme efflux can occur even when the light and electron microscope cannot detect with any certainty changesin cell structure. In the human disease Duchenne's muscular dystrophy, for example, the suggestion is that a change occurs in the structure of the sarcolemma at the molecular level that leads to a selective passage of cellular constituents based partly on molecular weight.[80] Under general circumstances the rate of efflux of an enzyme most likely depends not only on molecular weight and intracellular localization but also on its binding to various intracellular structures and its relative intracellular and extracellular concentration. No explanation for the significant differences in half-lives of the various plasma muscle enzymes reported for human beings and horses has been given.

9.6.6.1 Creatine Kinase

CK is the enzyme responsible for breaking down creatine phosphate to creatine and phosphate, releasing energy for muscular contraction. This reaction is the sole source of energy in muscle at the initiation of exercise. Thereafter, energy is supplied by oxidation of glucose and free fatty acids. Because this enzyme is responsible for the breakdown of creatine phosphate, it often is referred to mistakenly as creatine phosphokinase. This term is inappropriate, and one should use *creatine kinase*. In the horse, CK is found mainly in skeletal muscle, the myocardium, and the brain.[81] Little or no exchange of CK between the cerebrospinal fluid and plasma appears to occur. A significant increase in total plasma CK activity thereforeis caused by cardiac or skeletal muscle damage. CK (80,000 d) does not enter the blood stream directly after its release from the muscle cell but transits through the lymph via the interstitial fluid. The total quantity of circulating CK in the horse is estimated to be equivalentto the quantity of CK in approximately 1 g of muscle;a three- to fivefold increase in plasma CK activity corresponds to the apparent myolysis of approximately 20 g of muscle.[82] However, as described previously, making assumptions as to the amount of muscle damaged on the basis of serum CK activity is difficult, because muscle cells appear to be able to release significant quantities of CK without necessarily being lysed.

In human beings, two monomers of CK are known and are designated M and B. The enzyme is dimeric and three possible primary forms exist: MM, MB, and BB.In simplified terms, MM is found mainly in skeletal muscle, BB in the brain and epithelial tissues, and MB in the myocardium. In human beings the measurement of MB activities has been used as a sensitive test for the diagnosis of myocardial infarction.[83] Chronic skeletal muscle damage, however, has been suggested also to result in the characteristic myocardial infarction pattern.[84]

In the horse some confusion over CK isoenzymes exists with workers reporting different electrophoretic bands and tissue activities, perhaps because of the different techniques used.[85–88] In one study, the heart and skeletal muscle were found to contain predominantly the MM dimer; the brain, pancreas, and kidney, mainly the BB dimer; and the intestine, the MB and BB dimers.[87] This work suggests therefore that in the horse, CK isoenzymes on their own could not be used to differentiate between skeletal and cardiac muscle damage. This problem is alleviated largely by the current availability of tests for cardiac troponin I. Unfortunately, commercial tests for skeletal troponin I are not available. In the absence of clinical cardiac disease, one can attribute elevations in CK primarily to skeletal muscle. The plasma half-life of CK in the horse is short (108 minutes,[89, 123] ± 28 min with a plasma clearance of 0.36 ± 0.1 ml/kg/min)[82] in contrast to reports of 12 hours in human beings.[90]

9.6.6.2 Aspartate Aminotransferase

AST is found mainly in skeletal muscle, liver, and heart, although lower activities are present in several other tissues. Therefore AST is not tissue-specific.[78,81,91] Two isoenzymes have been identified by electrophoresis: MAST (found exclusively in the mitochondria) and CAST (originating from the cytoplasm or sarcoplasm). The ratio of cytosolic to mitochondrial enzyme in horse serum is significantly greater than that found in human beings and many other mammals.[92] In the horse, although the ratio of these two forms varies between tissues, no tissue-specificity is apparent for either isoenzyme. Therefore the conclusion is that the examination of sera for AST isoenzyme activities cannot indicate the tissue source, although large increases in MAST are unlikely to be found in the serum unless severe muscular injury has occurred. Some workers have reported an

apparently unique form of the enzyme in the sera from azoturia cases.[93] The plasma half-life of AST in the horse is 7 to 10 days,[89] far longer than the 11.8 hours in human beings.[90]

9.6.6.3 Lactate Dehydrogenase

LDH is a tetrapeptide made up of combinations of two different peptides, H (heart) and M (muscle), which form the five isoenzymes referred to as LDH_1 to LDH_5, that is, H_4, MH_3, M_2H_2, M_3H, and M_4, respectively. Like AST, LDH is found in most tissues and is therefore not organ-specific. However, tissues contain various amounts of the LDH isoenzymes, and the isoenzyme profile obtained by electrophoretic separation has been used to identify specific tissue damage.[94] For the most part, LDH_5 (plus some LDH_4) is found in the locomotor muscles; the liver contains mainly LDH_3 (with some LDH_4 and LDH_5); the heart contains mainly LDH_1 (with LDH_2 and LDH_3); and all types have been found in certain nonlocomotor muscles.[85,88] Training has been shown to increase the percentage of LDH_1 to LDH_4 and decrease that of LDH_5 in skeletal muscle.[95] The practitioner should use nonhemolyzed samples for LDH determinations because red blood cells contain large amounts of LDH.

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9.6.7 EXERCISE TESTS

Certain physiologic changes can result in a transient alteration in cell membrane permeability. Hypoxia, catecholamines, hypoglycemia, changes in pH, and altered ionic concentrations have been reported as causing such a change in membrane permeability.[91,96,97] Many of these changes are believed to act by decreasing the amount of ATP available for the maintenance of cell integrity, which becomes especially important during exercise.[98]

Measuring the CK and AST activities before and after a controlled period of exercise has been suggested as an aid to diagnosing certain muscle disorders. A major difficulty has been to establish exactly the normal enzyme response to exercise. Much confusion exists in the literature regarding this partly because of the differences inthe intensity and duration of the exercise undertaken, the varying sampling intervals used in the reports, and the inclusion of individuals with possible muscular problems. The majority of workers have suggested that an increase in CK activities occurs with hard exercise,[98–100] whereas with slower work, others have shown no significant increase. This difference suggests that intensity could be an important factor.[101,102] Such a conclusion is supported by work in dogs, which shows that CK activities correlate with the intensity of muscular activity.[100] Another study in the horse,[103] however, suggested that CK elevations did not vary according to the intensity of the work, and one researcher[104] proposed that the duration of exercise was a more important factor. A recent study suggested that when the duration of exercise was kept constant, the intensity of the exercise in fact did have an effect on the extent of CK activity increase.[105] However, this investigation did not look at the effects of exercise duration on the CK response.

Although no significant increase in CK activity was found following trotting exercise in conditioned animals by one worker, others recorded significant increases when horses performed the same exercise after 1 or more days of rest.[101] Increases in AST activities of 35% have been reported following a 1500-m canter[107] and of 50% following strenuous exercise in previously rested animals.[99] Most other workers have found little increase in AST following different types of exercise.[89,92,104,108]

Therefore the effects of exercise on plasma muscle enzyme activities may depend on the fitness of the animal and the intensity and duration of the exercise, as well as on the environment.[85,104,109] In horses, as in human beings, large intersubject variability may occur in the postexercise rise in CK activities that must be taken into account.[82] Plasma volume changes may affect the activities recorded, especially if measured immediately after exercise. A test that would enable fit and unfit horses to be tested equally has been proposed and involves riding the horse over a given distance at a speed that produces a steady heart rate of 200 beats per minute.[110] However, exercising the horse at an exact heart rate is difficult, and many veterinary surgeons do not have access to heart rate monitors. This means that veterinary surgeons tend to use the same exercise test in all animals, regardless of their fitness.

As discussed previously, the physiologic increase in CK activity following exercise is believed to be caused by a change in cell membrane permeability, possibly caused by hypoxia, although other factors likely are involved. Hypoxia may occur at lower workloads in unconditioned horses, and these may be expected to show higher postexercise activities than a fit horse given the same work. The suggestion has been made that the magnitude ofthe exercise-induced rise in enzymes decreases with training.[89,103,104] Some workers have found no significant changes in the AST and CK responses to exercise during a training program,[102] whereas others have found that following an endurance ride, the fittest animals (indicated by speed of the heart rate recovery following an endurance ride) had lower increases in CK activities.[108] The magnitude of exercise-induced changes in CK activities has been shown to increase with detraining. This study concluded that increases of more than 100% in AST activity following exercise likely are abnormal, regardless of the intensity of the exercise or the fitness of the animal. Also, if a short, submaximal exercise test is carried out, the serum CK and AST activities at 2 hours after exercise should not rise to more than 250% and 50% of the preexercise values, respectively, regardless of fitness.[105]

The point has been stressed that although exercise might result in statistically significant changes in CK and AST activity, these may not always be of biologic or clinical significance.[111,112] The practitioner always must take into consideration the clinical history and clinical presentation, for example, when interpreting enzyme values. 474
For example, a young racehorse given its first gallop often has activity changes greater than those described 475
(e.g., from a preexercise level of 40 U/L to a 2-hour postexercise level of 350 U/L), although this is unlikely to be clinically significant. However, in cases of recurrent exertional rhabdomyolysis (RER) or polysaccharide storage myopathy (PSSM), similar changes may indicate ongoing subclinical muscular dysfunction. For example, in the author's experience, horses with RER or PSSM may have 4-hour postexercise serum CK activities of 10,000 to 15,000 U/L without outward clinical signs of rhabdomyolysis. In human beings, the suggestion has been made that no relationship exists between CK activity and the amount of muscle damage after an eccentric exercise bout, reflecting that CK activity is a manifestation of muscle damage rather than a direct indicator of its severity.[82]

Currently, the author recommends a submaximal exercise test, which varies for each horse. The practitioner should choose the intensity and duration of this test according to the fitness of the horse and the exercise program it is undertaking, with the aim of giving the horse strenuous exercise without overexerting it. Box 9-1 illustrates a normal response to such a test. Horses that recently have ridden in a trailer a long distance for evaluation may require 24 hours of hospitalization to ensure a normal CK value before the examination.

9.6.8 OTHER FACTORS AFFECTING AST AND CK ACTIVITIES

9.6.8.1 Gender and Age

A group of Thoroughbreds was sampled over a 9-month period, and the 2-year-old fillies showed more significant fluctuations in AST and CK activities than the 3-year-old fillies and colts. Unfortunately, no 2-year-old colts were studied.[113] In a later study of 66 2- and 3-year-old Thoroughbred racehorses in training, the fillies were found to be more likely to have high CK and AST activities than colts, and 2-year-olds were more likely to have increased AST activity than 3-year-olds. The effect of age on the incidence of increased muscle enzyme activities was thought not to have been caused by the natural loss of2-year-olds from training with high enzyme activities, especially because several of these raced and won.[114] Certain animals may have physiologically higher plasma activities, or their muscle enzymes may be removed more slowly from the circulation. Alternatively, they may be more sensitive to the various insults that cause permeability changes in muscle fiber membranes. Age or training or both could have a dampening effect on muscle membrane changes. In dogs a significant decrease in CK activities has been reported with age, but no difference was found between males and females.[115] In one study no correlation was found between plasma progesterone concentrations and the fluctuations in CK and AST activities.[113] However, a later study showed that when fillies with high median AST activities were removed from the study group, a highly significant relationship was found between progesterone and AST, but not CK activities, and estradiol showed a significant effect on CK but not on AST activities. In rats, CK release after exercise orin vitro electric stimulation is greater in males than in females. Estradiol has been suggested to have a protective effect and to attenuate CK influx.[116] However, further work on the role of such hormones in CK and AST activities in the horse is necessary before one can draw conclusions. In addition, one must examine all studies in large groups of horse with some scrutiny when one considers that the incidence of some underlying myopathies is high within a population. Considering that AST and CK tend to rise exponentially with muscle damage, a few outliers in a study may skew results significantly. The incidence of RER in Thoroughbred horses may be as high as 5% of the population,[117] which may account for some of the increases in CK and AST activities observed in the studies cited.

9.6.8.1.1 BOX 9-1 CRITERIA FOR A NORMAL RESPONSE TO A SUBMAXIMAL EXERCISE TEST DESIGNEDFOR A GIVEN HORSE[*]

1. Pre-exercise:

   CK activity <100 IU/L (laboratory resting reference range, 0-49 IU/L)

   AST activity <300 IU/L (laboratory resting reference range, 150-230 IU/L)

2. Not more than a doubling of the resting CK activity at 2-4 hours after exercise
3. Return to baseline CK activities at 24 hours after exercise
4. Not more than a 50% increase in AST activity

5. No clinical signs of stiffness

* *CK*, Creatine kinase; *AST*, aspartate aminotransferase.

9.6.8.2 Time of Year and Training

Several workers have suggested that AST plasma activities increase in the early stages of training and then decrease as training progresses.[118,119] Time of year does not have a significant effect on the number of animals with normal or with high AST and CK activities.[114] However, researchers observed an increase in mean activities peaking in April and May and followed by a decrease to a lowin September in a group of Thoroughbred racehorses in the Northern Hemisphere.[114] The high mean activities in April, May, and June 475
were accompanied by high standard deviations, which made definitive conclusions difficult. Researchers also 476
found such large standard deviationsin another study of Thoroughbreds in training.[120] On an individual basis, a change in AST activities does not always seem to occur with training.[121] Therefore, decreases or increases in the serum levels of AST activity are probably not good indicators of peak fitness or overtraining.[92]

In a study of AST activities in a small number of barren and pregnant Standardbred mares, researchers apparently found evidence for a diurnal rhythm, with the lowest activities occurring in the early morning (4 to 6 AM) and the highest activities at night (10 to 12 PM). The mean activities increased from September to November until March. A circannual cyclicity was found, but the pattern differed between the two groups. In barren mares the arcophase appeared to occur in the second half of January, whereas for pregnant animals it occurredin September (approximately month 5 of pregnancy). The error fields, however, were broad.[122]

9.6.8.3 Relationship to Performance

Elevated CK and AST activities have been suggested to decrease the chance of a horse winning.[123] However, a group of 500 Standardbred trotters with a recent history of equine rhabdomyolysis and increased plasma muscle enzyme activities had a significantly better racing record compared with a large group of apparently unaffected horses.[25] Another study found that 50% of the horses with high median AST activities raced and won at least once.[114] However, determining whether these horses would have given better performances or won in better classes if they had not had such increased muscle enzyme activities obviously is not possible.

9.7 Classification of Myopathies

Coordinated movement and resting muscle tone rely on the interaction between the central nervous system and the motor units with input from the Golgi tendon organs (responding to muscle tension) and the muscle spindle apparatus (monitoring muscle length). Lesions in the neurons and their end plates (neurogenic myopathies) or in the muscle fibers themselves (myogenic myopathies) can induce pathologic alterations in skeletal musculature. The term *primary myopathy* has been given to any disorder attributable to fundamental morphologic, biochemical, or electric changes occurring in the muscle fibers independent of the central or peripheral nervous system.[124] Differences in the histologic appearance of neurogenic and myopathic myopathies have been reported in human beings[46,52] and horses.[125]

Classification of primary myopathies regarding pathologic changes is difficult, if not impossible, only because skeletal muscle has limited ways to respond to a variety of insults. The most useful basis for classification therefore may be cause. However, a firm etiologic basis for many causes of rhabdomyolysis in the horse has yet to be described. Therefore various systems have been suggested for the horse[109,126] in which disorders have been divided into congenital, exertional, neurologic, and endocrinologic categories. An alternative way is to classify accordingto the underlying pathophysiology, that is, disorders of excitation, action potential propagation, etc. Because knowledge of the pathophysiology of the various equine skeletal muscle disorders is limited, this system is of limited use. Practitioners may find approaching horses with muscle disorders from a problem-based approach most helpful. So horses are categorized into five categories: those having muscle cramping on exercise, those with gait alterations alone, those with muscle weakness, those with muscle wasting, and those with acute, severe rhabdomyolysiswith or without recumbency or death. These clinically based classifications are not mutually exclusive, and some disorders fall into more than one category. However, the author feels that this approach is the most comprehensive, considering the current volume of knowledge relating to muscle disorders in the horse.

Box 9-2 shows how the various equine skeletal disorders might be classified under the pathophysiologic system, using current information. Box 9-3 demonstrates a problem-based classification of the most common muscular disorders in the horse. One should note how many equine myopathies share common clinical presentations. Therefore a thorough genetic, environmental, and athletic history; physical examination; and laboratory workup are necessary to determine the most probable underlying cause in each case of muscle disease in the horse. Because the typical practitioner will be presented with a muscle-related problem, this chapter follows the classification set forth in Box 9-3. Before discussing the specific muscle diseases, the next section covers some general concepts and traditional thoughts concerning exertional rhabdomyolysis.

9.7.1 EXERTIONAL RHABDOMYOLYSIS

Exertional rhabdomyolysis refers to the syndrome of muscle cramping that occurs during physical exertion or exercise. Differential diagnoses for exertional rhabdomyolysis are extensive (Box 9-4). Terms used to describe the disorder include chronic exertional rhabdomyolysis, equine rhabdomyolysis syndrome, azoturia, Monday morning disease, tying-up, myositis, setfast, and chronic intermittent rhabdomyolysis.[65,92,127–129] Broadly, horses experience exertional rhabdomyolysis for two main reasons: the horse has an underlying myopathy (chronic exertional rhabdomyolysis) or the horse has been overexerted physically (sporadic exertional 476
rhabdomyolysis). Horses with underlying myopathies generally experience repeated episodes of rhabdomyolysis 477
after short bouts of exercise; therefore the condition may be characterized as chronic. However, horses that are overexerted may only experience a single episode in their lifetime because the bout of rhabdomyolysis is caused by physical and environmental circumstances as opposed to an underlying pathologic condition of the muscle.

9.7.1.1 BOX 9-2 CLASSIFICATION OF EQUINE MYOPATHIES ACCORDING TO CAUSE

I. Neurogenic: May be hereditary or environmental in origin, acquired, or congenital

- A. Disorders of anterior horn cells
- B. Disorders of motor nerve roots
- C. Peripheral neuropathies
- D. Disorders of neuromuscular transmission

1. Botulism
2. Tetanus

II. Myogenic

A. Traumatic
   1. Fibrotic myopathy
   2. Gastrocnemius muscle rupture
   3. Serratus ventralis muscle rupture
B. Inflammatory
   1. Sore or pulled muscles
C. Infectious
   1. Bacterial
      a. Clostridial myonecrosis
      b. *Streptococcus* spp.
         i. Abscessation
         ii. Autoimmune
         iii. Purpura hemorrhagica
         iv. Immunoglobulin G–mediated
         v. Immunoglobulin A–mediated (Henoch-Schönlein purpura)
      c. *Staphylococcus* spp.
      d. *Corynebacterium pseudotuberculosis*
   2. Viral
   3. Parasitic
      a. Sarcocystis spp.
      b. Trichinella spiralis
D. Toxic
   1. Cassia occidentalis
   2. White snakeroot

3. Ionophores

E. Hormonal

1. Hypothyroidism (unsubstantiated)

F. Circulatory

1. Postanesthetic myositis
2. Aortic-iliac thrombosis

G. Genetic

1. Mitochondrial enzyme deficiencies
2. Glycogen branching enzyme deficiency
3. Myotonias
    a. Hyperkalemic periodic paralysis
    b. Myotonia congenita
    c. Myotonia dystrophica
4. Glycogen storage disorders
    a. Polysaccharide storage myopathy in Quarter Horses
    b. Equine polysaccharide storage myopathy in Draft Horses
5. Recurrent exertional rhabdomyolysis in Thoroughbreds

H. Nutritional

1. Vitamin E deficiency

    Equine motor neuron disease

    Equine degenerative myelopathy

2. Selenium deficiency
3. Malnutrition
4. Carbohydrate overloading
5. Thiamine deficiency
6. Electrolyte deficiency

I. Exercise-related, overexertion, and the exhausted horse syndrome

J. Cachectic atrophy following chronic disease

K. Disuse atrophy

L. Malignancy: muscle tumors

M. Miscellaneous or idiopathic

1. Atypical myoglobinuria

2. Polymyopathy

Identifying horses with underlying myopathies that are characterized by chronic exertional rhabdomyolysisis a rapidly developing field of veterinary medicine. To date, three major syndromes have been described: RER in Thoroughbred horses, PSSM in Quarter Horsesand equine polysaccharide storage myopathy (EPSM) in Draft-breed horses. Horses that have chronic exertional rhabdomyolysis but that cannot be diagnosed with any of the three aforementioned disorders may be assumed to have a new or previously undescribed equine myopathy, provided no physical or environmental cause can be determined. Until more is known, horses with multiple bouts of rhabdomyolysis associated with minimal exercise that do not appear to fit into the categories ofRER, PSSM, 477
or EPSM are grouped and classifiedas having idiopathic chronic exertional rhabdomyolysis. One should not 478
assume that all Thoroughbreds, Quarter Horses, and Draft-breed horses demonstrating chronic or sporadic exertional rhabdomyolysis have RER, PSSM, or EPSM, respectively, simply because of their breed.

9.7.1.2

BOX 9-3 PROBLEM-BASED APPROACH TO HORSES WITH MUSCLE DISEASE

Disorders are grouped according to similar clinical appearance. (No category is mutually exclusive. Disorders are grouped by most common clinical presentation. The reader is directed to the text for more extensive discussion for each disorder/disease.)

I. Profound muscle cramping with exercise, tying-up syndrome, increased plasma/serum creatine kinase (CK) activity after exercise

A. Horses with underlying myopathy

1. Recurrent exertional rhabdomyolysis in Thoroughbreds

2. Polysaccharide storage myopathy in Quarter Horses

3. Equine polysaccharide storage myopathy in Draft Horses

4. Idiopathic chronic exertional rhabdomyolysis

5. Mitochondrial myopathy

B. Horses without underlying myopathy

1. Overexertion

2. Vitamin E or selenium deficiency
3. Electrolyte depletion

II. Horses with altered gait but without underlying myopathy and without muscle cramping, with or without elevated CK

A. Acute: muscle strain, sprain, tear

B. Chronic: fibrotic myopathy

III. Muscle weakness

A. Hyperkalemic periodic paralysis

B. Myotonia congenita and myotonia dystrophica

C. Equine motor neuron disease

D. Equine polysaccharide storage myopathy in Draft Horses (demonstrate primarily weakness)

IV. Muscle wasting

A. Generalized, may be accompanied by mild elevations in CK activity

1. Equine motor neuron disease
2. Streptococcal immune-mediated myositis: mediated by immunoglobulin G (IgG)
3. Cachectic atrophy
4. Disuse atrophy

B. Segmental

1. Neurogenic
2. Disuse atrophy
3. Fibrotic myopathy

V. Acute rhabdomyolysis, swollen painful musculature, with or without recumbency, with or without death

A. Severe, acute exercise-related rhabdomyolysis as from I.A. and I.B.

B. Malignant hyperthermia/postanesthetic myopathy

C. Clostridial myonecrosis

D. *Sarcocystis* spp.

E. Streptococcal immune-mediated myositis; Henoch-Schönlein (IgA) purpura

- F. Aortic-iliac thrombosis
- G. Toxic plants
  - 1 Cassia occidentalis
  - 2. White snakeroot
- H. Ionophore toxicity

VI. Disorders of the neonate

- A. White muscle disease/nutritional myodegeneration
- B. Foal rhabdomyolysis
- C. Glycogen branching enzyme deficiency
- D. Arthrogryposis

VII. Miscellaneous disorders

- A. Atypical myoglobinuria
- B. Postanesthetic myasthenia
- C. Polymyopathy
- D. Abscesses
- E. Tumors

In the past, exertional rhabdomyolysis in the horse was described in the literature under diseases of the liver, kidney, blood, or muscle. Exertional rhabdomyolysis also has been attributed to infection, cold weather, intoxication, nervous irritation, calcium deficiency, excess of glycogen, lactic acid poisoning, an increase in red blood cells, and an unbalanced alkali reserve.[130] Most theories have not undergone thorough scientific scrutiny to rule them in or out as an inciting cause, contributing factor, or primary cause.

Exercise is the common triggering factor in horses with exertional rhabdomyolysis. However, in horses with underlying myopathies the amount of exercise necessary to trigger muscle cramping is often minimal in contrast to healthy horses exhibiting a sporadic episode of rhabdomyolysis because of overexertion. Similarly, horses with underlying myopathies suffer from repeated episodes, whereas overexerted but otherwise healthy horses do not, provided they are not pushed repeatedly beyond their level of fitness. 478

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9.7.1.3

BOX 9-4 DIFFERENTIAL DIAGNOSIS OF EQUINE RHABDOMYOLYSIS

Acorn poisoning

Anthrax

Back problems

Castration sequelae

Colic

Cystitis

Hernia

Iliac thrombosis

Inguinal/popliteal lymphadenitis

Laminitis

Monensin poisoning

Nephritis

Pleuritis

Postexhaustion syndrome

Proximal limb lamenesses

Spinal cord disease (wobbles)

Tetanus

White muscle disease

Data from Arighi M, Baird JD, Hulland TJ: Equine exertional rhabdomyolysis, *Compend Cont Educ Pract Vet* 6:5726, 1984; Waldron-Mease E, Raker CW, Hammel EP: The muscular system. In Mansmann RA, McAllister ES, Pratt PW, editors: *Equine medicine and surgery*, ed 3, Santa Barbara, Calif, 1982, American Veterinary; Carlson GP: Medical problems associated with protracted heat and work stress in horses, *Compend Cont Educ Pract Vet* 5:542, 1985; Baldame GF: The "tying-up" syndrome, *Proc Am Assoc Equine Pract* 19:353, 1973; Gresswell JB, Gresswell AG: Azoturia. In Gresswell JB, Gresswell AG, editors: *A manual of the theory and practice of equine medicine*, ed 2, London, Bailliere Tindall, New York, 1890, William Jenkins; and Pritchard JT, Voss JL: Fetal ankylosis in horses associated with hybrid Sudan pasture, *J Am Vet Med Assoc* 150:871, 1967.

9.7.1.4 Classic Theories for Exertional Rhabdomyolysis

For centuries horses were believed to tie up for a single as yet undetermined reason. The most popular reason was inactivity (i.e., stall rest) accompanied by a full ration of grain. This scenario was most likely to occur in Draft Horses rested on Sunday and worked on Monday, hence the name Monday morning disease. To date, the influence of diet, specifically carbohydrates, on exertional rhabdomyolysis is an area of active research. Diet plays a role in horses that are *predisposed* to exertional rhabdomyolysis from several different underlying myopathies (see specific disorders in the following sections), but how diet may influence muscle function in normal, healthy horses is still largely unknown.[126,131,132]

9.7.1.4.1

Lactic Acidosis

From 1914 to 1915 history records that when oats were scarce and raw sugar was fed to horsesas a substitute, the incidence of metabolic diseases, especially azoturia, increased.[134] In two papers that are much quoted in the literature, excessive glycogen buildup was postulated to result in an overproduction of lactic acid during exercise.[135,136] These studies reproduced the condition by feeding horses 3 kg of molasses daily and then exercising them after a rest period. However, in these studies the levels of lactate measured in horses experiencing rhabdomyolysis were similar or lower than the values currently measured in normal, healthy horses exercising anaerobically. Therefore, lactate alone is unlikely to trigger exertional rhabdomyolysis in healthy horses.[137] The role of lactic acid in horses with underlying myopathy has been investigated only in Thoroughbred horses with RER. That study found no correlation between lactate levels and CK activity measured 4 hours after exercise.[131] In investigations of other breeds of horses with chronic exertional rhabdomyolysis the relationships between rhabdomyolysis and lactate were less clear. [40,129,138,139]

9.7.1.4.2

Vitamin E and Selenium and Exertional Rhabdomyolysis

A relationship between equine rhabdomyolysis and vitamin E and selenium deficiencies has been claimed because of the reported success of supplementation in preventing further attacks.[140] Certainly vitamin E and selenium are essential to normal muscle function in all mammals. However, assuming that all horses thatsuffer from exertional rhabdomyolysis do so because of vitamin E and selenium deficiency is inappropriate. Muscle biopsy samples from horses with equine rhabdomyolysis, for example, have shown no deficiency in vitamin E or selenium.[141] No relationship between low blood glutathione peroxidase (GSHPx) activity and high AST levels has been found,[142] and the feeding of additional vitamin E does not decrease the frequency of high AST activities.[143] The claim that vitamin E and seleniumprevent or cure equine rhabdomyolysis therefore has been refuted.[12] A group of horses fed apparently low dietary levels of vitamin E for 4 months demonstrated no apparent clinical signs, no clinically abnormal levels of CK or AST were recorded, and the performance of the animals in standardized exercise tests was not compromised.[144]

In a survey of 144 animals thought to suffer from chronic rhabdomyolysis, 5 had lowered GSHPx activities. [12,105] However, these five animals also had abnormal urinary fractional electrolyte excretion values. Appropriate oral electrolyte and selenium supplementation resulted in no further clinical episodes. However, determining if this recovery would have occurred if either of the measures had been undertaken alone or if therapy had been withheld is not possible.

The literature poorly documents how commonly neurologic or myopathic conditions occur that may be related to chronic vitamin E or selenium deficiency. Disorders that have been attributed to dietary 479
deficiencies include equine motor neuron disease, equine degenerative myelopathy, neonatal nutritional 480
myodegeneration (white muscle disease), neonatal rhabdomyolysis, a predisposition to postanesthetic myopathy, and adult cases of exertional rhabdomyolysis. A case of severe masseter myonecrosis also has been attributed to selenium and vitamin E deficiency.[145]

Supplemental vitamin E may be beneficial in horses with underlying myopathies or healthy horses as an additional source of antioxidants in the diet. In fact, horsesin heavy work do appear to need more vitamin E

in their diet than many commercial feeds provide.[146] However, how chronic low vitamin E or selenium intake may affect performance in horses predisposed to rhabdomyolysisor in healthy horses is unknown. Therefore a common recommendation is to increase vitamin E intake in horses with chronic exertional rhabdomyolysis and healthy horses performing hard work even though the scientific work to uphold this recommendation has not been performed.

9.7.1.4.3 Role of Vitamin E and Selenium in Muscle Function

GSHPx, a selenium-containing enzyme, and vitamin E help protect cells against free radicals,[147] which are formed by the reduction of molecular oxygen during normal oxidative processes. The following is an example:

$$O_2 \rightarrow O^- \text{ (superoxide) enzyme}$$

A free radical (or reactive oxygen species) is any chemical substance that has an unpaired electron in its outer orbit. The presence of this electron makes the free radical inherently unstable. Superoxide radicals, for example, in solution form hydrogen peroxide by dismutation:

$$2O_2 + 2H^+ \rightarrow H_2O_2 + O_2 \text{ (superoxide dismutase)}$$

The superoxide radical also can react with hydrogen peroxide and hydrogen ions to produce the potent hydroxyl radical ($HO^\bullet$).

$$H^+ O_2^- + H_2O_2 \rightarrow O_2 + H_2O + HO\bullet$$

The hydroxyl radicals in turn can react with almost any organic molecule to produce other organic radicals via chain reactions.

Free radical reactions are responsible for a number of key biochemical events, including mitochondrial electron transport, prostaglandin synthesis, phagocytosis, and degradation of catecholamines. Under controlled circumstances free radicals are therefore necessary for life, but when uncontrolled they may result in a number of degenerative disease processes. For example, free radicals can cause irreversible denaturation of essential cellular proteins and lipid peroxidation of exposed polyunsaturated fatty acids within the phospholipid structure of cellular membranes, resulting in structural damage and the formation of further hydroperoxides. The cellular membrane damage in turn may result in the release of liposomal enzymes and further damage. The free radicals also can cause damage to hyaluronic acid and collagen, resulting in enhanced prostaglandin production, and they can uncouple oxidative phosphorylation and inactivate certain enzymes.

A system of natural antioxidant defenses is present within the body to counteract such free radical–induced damage, including GSHPx and vitamin E. GSHPx acts to reduce the production of hydroxyl radicals by reducing hydroperoxides to alcohols as part of a cyclic system re-forming glutathione. Within red blood cells the enzyme acts as an integral part of the system, protecting against the conversion of hemoglobin to methemoglobin by hydroperoxides. Vitamin E acts as a scavenger of free radicals, and vitamin C may assist by reducing the tocopheroxyl radicals formed by this scavenging. In addition, vitamin E helps block lipid peroxidation and also may form an important part of the membrane structure because of its interaction with membrane phospholipids. Other naturally occurring antioxidant defenses include chelators that bind to and decrease the concentrations of transition metals (e.g., iron).

Vitamin E and selenium deficiencies have been implicated in muscular problems in several species, especially sheep and pigs.[124,148] In the horse a myopathy attributed to vitamin E and selenium deficiency has been reported in foals from birth to 9 months of age.[149] The myopathy often is referred to as white muscle disease. In older animals, maxillary myositis, polymyositis, and dystrophic myodegeneration also have been attributed to such deficiencies.[150] However, some controversy exists over the role of vitamin E and selenium in such conditions. In foals the syndrome apparently has been well documented in geographic areas of known selenium deficiency and inadequate vitamin E intake,[151] although many foals raised in such areas may have decreased GSHPx activities and low blood selenium concentrations without being affected.[152]

Two common sources of free radicals during exercise are increased activity of xanthine oxidase during anaerobic degradation of purine nucleotides and the partial reduction of oxygen during oxidative phosphorylation within mitochondria. The precise mechanisms leading to muscle damage and fatigue during exercise are uncertain, although free radicals or reactive oxygen species possibly may play an important role. Following strenuous exercise, free radical production may exceed the capacity of the natural defense mechanisms of the cells, leading to muscle cell damage.

For many years scientists have appreciated that the human genetic muscular dystrophies share many
characteristics with the vitamin E and selenium deficiency myopathies in animals, although treatment of 480
patients with vitamin E so far has not been shown to be beneficial. However, with increased speculation that 481
free radical–mediated damage may be a factor in the cause or pathophysiology of the processes involved, a wide range of techniques has been developed to evaluate free radical damage. To date, no single method has been developed as the optimal way to study free radical processes. Perhaps because of the various techniques and differing criteria used for the choice of patients, few consistent abnormalities have been reported, especially respecting the measurement of antioxidant levels in blood or tissue.[153] The glutathione redox ratio has been suggested to be one of the most sensitive indices of oxidative stress, although reports have been inconsistent on the effects of exercise on the glutathione system in human beings, perhaps because of the varying fitness of the subjects and differences in the exercise intensity used. A study in the horse has suggested that exercise can induce changes in biochemical parameters (including the glutathione redox ratio) that indicate oxidative stress and the changes exacerbated during exercise at high temperatures and humidity.[154]

Vitamin E has an apparent protective effect on experimental damage to rat skeletal muscle and may act via a non–antioxidant mechanism, with the hydrocarbon chain being an important mediator of the effects seen.[155] An apparent protective effect occurs from supranormal extracellular vitamin E concentrations on normal rat muscle, and exacerbation of exercise-induced damage to skeletal muscle by vitamin E deficiency has been reported. Nutritional modification of skeletal muscle, perhaps by reduction of the unsaturation of skeletal muscle membranes or enhancement of the antioxidant status of muscles, or a combination of both, may help prevent or reduce calcium-induced skeletal muscle damage.[42] Artificial methods to reduce oxidant damage during exercise include the administration of exogenous sources of naturally occurring antioxidant defenses (e.g., vitamin E) and limiting the formation of free radicals by minimizing the formation of inosine monophosphate from purine degradation during exercises.

Type I and type IIA fibers are most likely to participate in excess free radical formation, because they are the most oxidative fibers and contain the most intracellular lipid stores. Selective type I fiber degeneration is found in nutritional myopathies of sheep and pigs that are responsive to vitamin E and selenium.[40]

Foals affected by exertional rhabdomyolysis have a significantly higher proportion of type 2C fibers and lower proportions of type 1 and type 2A fibers than healthy foals of comparable age.[156] These type 2C fibers include the undifferentiated fibers normally seen in foals and a proportion of previous type 1 and type 2A fibers, which appear as type 2C because of degenerative or regenerative processes. Some differences in the pathologic processes do appear to exist, however, between the equine conditions associated with vitaminE and selenium deficiencies and those in other species. Vitamin E deficiencies in calves and chicks show mitochondrial alterations early in the condition. In vitamin E– and selenium-deficient lambs and chickens, the earliest changes occur in small vessels, connective tissue, and neuromuscular elements. In the horse the changes appear to affect the myofibrilsfirst. Morphologic changes in mitochondria and the sarcoplasmic reticulum seem only to occur in fibers with advanced lesions.[156,157]

Factors that may predispose horses to a vitamin Eand selenium deficiency include rancid feed, the addition of fish or plant oil to the feed, prolonged storage of grain (e.g., dry grain loses tocopherol activities far more slowly than moist grain), poor-quality hay, and lush pastures.[126] Pastures associated with acid, poorly aerated soils, soils with a high sulfur content, or volcanic rock are more likely to be selenium deficient. Cases of deficiency are more likely to occur in areas with low (<0.05 ppm) plant selenium content. Dietary levels of selenium may be misleading, however, not only because other factors are likely to be involved in the pathogenesis of the condition but also because the biologic availability of the various types of selenium have not been determined. In addition, high but nontoxic levels of copper, silver, tellurium, and zinc possibly could interfere with selenium availabilityand therefore induce typical lesions in animals on diets containing amounts of selenium usually considered to be adequate.

Probably other factors such as stress (including managemental regimens and environment) and increased physical activity may be important for expression of clinical signs in the face of a deficiency and may explain why two similar groups of animals receiving the same dietary intake can show different clinical responses to the amounts of vitamin E and selenium. Acute selenium toxicity in the adult horse also may be associated with myodegeneration plus pulmonary edema, gastroenteritis, hepatic degeneration, and necrosis.[126,157]

9.7.1.4.4 Hypothyroidism

Muscular problems are a common symptom of hypothyroidism in human beings.[158,159] In mild human cases, fatigue may be the only presenting symptom, although more severe cases may be accompanied by overt muscle cell damage, increased resting plasma CK activities, and sometimes increased myoglobin levels.[160] Poor racing performance and certain myopathies have been related to mild secondary hypothyroidism,[161] although this conclusion has been challenged.[162] Oral thyroxine supplementation may improve performanceor decrease the incidence of myopathies in cases of hypothyroidism.[109,161] However, considerable debate still exists as to whether hypothyroidism is important in the pathogenesis of exertional myopathies in the horse.[65] 481

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Resting thyroxine concentrations did not differ significantly between animals believed to be suffering from chronic rhabdomyolysis and those suffering from a variety of other conditions. However, some rhabdomyolysis sufferers may have a lowered response to thyrotropin-releasing hormone,[105] which may reflect a decreased thyroid reserve, but too few cases have been investigated to date. A recent study examined exercise in thyroidectomized horses. Exercise intolerance was documented; however, increases in

CK activity were not.[163] Conversely, in the author's experience, oversupplementation may lead to iatrogenic hyperthyroidism and symptoms of mildly increased CK activity and muscle wasting.

9.7.1.4.5

Viral Causes

Muscle pain or myalgia is a common symptom in the acute phase of influenza and other viral illnesses in human beings, although few reports of severe muscular problems are available.[51,164,165] Myalgia and myoglobinuria have been observed with herpesvirus infections.[51] Some horses are stiff and unwilling to work during or after an attack of the virus.[166] Viral infection has been reported as being one of the predisposing factors to equine rhabdomyolysis.[167]

A clinical investigation of an outbreak of muscle stiffness and poor performance in a flat-racing stable of Thoroughbreds revealed that more than one third of the horses demonstrated signs of muscular stiffness over2 months, and 64% at one or more of the sampling times had increased CK and AST activities.[168] Serologic tests were highly suggestive of an equine herpesvirus type 1 infection. The possibility of a viral cause, at least in outbreaks of stiffness in racing yards, needs more in-depth evaluation. The virus could affect muscle directly, resulting in an increased susceptibility to exercise-induced damage. Alternatively, the increase in blood viscosity observed after infection could result in an impaired blood flow and decreased oxygen delivery to the peripheral tissues.[169] A recent study of horses exercised after experimental infection with equine influenza virus failed to demonstrate significant effects on muscle function or CK activity or evidence of muscle stiffness.[170]

9.7.1.5

Treatment of Rhabdomyolysis

Treatment of rhabdomyolysis depends on the underlying cause. For example, horses experiencing muscle damage because of sepsis or trauma require different approaches than horses experiencing rhabdomyolysis caused by underlying, heritable myopathies. Horses with rhabdomyolysis often have colic or nonspecific signs. Other differential diagnoses include disorders that frequently are associated with depression or a reluctance to move and include but are not limited to anthrax infection, postcastration abscesses, colic, cystitis, neurologic disorders, lymphadenitis, laminitis, nephritis, pleuritis, acorn poisoning, tetanus, and skeletal lamenesses.

The practitioner should perform a thorough physical examination and obtain a detailed history. The initial workup of a horse suspected of having rhabdomyolysis should include a complete blood count, chemistry profile, and urine collection. Treatment should follow the observations made on physical examination and changes observed in the blood count and chemistry profile. The practitioner should rehydrate horses with significant dehydration with fluids that reflect any deficiencies identified on the chemistry profile and should treat horses in significant discomfort with nonsteroidal antiinflammatory drugs (NSAIDs), provided renal perfusion and function are supported. The practitioner should not treat horses experiencing rhabdomyolysis in the face of extreme exhaustion and dehydration with nephrotoxic medications (aminoglycosides and NSAIDs) until ascertaining renal function. Horses that typically fall into this category include those with capture myopathies (e.g., horses that are trapped and struggle in trailer accidents, get wrapped up in fences, are being taught to tie to a fixed object, or get cast in a stall) and endurance horses. Treatment for horses with mild cases of rhabdomyolysis includes keeping them warm, minimizing movement, and providing stall rest and NSAIDs as appropriate. Muscle enzyme activity and the physical response of the horse to being asked to move and exercise can guide the practitioner as to when returning the horse to normal physical activity is appropriate.

Therapies specific to each type of rhabdomyolysis are discussed in their respective sections.

9.8 Horses With Underlying Myopathy

9.8.1 RECURRENT EXERTIONAL RHABDOMYOLYSIS

Racing Thoroughbreds share a high degree of relatedness. As much as 80% of the genome of the modern Thoroughbred directly traces to 31 animals.[171] This extensive amount of linebreeding makes associating diseases with a particular ancestor difficult because so many horses healthy or not may be related to that individual. The suggestion has been made that many Thoroughbred horses share a similar genetic trait that leads to RER.[132,172] Epidemiologic surveys of racing Thoroughbreds support that exertional rhabdomyolysis is so common, it may affect as much as 5% of the racing Thoroughbred population, and that the majority of these horses likely have the same condition referred to as recurrent exertional rhabdomyolysis.[117]

9.8.1.1 Clinical Signs and Laboratory Findings

Thoroughbred horses with RER may have mild to moderate signs of muscle cramping. Clinical signs of RER are similar to other horses exhibiting exertional rhabdomyolysis. The predominant clinical signs are mild to 482

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severe muscle cramping of the major muscle groups of the hindquarters including the gluteal, semimembranosus, semitendinosus, biceps femoris, and quadriceps femoris muscles. Involvement of these muscle groups is not exclusive, and muscle cramping of the forelimb musculature may occur. Cramping is associated with mildto severe pain, manifested by anxiety, profuse sweating, refusal to move, and increased heart and respiratory rates. Affected muscles are firm and painful on palpation. The practitioner can palpate generalized or focal areas of flocculation that may be associated with focal areas of muscle tearing or edema. In mild to moderate cases muscle contractures abate within several hours, and the horse moves about more comfortably. In severe cases, rhabdomyolysis may lead to recumbency, necessitating a prolonged convalescence, which in turn may lead to further complications. Recovery is related to the severity of muscle fiber necrosis and may take days to weeks in mild and moderate cases and months in horses with severe rhabdomyolysis. Repair begins with migration of satellite cells from their position under the basement membrane and their transformation into myoblasts. Disruption of the muscle cell basement membrane rarely occurs even in severe cases of exercise-induced rhabdomyolysis. However, should damage to the basement membrane occur, the defect would heal largely with connective tissue.[173] In most cases, horses readily return to athletic function.

Damaged muscle fibers release myoglobin and the intracellular enzymes LDH, AST, and CK into the circulation. Myoglobin is filtered by the kidneys and is excreted in the urine. With moderate to severe muscle necrosis myoglobin causes the urine to be orange to brown. In horses with systemic acidosis and dehydration that suffer significant muscle damage, myoglobin may cause tubular nephrosis with acute or delayed renal damage or failure.[174] In Thoroughbreds with RER, onset of rhabdomyolysis occurs early, during aerobic exercise, and for this reason no accompanying systemic acidosis or dehydration occurs and renal damage is rare. Tubular nephrosis rarely occurs, presumably because myoglobin does not precipitate out in the urine of the adult horse.

Elevations in serum enzymes and myoglobin are proportional to the amount of muscle damage, with serum myoglobin levels rising most rapidly. Myoglobin assays are not used frequently in clinical situations because

of the difficulty of measuring equine myoglobin and because the half-life of myoglobin in the serum is short, with peak levels occurring minutes after exercise. LDH and AST activity are less specific indicators of muscle damage than CK or myoglobin because they also are released with liver damage. In clinical situations, CK and AST activity are the most frequently measured enzymes used to evaluate muscle necrosis. CK and AST are included in conventional serum or plasma chemistry profiles and can provide clues as to the length of time since the initial insult. Serial elevations of CK and AST indicate ongoing muscle necrosis. After an episode of rhabdomyolysis, CK levels increase exponentially from the reference range to levels as high as 100,000 U/L. CK is a sensitive indicator of muscle damage, with activity peaking 4 to 6 hours after insult. If muscle necrosis is not ongoing, CK levels decrease rapidly over the next 24 to 48 hours because of the relatively short (108-minute) half-life. Serum AST activity takes 12 to 24 hours to peak, and activity may remain elevated for 7 to 14 days after insult.[39]

In Thoroughbred horses with RER, CK activity appears to peak 4 to 6 hours after activity and affected horses have intermittent elevations in CK activity that are not associated with clinical signs of muscle cramping (subclinical exertional rhabdomyolysis).[175] Subclinical exertional rhabdomyolysis is common in Thoroughbred horses with RER, and CK activity may be as high as 10,000 U/L. Normal healthy horses participating in race trainingalso may have occasional increases in CK activity butdo not appear to have them as frequently as horseswith RER.

9.8.1.2 Causes

A group of Thoroughbreds with a history of chronic exertional rhabdomyolysis recently were studied and were found to have similar abnormalities.[132,176] Muscle biopsies of the middle gluteal muscle did not identifyany abnormal accumulations of polysaccharide, but an increased number of centrally located nuclei were found in mature muscle fibers. In vitro contracture studies demonstrated increased sensitivity to caffeine and halothane and faster times to peak contraction and 50% and 90% relaxation compared with healthy Thoroughbred horses or Quarter Horses with PSSM.[177] The in vitro contracture study now is considered the gold standard for diagnosing RER in the Thoroughbred horse.

The muscle contracture results are similar to pigs with malignant hyperthermia.[178] However, further testing has demonstrated that the cellular defect in Thoroughbreds with RER and pigs with malignant hyperthermia is different. Therefore RER represents a disorder similar but not identical to malignant hyperthermia in swine.[179]. Of interest is that occasional incidences of malignant hyperthermia in Thoroughbred horses with chronic exertional rhabdomyolysis have been reported.[180–185] This may indicate that Thoroughbred horses with RER may be more susceptible to episodes of malignant hyperthermia while under general anesthesia, but the incidence is far less common than in swine.

The exact cellular mechanism that leads to RER in Thoroughbred horses has yet to be elucidated, and genetic studies are currently under way. Possibly the defect lies in calcium regulation within the cell at the level of the dihydropyridine or ryanodine receptor, but this has yet to be confirmed. 483 484

9.8.1.3 Pathophysiology

RER affects as much as 5% of the racing Thoroughbred population and likely is inherited as an autosomal dominant trait.[117,172] However, expression of the disorderis multifactorial and depends on the fitness level and exercise schedule of the horse, diet, age, gender, temperament, and the presence of any skeletal lameness.[117]

Expression of RER in Thoroughbred horses has been postulated to be related to the physicochemical changes experienced by the body during a high level of stress or anxiety. Such a trigger would be similar to that seen in swine with malignant hyperthermia. However, the exact mechanism of how anxiety may trigger exertional rhabdomyolysis in susceptible horses remains unknown.

Many of the factors listed previously may influence the mental and physical state of the horse. For example, the typical Thoroughbred horse with RER has longbeen described as a 2- or 3-year-old filly with a nervous temperament.[65,66,117,186] A recent epidemiologic survey carried out in the United Kingdom showed that females are more likely than males to suffer from this condition, particularly in the birth to 2-year-old and 5- to 6-year-old age groups.[105,187] However, an equal sex ratio was reported in another study.[188] Genetic studies support that RER likely is inherited as an autosomal dominant trait.[172] The increased expression of RER in female Thoroughbreds and in younger horses might be because of temperament. Fillies are more likely to be describedas having a nervous temperament by their trainers, and young horses may find the race track environment stressful.[117]

Diet also influences expression of RER, and affected horses often consume quantities of grain in excess of10 pounds (4.5 kg) per day. Higher CK activity has been documented in horses with RER compared with control horses when both groups were fed diets high in carbohydrates. In addition, dietary studies have shown that feeding horses diets high in carbohydrates at levels at which the caloric intake exceeds body demands may accentuate nervousness. This effect has been observed in Thoroughbreds with RER and in normal healthy horses.[175] Conversely, horses with RER that are fed diets higher in fat demonstrated a decrease in CK activity, and horses with RER and healthy horses consuming diets high in fat often are regarded as being more calm and less fearful.[131] A number of high-fat, low-carbohydrate commercial grain mixes are now available for the horse owner, and anecdotal reports support laboratory findings that the number of incidences of rhabdomyolysis in horses with RER consuming these diets are fewer. Whether the influence of diet on the expression of RER is caused by temperament alone, another factor, or a combination of factors has yet to be determined.

Racehorses with RER are more likely to have an episode of rhabdomyolysis when exercised at a medium gallopas opposed to walking, trotting, breezing, or racing. Horses with RER that are exercised at the trot were more likely to have increased CK activity than when galloping at high intensity. The influence of temperament also may be a factor. Thoroughbreds at the race track are often trotted against traffic as a warm-up and then are turned around to gallop back home at a controlled rate of speed. This training scenario was the most frequent form of exercise associated with episodes of rhabdomyolysis and may result from excitement or anxiety that triggers the episode of rhabdomyolysis in susceptible horses. Horses that are fit for racing may find this training scenario especially exciting because they often pull and try to run faster. When racehorses were allowed to gallop full out as in breezing or racing, episodes of rhabdomyolysis were infrequent.[117]

Horses with an underlying lameness are predisposed to episodes of exertional rhabdomyolysis. Whether changes in gait may be related to muscle pain or soreness or whether underlying skeletal disorders may alter a horse's way of going, precipitating an episode of rhabdomyolysis, or whether running in pain from a skeletal disorder may trigger an episode of rhabdomyolysis is unclear.

Inclement weather often has been cited as causing an increase in the incidence of RER, as has the time of year.[127,130] In a 2-year survey in the United Kingdom, significantly more episodes of rhabdomyolysis were reported from November to February than during other times of the year.[105,187]

At the race track, large groups of horses are fed, exercised, and housed under similar conditions. Yet some are particularly predisposed to chronic exertional rhabdomyolysis, which underscores the likelihood of a genetic basis for the disorder. One is tempted to assume thatall affected Thoroughbreds at the racetrack have RER. However, the practitioner must be wary about diagnosing all Thoroughbreds with chronic exertional rhabdomyolysis as having RER until the genetic fault is identified and a genetic test is available.

9.8.1.4 Diagnosis

Currently, the gold standard for diagnosing RER is the caffeine or halothane muscle contracture test.[176] However, this test requires a biopsy sample from intercostal muscle and is not readily available. Therefore diagnosis of RER is based on history, laboratory documentation, and histopathologic examination of muscle. Horses with RER should be Thoroughbreds or Thoroughbred crosses, because of the likelihood the disorder is inherited as an autosomal dominant trait. The horse should have experienced multiple bouts of muscle cramping after nonintensive exercise accompanied by documented elevations in CK activity. Muscle biopsy reveals an increased number of central nuclei without evidence of PSSM.

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9.8.1.5 Treatment

Treatment of horses with RER aims at managing the frequency of the clinical episodes of rhabdomyolysis, considering that the disorder is likely a primary myopathy. Environmental and dietary factors appear to influence the expression of RER. Such factors include dietary fat and carbohydrate content, dietary electrolytes, exercise regimens, housing and turnout conditions, and underlying skeletal lameness. The practitioner must pay particular attention to the temperament of the individual horse. The most successful trainers modify the affected workout schedule of the horse to minimize stress. For example, horses that prefer to work alone or with company should do so. If a horse is calmer to work when the track is empty, the trainer should exercise it early in the day; others may benefit from a long, slow warm-up to minimize stress. One should make similar stress-reducing modifications for affected horses used in other disciplines.

One should modify the diet to provide adequate but not excessive amounts of calories for the level of work being performed such that a greater proportion is supplied from fats as opposed to carbohydrates. High-calorie diets composed primarily of fat are effective in decreasing postexercise CK activities in affected horses.[189] The optimal fat source and percentage of fat in the diet have yet to be determined, but several commercially available feeds on the market have been found to be useful. The exact mechanism by which increased dietary fats lower CK activity in horses with RER has yet to be determined.

Dantrolene has been used in human medicine to treat and help prevent malignant hyperthermia. In this condition an abnormal release of calcium from the sarcoplasmic reticulum appears to occur, resulting in prolonged contracture, hyperthermia, metabolic and respiratory acidosis, and muscle damage. Dantrolene is thought to decrease the rate of calcium release from the sarcoplasmic reticulum and to affect charge movement in the T tubule system. Dantrolene has been used in human beings to treat chronic exertional rhabdomyolysis.[190] Controlled studies examining the use of dantrolene in RER are being performed. Until these are reported, the guidelines cited next have been shown anecdotally to be efficacious.

Although dantrolene sodium has been suggested for treating equine rhabdomyolysis, the drug is used more commonly in prevention. Giving 2 mg/kg/day diluted in normal saline by a stomach tube for 3 to 5 days and then every third day for a month has been recommended.[65,191] A lower daily dose (300 mg) may be equally

beneficial[191] and perhaps preferable because the drug is hepatotoxic.[65] Another recommendation is 500 mg orally for 3 to 5 days and then 300 mg every thirdday for a time. The time period depends on the circumstances and the effect on the liver, but prolonged treatment is not recommended. Monitoring of hepatic status and function is recommended because the effects on the liver seem to be individually variable. Up to 1 g of dantrolene, given with a small feed 1½ to 2 hours before exercise for 3 to 5 days has been used with apparent success in racehorses in the United Kingdom, but in some cases the horses may have been suffering from overexertion, for example, rather than RER. Such doses have been suggested to be unlikely to reach therapeutic levels. The drug appears not to be as bioavailable in the horse as in human beings and is cleared more quickly.[181] One must take care with dantrolene because its use and efficacy remain uncertain.

9.8.2 POLYSACCHARIDE STORAGE MYOPATHY

PSSM is a recently described and apparently common disease of Quarter Horses and Quarter Horse–related breeds such as the Paint Horse and Appaloosa.[132] The disease is characterized by repeated episodes of exertional rhabdomyolysis that in some cases may be inducedwith little exercise. Increased CK activity in the plasma or serum accompanies episodes of rhabdomyolysis. The most distinctive characteristic of the disease is a mild to severe accumulation of abnormal polysaccharide within the myoplasm. Studies are ongoing to characterize the disorder and to describe a likely candidate gene and hopefully the development of a genetic test.

9.8.2.1 Clinical Signs and Laboratory Findings

Horses with PSSM have frequent episodes of muscle cramping and rhabdomyolysis. Clinical signs and laboratory changes (increased muscle enzymes, myoglobinuria) associated with an episode of PSSM rhabdomyolysis look similar to other causes of rhabdomyolysis. Mild episodes are characterized by a stiff gait, anxiety, and stretching out in stance. More severe episodes are characterizedby anxiety, painful behavior, sweating, and reluctance to move or recumbency. Horses appear to vary in the severity or expression of the disorder. Some horses also appearto tolerate exercise better than others despite similar changes in CK activity after exercise. This may reflect a tolerance of the individual horse to muscular discomfort associated with exercise. Episodes are especially prevalent when exercise intensity increases.

Horses that experience frequent episodes of rhabdomyolysis and have considerable accumulations of abnormal
polysaccharide storage within the muscle fibers may have persistent elevations in serum or plasma CK 485
activity. Whether this represents ongoing subclinical rhabdomyolysis or an alteration in the permeability of the 486
muscle cell membrane is unknown.

Muscle biopsy of horses with a history of chronic exertional rhabdomyolysis is indicated. Horses with PSSM have subsarcolemmal accumulations of an abnormal polysaccharide that is visible with a periodic acid–Schiff stain.

9.8.2.2 Causes and Pathophysiology

Evidence supports that PSSM is heritable.[192,193] In addition, the accumulations of abnormal polysaccharide observed in periodic acid–Schiff stained muscle sections appear to increase with age and therefore may be a secondary feature of the disease. Significant accumulations of abnormal polysaccharide may not be visible until3 years of age in some horses, despite documented intermittent exercise-associated increases in serum and plasma CK activities at a younger age. Affected horses show great variability in expression of the disorder as

reflected in the amount of abnormal polysaccharide observed in muscle sections, desire and ability to exercise, frequency and severity of episodes of rhabdomyolysis, and degree of elevations in CK activity.

The cause of PSSM appears to be related to glucose metabolism as opposed to calcium regulation as has been documented in Thoroughbred horses with RER. Overall, affected horses demonstrate greater total muscle glycogen content compared with control horses. In addition, when fasted, horses with PSSM have consistently lower blood glucose concentrations than healthy controls. When given intravenous or oral glucose, horses with PSSM cleared the glucose from the blood stream at a faster rate than healthy controls. When administered insulin, horses with PSSM became severely hypoglycemic and remained so longer than healthy control horses. Why horseswith PSSM have an increased sensitivity to insulin is unknown. However, this increased sensitivity results in increased uptake of glucose into the muscle cell. When the muscle cell takes up glucose from the blood, glycogen synthase but not glycogen branching enzyme is stimulated. Glycogen synthase is responsible for forming the straight chains of glucose in glycogen, whereas glycogen branching enzyme is responsible for the branching points. The abnormal polysaccharide within the muscle cell appears to be long straight chains of glucose molecules compared with the normal branched configuration of glucose molecules that compose glycogen. Horses with PSSM can break down the abnormal glycogen for energy. The accumulation of abnormal glycogen appears to lie in the fact that with few branches, only a few points exist on which the enzymes responsible for breaking down glycogen can act, coupled with increased formation resulting from the increased cellular uptake of glucose. Horses with PSSM have glycogen branching enzyme with apparently normal activity.

Exercise influences glucose uptake into muscle cells. The physiologic changes associated with exercise result in changes in the number and activity of glucose transporters within the muscle cell membrane. Horses with PSSM have glucose tolerance curves more similar to healthy controls when they are exercised regularly,[194,195] which may explain why regular exercise and maintaining a certain level of fitness appears to help affected horses. Diets high in fat also appear to help these horses and may be related to a less severe postprandial glycemic response and decreased uptake of glucose by muscle cells. The exact cellular defect that results in increased uptake of glucose into the muscle cell is undetermined.

9.8.2.3 Diagnosis

A genetic test has yet to be developed to diagnose horses with PSSM. Therefore diagnosis generally is based on appropriate genetic heritage, repeated bouts of exertional rhabdomyolysis, and the presence of abnormal polysaccharide on a periodic acid–Schiff stained muscle biopsy. Because the accumulation of abnormal polysaccharide appears to be a secondary feature of the disease, accurately diagnosing affected horses until 3 years of age or more may not be possible. Some anecdotal evidence supports that mildly affected horses fed diets low in carbohydrates may accumulate polysaccharide in smaller amounts or more slowly than other affected horses. Details concerning what factors influence the expression of the disorder and the role different diets have on the accumulation of abnormal polysaccharide have yet to be investigated.

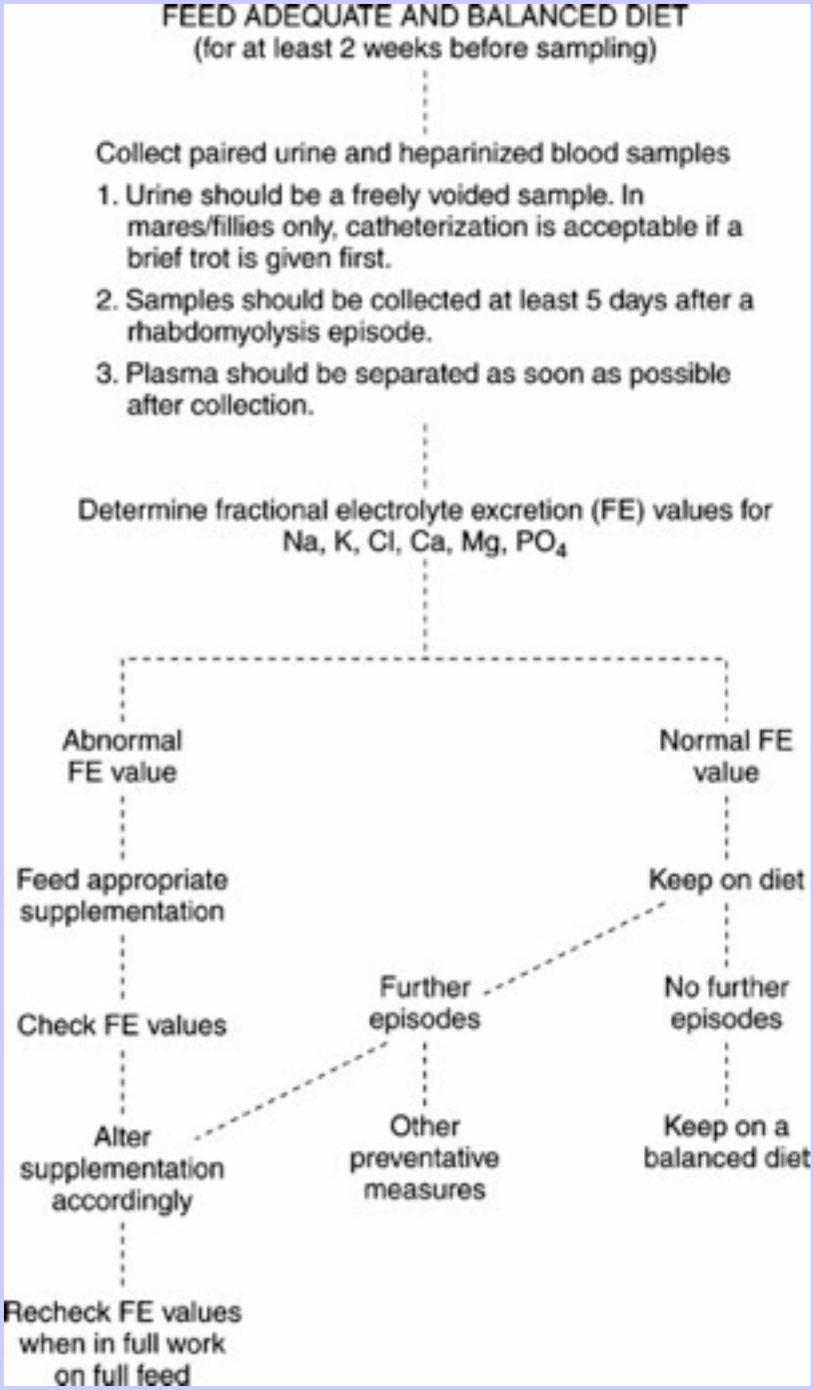
An exercise tolerance test can be a useful diagnostic tool in horses with PSSM. Affected horses commonly experience a doubling or more of baseline CK activity after 15 minutes of trotting on a lunge line. The practitioner draws blood before and 4 to 6 hours after the exercise. The practitioner also should have fractional excretions of electrolytes (Figure 9-4), measurements of serum selenium (or GSHPx activity), and vitamin E concentration determined on horses with PSSM. One should supplement dietary electrolytes and selenium and vitamin E intake if these are abnormal.

9.8.2.4 Treatment

Horses with PSSM have a heritable myopathy that in many horses can be managed readily. Because of the nature of the disorder, the practitioner should counsel owners, trainers, and riders of affected horses that the horse may not be able to perform at elite levels of competition if the condition is severe. However, many horses do perform well and only have occasional episodes of rhabdomyolysis. One should exercise horses regularly because a good level of basal fitness appears to protect against episodes and should increase the 486
exercise intensity slowly. Horses may live in stalls, but living outside where the horse may move around 487
readily appears to lessen the frequency of episodes of rhabdomyolysis.

The practitioner should modify the diet to provide adequate calories for the level of activity of the horse and moreover should limit the intake of carbohydrates. Many horses can do well consuming only a grass or alfalfa hay and a simple vitamin and mineral supplement. If additional calories are necessary, they can be supplied as fats. Many different fat sources are available and include corn or vegetable oils, linseed oil, or rice bran. Commercial grain mixes that are high-fat, low-carbohydrate feeds are available and may be a good source of fat for the horse without the concern associated with other sources of fat. Oils can spoil, and larger quantities of oils or rice bran may not be palatable for some horses.

Figure 9-4 Protocol for using the fractional electrolyte excretion test in clinical cases of the equine rhabdomyolysis syndrome. One should not evaluate the electrolyte statuswithout also evaluating Vitamin E and selenium status.



9.8.3

EQUINE POLYSACCHARIDE STORAGE MYOPATHY

EPSM is the term currently used for a disorder described in Draft-breed horses.[196,197] Although histopathologic abnormalities of muscle biopsy samples are similar with these two disorders, clinical signs and apparent incidence in the two breeds are different.

9.8.3.1

Clinical Signs and Laboratory Findings

EPSM has been described in Draft Horse breeds including Belgians, Percherons, Clydesdales, Shires, Haflinger, Norwegian Fjord, Suffolk, Irish Draft, Draft crosses, and a Draft mule. Depending on the description of the abnormal periodic acid–Schiff positive staining polysaccharide on muscle biopsies, the incidence of EPSM in the general Draft Horse population has been estimated to be 45% to 66%. Whether this form of polysaccharide storage disease occurs in other breeds has been debated.

Clinical signs of EPSM appear to take two forms. Draft Horses with EPSM may exhibit exercise-associated muscle cramping similar to that observed in other types of exertional rhabdomyolysis. Complications from rhabdomyolysis are similar to those described in other horses with severe rhabdomyolysis and include postanesthetic recumbency, renal failure, and laminitis. EPSM is associated with a second clinical syndrome in Draft Horses characterized by progressive poor performance, shivers or a shiverslike gait, progressive muscle wasting, muscle weakness, recumbency, and death (Figure 9-5). Serum or plasma CK activities are increased in horses with EPSM demonstrating either syndrome and vary from mild to severe depending on the degree of rhabdomyolysis.

Figure 9-5 Draft Horse with equine polysaccharide storage myopathy standing with a base-narrow, weak stance.

9.8.3.2
Causes and Pathophysiology

The underlying defect causing EPSM has not been determined. Glucose tolerance tests similar to those reported in Quarter Horses with PSSM have not been performed. Therefore whether the defect in Quarter Horses with PSSM and Draft Horses with EPSM are similar or distinct is unknown. Similarly, detailed familial studies have not been performed. As much as 66% of the Draft Horse population has been described as having abnormal polysaccharide within muscle cells on biopsy. The finding has been described in a referral hospital necropsy population and from muscle biopsies from apparently healthy horses without a history of muscle-related problems. This raises the question of whether Draft Horses as a population are linked closely genetically and therefore a large percent of the population has a similar genetic defect or whether the clinical syndrome results from another cause and the high incidence of abnormal polysaccharide within the muscle fibers is an innocent finding. No other species have been described as having innocent accumulations of polysaccharide intracellularly, so probably the aforementioned explanation is plausible. Further investigation is necessary to define this disorder in Draft Horses more clearly.

9.8.3.3
Diagnosis

Diagnosis of EPSM is based on appropriate genetic heritage, presence of abnormal glycogen accumulation in muscle biopsy specimens, increased serum and plasma muscle enzyme activity, and presence of appropriate clinical signs. Review of muscle biopsies obtained from Draft Horses at postmortem examination suggests that EPSM may affect as many as 45% to 66% of Draft Horses.

9.8.3.4
Treatment

Treatment of Draft Horses with EPSM involves dietary changes, primarily increasing fat intake. Increased fat intake was supported in one study that used spot checks ofCK activity demonstrating a generalized lowering of CK values and a history of fewer episodes of rhabdomyolysis.[196] As with other causes of chronic rhabdomyolysis, the practitioner should optimize the electrolyte, vitamin E, and selenium intake of the horse. Because of the high incidence of abnormal polysaccharide within the muscle, the suggestion has been made that all Draft Horses should consume diets that are low in carbohydrates and highin fats.

9.8.4
IDIOPATHIC CHRONIC EXERTIONAL RHABDOMYOLYSIS

Horses of many breeds and types other than Thoroughbreds, Draft Horses, and Quarter Horses may experience recurrent episodes of exertional rhabdomyolysis. Of special note are Standardbred and Arabian horses. These breeds share some common genetic heritagewith Thoroughbred horses, and individuals with chronic exertional rhabdomyolysis may have muscle biopsy findings consistent with RER. These individual horses may respond favorably to management changes (high-fat diet and regular exercise) recommended for Thoroughbreds with RER. However, muscle contracture testing of affected Standardbred and Arabian horses has not been performed to confirm similar muscle pathophysiology.

Some Warmblood breeds with chronic exertional rhabdomyolysis have abnormal polysaccharide evident within muscle fibers on muscle biopsy. Clinically, these horses resemble Quarter Horses with PSSM as opposed to Draft Horses with EPSM. However, many Warmblood horses share genetic history with Draft breeds. Glucose tolerance testing of Warmblood horses with abnormal polysaccharide has not been performed. Affected

Warmblood horses often are managed with high-fat diets and regular exercise as described for Quarter Horses with PSSM.

Some horses with idiopathic exertional rhabdomyolysis do not respond to management and dietary recommendations described previously. Practitioners should counsel owners of horses that suffer from repeated episodes of rhabdomyolysis despite multiple management changes to decrease the exercise intensity the horse is performing or retire the horse from regular work.

9.8.5 MITOCHONDRIAL MYOPATHY

A small family of horses has been described with a syndrome of severe exercise intolerance. In this family one mare was found to have a deficiency of complex I respiratory chain enzyme. She had severe exercise intolerance and muscle stiffness with extreme lactic acidosis in relationship to the degree of exercise, but without rhabdomyolysis. Muscle biopsy demonstrated large accumulations of mitochondria with bizarre cristae formations. Biochemical analysis revealed low activity of nicotinamide adenine dinucleotide coenzyme Q reductase, the first enzyme complex of the mitochondrial respiratory chain, which resulted in impaired oxidative capacity for exercise and heavy reliance on anaerobic metabolism for muscular energy.[198] Examination of related horses provided evidence that the condition was likely familial.

9.9 Horses Without Underlying Myopathy

9.9.1 OVEREXERTION

Overexertion may be defined as physical exertion to a state of abnormal exhaustion. Many horses willingly work to a point where they are too exhausted to go on. If a horse is worked to a point of exhaustion, especially in an environment of heat and high humidity, multiple organ failure may result. Rhabdomyolysis is a frequent 488
component of overexertion. Rhabdomyolysis may be precipitated from glycogen depletion and electrolyte 489
depletion. Because horse sweat is hypertonic, profound and prolonged exercise leads to significant electrolyte depletion. Lactic acidosis induced by dehydration and anaerobic glycolysis further complicates electrolyte depletion. Laboratory tests typically reveal muscle enzymes 10 to 1000 times greater than normal; depletion of sodium, potassium, and chloride; and dehydration. Muscle biopsy demonstrates acute rhabdomyolysis without evidence of previous rhabdomyolysis. Horses require emergency resuscitative therapy including intravenous fluids and restoration of electrolytes. Horses in anuric or oliguric renal failure may respond favorably to furosemide or dopamine therapy.

The exhausted horse syndrome tends to occur when horses have been pushed past their performance limits. The syndrome is believed to have a complex etiology involving a combination of fluid and electrolyte losses, depletion of energy stores, and extremes of environmental conditions. The syndrome therefore usually occurs in association with Three-Day Events and long-distance rides.[167,188] A brief summary of some of the possible contributing factors follows. More detailed information is available.[169,199–201] The amount of sweat produced by an exercising horse depends on the environmental conditions, nature of the work, and the fitness of the animal. Under favorable climatic conditions, sweat loss can be on the order of 5 to 8 L/hr on long-distance rides. In hot, humid conditions, where sweating is partially ineffective, sweat production can be as high as 10 to 15 L/hr. An endurance horse can lose at least 25 to 30 L or more if conditions are unfavorable, which far exceeds the 1 to5 L lost by a racehorse performing a sprint at top speed. Early on in exercise and when the sweat losses are low, much of the water loss can be made up by absorptionof water from the large intestine. However, if water losses

via sweat are between 5% and 10% of body weight, a decrease in circulatory volume occurs. The decrease tends to occur after about 3 hours of exercise with moderate sweating but occurs much sooner during exercise in high heat and humidity.

Sweat contains low concentrations of calcium and phosphate but is hypertonic to plasma with respect to sodium, potassium, and chloride. During exercise, the horse appears to be able to maintain, initially at least, its plasma electrolyte concentrations at the expense of other body compartments. This is especially true for sodium, for which the contents of the gastrointestinal tract are believed to provide an important reservoir. Decreases in plasma sodium of up to 6 mmol/L occur during endurance rides in hot weather. The decreases are likely to result from significant sodium and water losses followed by partial replacement of the water deficit by drinking. Sodium concentrations may in fact increase in those animals that are not allowed to drink or that refuse to drink.

Potassium losses in sweat are also significant during prolonged exercise. Potassium is lost in sweat and is exchanged for sodium and lost into the gastrointestinal tract. During heavy exercise and sweating, muscle tissue is the major source of potassium replacement. Hypochloremia is observed commonly after endurance rides, reflecting chloride losses in sweat. Chloride is the major anion absorbed in the kidney, and in its absence bicarbonate is resorbed.

The most consistent acid-base alteration associated with endurance in hot environments is metabolic alkalosis, probably related to hypochloremia and hypokalemia caused by heavy sweating, and therefore may not be seen in cooler conditions. Hypocalcemia sometimes has been observed in endurance horses and might result from calcium losses in sweat or from disturbances of calcium homeostasis. These have been suggested to include energy deficiency, alkalosis, or competition with sodium and potassium exchange in the skeleton.

Even with frequent access to water, many endurance horses may develop clinical signs of slight to moderate dehydration during the course of a ride, as well as signs caused by some disturbance in electrolyte status. The combination of dehydration and sodium depletion results in a decreased plasma volume as determined by raised packed cell volume, total protein, and blood viscosityand in turn may result in inadequate tissue perfusion and inefficient oxygen and substrate transport, which can result in impaired renal function. Alterations in acid-base balance plus calcium, magnesium, and potassium depletion may contribute to the development of gastrointestinal tract stasis or ileus, as well as muscle cramps, synchronous diaphragmatic flutter, and rhabdomyolysis. If severe losses of water and electrolytes occur, then sweat production may decrease, resulting in even less effective thermoregulation.

Severe cases of dehydration coupled with electrolyte depletion may result in decreased renal perfusion and oliguria or anuria. Rhabdomyolysis may result from a combination of electrolyte depletion, poor perfusion, and resulting membrane instability. Myoglobinemia in human beings with decreased renal perfusion leads to kidney failure. Myoglobinemia is less common in the horse, possibly because metabolic alkalosis occurs more commonly than metabolic acidosis in heavily exercised horses. If volume depletion is severe enough, metabolic acidosis may occur in the horse because of anaerobic glycosis and may contribute to kidney failure in some horses.

9.9.1.1 Clinical Signs and Laboratory Findings

Box 9-5 shows some of the signs seen in exhausted horses and the associated laboratory findings. Signs may 489
last for several days. Various guidelines have been given in the literature to help distinguish the exhausted 490
horse from the nonexhausted, tired horse. The pulse and respiratory rates may be similar in both cases at the end of an endurance ride, but in the nonexhausted horse the rates tend to return to normal far quicker, for

example, to a heart rate of less than 60 to 70 beats per minute with a respiratory rate of less than 40 per minute within 25to 30 minutes, unless the ambient temperature is high. In the exhausted horse the pulse rate tends to remain greater than the respiratory rate, for example, greater than 70 beats per minute 30 minutes after exercise, with a persistently elevated rectal temperature.[126,188,201]

9.9.1.1.1

BOX 9-5 CLINICAL SIGNS AND LABORATORY FINDINGS IN THE EXHAUSTED HORSE

Clinical Signs*

Depression

Dehydration, anorexia, decreased thirst

Elevated respiratory and heart rates

Raised temperature

Poor sweating response

Poor jugular distention, increased CRT, decreased pulse pressure

Decreased intestinal sounds, laminitis

Muscle cramps

Synchronous diaphragmatic flutter

Laboratory Findings

Metabolic alkalosis

Paradoxical aciduria

Hypokalemia, hyponatremia, hypochloremia

Increase in CK, AST, LDH activities

Proteinuria

Azotemia

Lipidosis

Signs of hepatic failure

CRT, Capillary refill time; *CK*, creatine kinase; *AST*, aspartate aminotransferase; *LDH*, lactic dehydrogenase.

* Not all signs are present in all cases.

9.9.1.2 Treatment

Horses that appear depressed with persistently elevated heart and respiratory rates may respond to rest, cooling out, and access to salt, feed, and water. If one sees no improvement within 30 minutes, the horse often needs fluid therapy. In the more severe case of a horse that refuses to eat or drink, the horse needs prompt and vigorous fluid therapy. Fluids restore circulating blood volume, correct electrolyte deficits, and provide a source of readily metabolizable energy. One may administer fluids orally or intravenously. One can give 5 to 8 L orally every 30 minutes to 1 hour, if required, but one should stop if any discomfort or gastric reflux becomes apparent. One should avoid hypertonic solutions given orally. In severely affected animals, one can give up to 10 to20 L/hr of fluids intravenously; in addition, insertinga stomach tube may be necessary. One can use saline or Ringer's solution with potassium chloride added to provide approximately 10 mEq/L. After the horse starts eating hay, one may discontinue potassium supplementation. One can administer glucose as 5% dextrose saline, or one can add 50% dextrose to Ringer's solution to deliver between 50 and 100 g/hr (concurrent insulin administrationhas been recommended). Slow administration of calcium also may be of value. Sodium bicarbonate administration is not indicated, however, because acidosis, if present, resolves with fluid volume expansion and is contraindicated if the horse is in a state of metabolic alkalosis. The horse may require other therapeutic agents to control pain and anxiety, although one should use the NSAIDs and phenothiazine derivatives with extreme caution in the face of dehydration (low blood pressure) and possible renal dysfunction. Avoiding corticosteroid administration has been recommended unless absolutely necessary.

One can use cold water body sprays and alcohol baths, particularly over the head and neck region, to cool the patient. A common myth is that ice water bathing may lead to muscle cramps. However, whole body ice water bathing and cold water spray fans have been used successfullyin major international events under hot and humid conditions without adverse effects. In recumbent, hyperthermic patients, one should give cold water enemas delivered via a handheld gravity-feed rectal tube. Further information concerning exercise physiology under environmental conditions of high heat and humidity is available.[167,199–202]

9.9.1.3 Prevention

Many endurance competitors train their endurance horses to drink electrolyte-rich water. This may help partially to restore electrolyte concentrations in addition to water during a race. However, plain water always should be available as an alternative. The proper amount of water intake during competition is unknown because the maximum absorption of water from the gastrointestinal tract is unknown, considering that exercise draws circulation away from the intestine. The amount of water contained in the intestinal tract depends on the amount of fiber contained therein. Research is ongoing to determine the optimum combination and time of feeding of grains, forage, electrolyte supplements, and water before and during long-distance competition. These findings also vary depending on the type of competition (endurance versus Three-Day Event) and length of course (10, 50, or 100 miles). 490

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Various suggestions have been made as to the optimal nature of any electrolyte supplement. Supplements containing from 1 to 4 times more sodium than potassium have been recommended. Sodium bicarbonate often has been suggested as part of an electrolyte mix, but because most endurance horses tend to become alkalotic, this in fact may be counterproductive and is not recommended. Additional sodium and potassium chloride supplementation for a few days after competition also may be helpful in replenishing body stores but often is unnecessary if the basic diet is acceptable.[200,201,203]

Many workers have reported beneficial effects from the oral or parenteral administration of vitamin E and selenium.[140,204] The beneficial effects of these compounds have been noted as often being difficult to assess because they often are given in addition to changesin diet and exercise.[205] Possibly any effect is caused by limitation/prevention of free radical–induced muscle damage. Oral administration is preferable to intermittent injection. The sections on equine motor neuron disease and white muscle disease give additional information concerning vitamin E and selenium deficiency.

9.9.2 ELECTROLYTE DEPLETION

Alterations in the intracellular or extracellular concentrations of electrolytes can occur in response to dietary deficiency and sweat losses or as a normal response to high-intensity exercise. The best way to assess the total body level of electrolytes is often to use fractional urinary excretions.[105,206,207] Table 9-2 lists recommendations for correcting abnormal dietary electrolytes. For example, in endurance exercise, horses may lose significant amounts of sodium, potassium, and chloride in sweat, which may contribute to fatigue, exertional rhabdomyolysis and synchronous diaphragmatic flutter. During anaerobic exercise, cellular acidosis occurs because of incomplete oxidation of pyruvate and the resulting accumulation of lactate. Volume depletion and poor circulation to muscle exacerbate acidosis. Systemic acidosis increases theserum fraction of ionized calcium and induces extrusion of potassium from muscle cells,[208] which may lead to changes in the action potential across the sarcolemma.

TABLE 9-2 Examples of Supplementation Given to 400- to 500-kg Horses Based on Fractional Electrolyte (FE) Excretion Test Results

FE VALUES	SUPPLEMENTATION
Low Na^+	2 oz (56 g)/day NaCl
Low Na^+ and K^+	2 oz (56 g) NaCl plus 10 oz (28 g) KCl/day for 2 weeks, then 2 oz (56 g) NaCl/day
High PO_4	Decrease bran intake if horse is fed, plus 2 oz (56 g) $CaCO_3$/day or alternative source(s) of calcium
High Na^+	Lower Na^+ intake (e.g., change source of hay if grown near the coast)
	Normal values: $FE_{Na}{}^+$ = 0.04–0.8; $FE_K{}^+$ = 35–80; FE_{PO4} = 0–0.2

Data from Harris P, Colles C: The use of creatine clearance ratios in the prevention of equine rhabdomyolysis: a report of four cases, *Equine Vet J* 20:459, 1988.

Aside from its role intracellularly in Mg^{2+} ATP, extracellular magnesium influences muscle cell function. Extracellular magnesium concentration is inversely proportional to acetylcholine concentration at the neuromuscular junction; therefore small changes in the serum concentration of magnesium reflect proportionately large shifts in whole body magnesium levels. Low blood levels of magnesium lead to increased membrane irritability and dysfunction of many enzymes necessary for protein, carbohydrate, and lipid metabolism. The most dramatic example of hypomagnesemia is grass tetany, which occurs in cattle grazing

magnesium-deficient pasture.[209] Whole body calcium balance is also important in muscular function, and hypocalcemia can be life threatening in most species. In horses, hypocalcemic tetany may be associated with heavy lactation, intestinal disorders, endurance exercise, and malnutrition.[210]

Sodium, chloride, potassium, calcium, and magnesium play key roles in muscle fiber contractility and irritability. Therefore small imbalances may lead to muscle dysfunction and possibly exertional rhabdomyolysis. Diagnosis of whole body electrolyte deficits can be difficult to confirm because of the rigorous homeostatic mechanisms in place that serve to maintain serum concentrations. Therefore a lack of correlation exists between intracellular ion concentrations and serum ion concentrations. In addition, electrolyte balance may shift in the body as a direct response to disease processes. For example, a whole body deficit of potassium may exist, but plasma potassium may be normal because of efflux of potassium from damaged or acidotic muscle.[65]

Kinslow, Harris, Gray, et al.[211] performed one study examining the role of total body electrolyte balanceand its effect on exertional rhabdomyolysis in 1995. The authors followed four mature Thoroughbred mares through their estrous cycles. Group mean percent fractional excretion of electrolytes in urine showed cyclical trends that appeared to fluctuate in relation to the stage of the estrous cycle. They saw large increases in the urinary excretion of sodium, calcium, and magnesium in samples collected during the period from 2 days preceding to 4 days following estrus. The authors found significant correlations in individual animals between low progesterone 491
concentrations and 24-hour fractional excretions of sodium, potassium, chloride, calcium, and phosphorus and 492
between serum estradiol 17β and calcium, magnesium, and chloride. These results suggest that ovarian steroid hormones may exert an influenceon urinary excretion of electrolytes in mares. Therefore estrous cycle–induced losses of sodium, calcium and magnesium in mares consuming a diet marginal or deficient in electrolytes could predispose them to exertional rhabdomyolysis.

To examine the effect of total body potassium on exertional rhabdomyolysis, Bain and Merritt[212] measured potassium concentrations within red blood cells and plasma in Thoroughbred horses with and without exertional rhabdomyolysis. The authors found that in fillies with exertional rhabdomyolysis, potassium concentration within red blood cells was lower than that of controls within 48 hours after an episode of exertional rhabdomyolysis. Whether decreased red blood cell potassium is an inciting cause of exertional rhabdomyolysis or results from exertional rhabdomyolysis is unknown. Beech and Lindborg[213] did not repeat these findings; they foundno difference in red blood cell potassium concentrationin horses with and without exertional rhabdomyolysis. However, the authors did find a significantly lower concentration of muscle potassium on a dry weight basis in horses with exertional rhabdomyolysis. Unfortunately, lower concentrations of muscle potassium did not correlate with the histologic appearance of the muscle. The authors concluded that low red blood cell potassium concentration may not be a valid indicator of reduced muscle potassium, and they did not associate potassium concentration with a predisposition for exertional rhabdomyolysis.

A study using potassium-depleted horses further clouds the role of potassium in exertional rhabdomyolysis.[214] In this study, the authors depleted healthy horses of potassium by withdrawing feed and by administering sodium bicarbonate and furosemide. The result was decreased plasma potassium (2.9 ± 0.3 mEq/L, treatment group; 3.3 ± 0.5 mEq/L, control), chloride, calcium, and magnesium compared with control horses. Red blood cell potassium concentration remained unchanged. A significant increase in CK activity occurred in the potassium-depleted horses compared with controls 240 minutes after exercise (296 ± 211 U/L, potassium depleted;86 ± 20 U/L, control). After exercise, three of the six horses developed diaphragmatic flutter, and two horses had CK activity beyond the normal range (685 U/L and 1374 U/L), and one horse was reported to walk with a stilted gait after exercise. The authors postulated that potassium depletion may play a role in exercise-induced muscle damage but could not conclude that it was the sole cause of increased serum CK activity. Unfortunately, a

similar study has not been performed in horses with underlying myopathies such as RER or PSSM to determine if potassium depletion influences rhabdomyolysis in a susceptible population.

Harris and Snow[215] also investigated the role of electrolyte imbalance in the pathophysiology of exertional rhabdomyolysis in 1991. The authors found that 100 of 144 Thoroughbred horses with exertional rhabdomyolysis had fractional excretions of electrolytes outside the normal range. Of those horses with abnormal fractional excretions, 72 of 100 responded favorably to additional electrolytes in their diet. However, the normal ranges for fractional excretions used in this study were more narrow than other published normal ranges, and other investigators have not duplicated the authors' results.[216] The intermittent nature of exertional rhabdomyolysis in racehorses also makes the verification of a real response to therapy difficult to confirm.

One must consider the relative concentration of electrolytes in the diet when examining their influence on exertional rhabdomyolysis. Poorly balanced electrolyte supplements may exacerbate dietary deficiencies of other ions. For example, high concentrations of serum potassium may aggravate the effects of low serum calcium, resulting in muscular hyperactivity.[217] Such increased levels of serum potassium are found during normal exercise. In 1998 Harris and Colles[218] described a case study that may support this theory. In this study, low dietary calcium and increased phosphate excretion were found in two of four horses experiencing exertional rhabdomyolysis. Urinary clearance ratios were low for potassium in one case and low for sodium in another. All horses were reported to improve with dietary changes, according to the investigators.

Electrolyte balance within the body and interactions between the major electrolytes play important roles in normal muscular function. In healthy horses, imbalances and deficiencies may occasionally cause muscular dysfunction. The fractional electrolyte test is the best test available to assess electrolyte state but must be applied and interpreted with care,[219] and the different feeding practices among countries may mean that electrolyte supplementation based on the fractional electrolytetest may have more or less relevance. In horses predisposed to exertional rhabdomyolysis, identical imbalances or deficiencies may lead to more severe rhabdomyolysis because of preexisting abnormalities in muscular function.

9.10 Horses With Altered Gait

Many abnormalities can affect the gait of horses. These problems may be related to musculoskeletal, cardiovascular, neurologic, or metabolic abnormalities. This section discusses muscle diseases that may result in altered gait as the presenting complaint. 492

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9.10.1 ACUTE MUSCLE INJURY

9.10.1.1 Gastrocnemius Muscle Rupture

Rupture of the gastrocnemius muscle occurs in horses attempting to get up after a long period of recumbency (e.g., after anesthesia or postpartum paralysis). Rupture also occurs in animals that rear and fall over backward and occurs occasionally following overextension. In foals, ruptures have been reported following the first attempt of the foal to rise, especially if the foal has poor muscle tone or coordination. The condition also occurs in foals after an assisted delivery (dystocia) and in foals following manual attempts to straighten a fixed tibiotarsal joint. A rupture occurring in the tendon of insertion may result in avulsion of a portion of the tuber calcanei. If rupture occurs in the muscle belly, a hematoma may formand may calcify. The condition can occur unilaterally or bilaterally; however, the animal will be unable to standif rupture is bilateral or if complete

Achilles' tendon rupture occurs. The affected hock(s) may be flexed excessively because of the loss of the extensor influence of the gastrocnemius, giving the horse a squatting appearance. Heat, swelling, and pain are normally evident in the initial stages. The plasma muscle enzyme activities also tend to be elevated, at least initially.[220,221] Treatment involving support in a sling with the affected limb immobilized in an extended position has been recommended,[220] but the prognosis is guarded for a return to normal function.

9.10.1.2 Serratus Ventralis Rupture

Rupture of the serratus ventralis is rare and occurs as a consequence of dorsal impact trauma over the withers and neck region or after jumping a high fence or jumping off a raised platform. Animals usually are affected bilaterally. The thorax drops between the paired scapulae so that the dorsal borders are above the thoracic spinous processes. The croup is often higher than the withers. The condition is painful, and radiographs are needed to eliminate the possibility of dorsal spinous process fracture. The prognosis is poor. Recommended treatment is a prolonged period in a sling if the horse is suited temperamentally to such a restriction.[222]

9.10.1.3 Sore and Pulled Muscles

Pulled muscles (i.e., muscle tears or strains resulting in some disruption of muscle architecture and occasionally the formation of a hematoma) often are presented clinically during or immediately following exercise. Depending on the extent of the trauma and the muscle groups involved, obvious swelling and apparent pain on palpation may or may not be present. Plasma CK and AST activities may or may not be elevated significantly. Definitive diagnosis may be difficult, especially in chronic cases. Faradic stimulation, scintigraphy, thermography, and diagnostic ultrasound are some of the more common techniques used to diagnose a pulled muscle. Treatment tends to be a combination of rest and physiotherapy (e.g., manipulation, faradism, and ultrasound). Perhaps one should differentiate pulled muscles from the overexerted or sore muscle in which the associated damage tends to be at the cellular level involving structural damage of the contractile elements. Delayed onset muscular soreness (DOMS) is a commonly recognized condition in human beings. The soreness tends to increase in intensity over the initial 24 hours after exercise and peaks around 24 to 72 hours, hence its name. Whether DOMS occurs in the horse is not certain, although the diagnosis has been suggested when a stiff gait, palpable soreness, and a reluctance to move, as well as increased hydroxyproline concentrations, are observed 24 hours after exercise. Free radicals may be involved in the pathophysiology of acute and delayed onset postexercise muscular soreness. Treatment of DOMS in human beings varies from rest to light exercise with or without analgesia and the external application of heat. In the horse continued exercise may be contraindicated if the horse has experienced significant exertional rhabdomyolysis as diagnosed by moderate to high CK activity and in horses with an obvious muscle tear as indicated by the presence of heat, pain, swelling, and abnormal gait. In human beings the best prevention for DOMS is moderate daily increases in the degree of training.

9.10.2 CHRONIC MUSCLE INJURY: FIBROTIC MYOPATHY

Fibrotic myopathy tends to be a chronic, progressive disorder and may occur following excessive exercise over a long period of time that has resulted in the repeated tearing and stretching of muscle fibers.[59] Fibrotic myopathy also has been observed in Quarter Horses and stock horses following maneuvers such as sliding stops, in which the large thigh muscles are contracting while the stifle and hock are extending,[126,188] and in horses tied by a halter and shank and that pull back and fight strongly. Fibrotic myopathy also has been described as congenital, perhaps caused by periparturient trauma,[223] and following intramuscular injection.[224] The semitendinosus

muscle is affected most frequently, but adhesions to the semimembranosus and biceps femoris are common. Myopathy also may involve the gracilis or biceps brachii muscles.

The altered gait seen in horses with fibrotic myopathy of the hamstring muscles is characteristic. The horse walks with a shortened anterior phase of stride and then snaps the foot to the ground with a slap. The pathophysiologic process appears to involve trauma (external or work-related) followed by inflammation, muscle fiber atrophy, and replacement by fibrous tissue. Occasionally, mineral deposits form in the affected tissues, in which case the condition has been referred to as an ossifying myopathy.[224] The characteristic gait likely results 493
from a combination of scar tissue preventing normal extension and contraction of the muscle belly and possibly 494
an altered γ-efferent loop with an abnormal setting of the muscle spindle trigger, which may allow the early unchecked contraction of caudal thigh muscles during the late swing phase of the stride.[56]

9.10.2.1 Clinical Signs

Fibrotic myopathy has been described as a nonpainful mechanical lameness associated with a distinct gait abnormality. The condition can be unilateral or bilateral, and the horse pulls the affected limb(s) back and down before the end of the protraction phase so that the foot slams down, resulting in a louder sound on impact than that of an unaffected limb and in a shortened cranial or swing phase and a lengthened weight-bearing phase,[223] which has been referred to as a goose-stepping gait. The condition tends to be most obvious at the walk. If the condition involves the biceps brachii muscle, the horse shows a shortened cranial phase of the thoracic limb with a tendency for the foot to land at the toe in a way similar to those animals affected by navicular syndrome. Occasionally, one can identify the area of muscle damage because of a dimpling or depression in the skin overlying the muscle. In cases in which significant fibrous tissueor calcification of the muscle occurs, the site is readily palpable. The lameness tends to be unresponsive to routine antiinflammatory therapy.

9.10.2.2 Diagnosis

Diagnosis of fibrotic myopathy is based on clinical signs and history. In one report, two horses with congenital fibrotic myopathy did not have palpable thickening of the muscle or tendon and had no evidence of scar formation.[225] Histologically, one may observe a band of dense collagenous connective tissue or irregular, jagged pieces of mineral. Diagnostic ultrasound has been useful in some cases to confirm the diagnosis and to determine whether mineralization is present in addition to the fibrosis.

Fibrotic myopathy may be distinguished from stringhalt by closely observing the gait. In horses with stringhalt, the rear limb hyperflexes during the cranial or swing phase and a stepwise caudal jerking movement does not occur just before the foot hits the ground.[188]

9.10.2.3 Treatment and Prognosis

Surgical treatments described for fibrotic myopathy include resection of the fibrotic band, semitendinosus myotenectomy, and simplified semitendinosus tenotomy.[109,224,225] Some improvement in gait may occur in some horses after surgery, but complete absence of lameness is an unlikely result. Long-term prognosis is poor because the condition tends to recur after surgery. Postoperative problems with wound healing are common.[224] Transection of the semitendinosus tendon insertion on the tibia distal to the myotendonous junction has been said to resultin much less postoperative trauma, which may help to decrease the potential for recurrence,

[225] and full recovery has been reported anecdotally in a few cases. Performing a less radical excision and combining this with passive postoperative flexion-and-extension physiotherapy therefore may be preferable. Surgery is unlikely to be of value if the biceps brachii is involved.[224]

9.11 Disorders Characterized by Weakness

Disorders discussed in this section have in common a major clinical presentation of intermittent or continuous weakness. The major differential diagnoses for weakness include a variety of lower and upper motor neuron disorders. Hyperkalemic periodic paralysis, myotonia congenita, and myotonia dystrophica are characterized by an alteration in electric conduction across the muscle cell membrane. Equine motor neuron disease results from chronic vitamin E deficiency and consequential long-term oxidative stress. PSSM of Draft-breed horses may be characterized by progressive weakness and is discussed under the heading of disorders characterized by muscle cramping and elevated CK activity.

9.11.1 HYPERKALEMIC PERIODIC PARALYSIS

In human beings, three types of periodic paralysis are described: hypokalemic, hyperkalemic, and normokalemic. These disorders are caused by abnormalities in membrane permeability or a defective cation pump, resulting in an altered electrochemical gradient across the sarcolemma. The alteration in turn results in a change in resting membrane potential and threshold potential, resulting in a muscle fiber that is more or less excitable.[6] Together these disorders are classified as paramyotonias.

9.11.1.1 Clinical Signs and Laboratory Findings

In horses, hyperkalemic periodic paralysis (HYPP) occurs in Quarter Horses, Paint Horses, Appaloosas, and other horses carrying bloodlines that trace back to the sire Impressive. Affected horses tend to be well muscled. Because of this phenotype the defect has been postulated to confer or to be linked closely with other genes that are associated with a heavily muscled phenotype. Between episodes horses appear to be clinically normal and can be highly successful show horses.[226] In fact, many breeders believe that being a heterozygote greatly enhances the physique of horses and is therefore necessary to compete in the show ring. Scientific research confirming or refuting this has not been performed.

Prolapse of the membrana nictitans may be the initial sign at the onset of an episode of HYPP with or without 494
facial muscle spasm and generalized muscle tension. One may observe sustained contraction of the muscles of 495
the muzzle and drooling. Heart rate and respiratory rate are often normal or only slightly increased. Whole body sweating often has been reported. Muscle fasciculations are common, especially in the shoulders, flanks, and neck area. One may observe myotonia (sustained muscle contraction in response to percussion).[25,226,227] Although the horse may remain standing, it may not be able to lift its head and may show intermittent buckling at the knees and hocks. In mild cases, however, the only signs observed may be mild muscle fasciculations or twitching similarto signs of shivering. Recumbency may occur in some horses with diminished tendon reflexes, although affected animals tend to remain conscious and alert. Duration of episodes is usually short (20 minutes to 4 hours, typically 30 minutes to 1 hour). Death can occur during an episode, usually from cardiac arrest or respiratory failure.

Some horses demonstrate altered vocalization and audible respiratory stridor, which may indicate laryngeal spasm or paralysis.[228] In a survey of 69 homozygous horses, more than 90% were reported to suffer some

degree of abnormal airway noise, often between episodes, which tended to be an inspiratory stridor and was continuous in 21% of those evaluated. Exercise, excitement, and stress were possible triggering factors. Upper airway endoscopy of 24 horses revealed 9 with pharyngeal collapse, 10 with laryngeal spasm, 9 with pharyngeal edema, and 6 with a displaced soft palate. Clinically, homozygous affected animals tend to show exercise intolerance, whereas heterozygous animals may tolerate exercise well.[229]

In affected horses, one usually observes clinical episodes before 3 years of age.[230] However, a wide variation in clinical expression of the condition occurs (incomplete penetrance of the gene), with some affected animals showing minimal clinical signs. The variability in clinical signs may result from higher expression of mutant channels in the muscle of horses with symptoms than in those that are asymptomatic. Although variability in the severity of disease occurs between horses, in general, homozygous animals tend to have more severe clinical signs compared with heterozygotes. Muscle fiber diameter or fiber type is related to clinical expression. Management factors such as diet (high versus low potassium intake) and environmental stress may be more influential in the expression of the disorder.[229]

Between episodes, serum potassium concentration is usually within normal limits but may be increased slightly. During an episode, serum potassium concentration usually is increased (5.0 to 11.7 mmol/L),[231] although episodes without associated hyperkalemia have been reported.[232,233] Therefore the absence of hyperkalemia during an episode does not preclude a diagnosis of HYPP. Sodium and calcium concentrations may be decreased and hemoconcentration also may be observed during an episode. Serum CK and AST activities may be normal or mildly increased.[188,226,227] Recovery from an episode is associated with a decrease in serum potassium concentration.

Acute death from hyperkalemia-induced cardiac standstill may occur and is more likely in horses that are homozygous or poorly managed. Poor management may include feeding of diets high in potassium and irregular feeding, traveling, or strenuous exercise schedules.

9.11.1.2 Causes and Pathophysiology

HYPP in human beings is inherited as an autosomal dominant trait and has been studied extensively, as has paramyotonia congenita. To date, only HYPP has been diagnosed in horses, and the disorder also appears to be inherited as an autosomal dominant trait.[229,231] In human beings and horses, HYPP is characterized by intermittent attacks of weakness or paralysis, which are precipitated by a number of factors, including potassium intake, fasting, cold, and rest after exercise. Paramyotonia congenita typically presents as a cold-induced myotonia. Both conditions have been linked to the human adult skeletal muscle sodium gene on chromosome 17q. The genetic mutation responsible for HYPP in human beings affects a gene at the SCN4A locus that encodes the α-subunit of the adult human skeletal muscle voltage-dependent sodium channel.[235] In the horse the mutation is a phenylalanine to leucine mutation in the transmembrane domain IVS3 of the α-subunit of the sodium channel.[236] In affected individuals an increase in membrane sodium conductance occurs because of the defective subpopulation of the voltage-dependent sodium channels failing to inactivate and remaining open or repeatedly opening. A small rise in the serum potassium concentration because of clinical variation, ingestion of potassium, or muscular activity may trigger a further increase in membrane sodium conductance, depolarization of the muscle membrane, and movement of potassium out of the muscle cells. As the membrane depolarization develops, the membrane initially becomes hyperexcitable and shows myotonic behavior. With further depolarization of the muscle, the muscle cell membrane becomes unexcitable and paralysis occurs.[235,237] In Quarter Horses, all affected horses can trace their lineage to a stallion named

Impressive.[236,238] For this reason the disorder occasionally is referred to as Impressive disease. Whether the mutation in Impressive was a spontaneous mutation or was inherited is unknown. The mutation has become common in the Quarter Horse population because of the success of Impressive and his offspring in the show arena and the consequential extensive and long breeding career of Impressive. Of 20,000 plus samples collected between 1992 and 1995 approximately 63% were homozygous normal (N/N), 36%were 495
heterozygous affected (N/H), and 1% were homozygous affected (H/H).[229] These data translate to a gene- 496
positive frequency of 4.4% of the Quarter Horse population.

Plasma potassium concentrations of normal horses undergoing intensive exercise may reach the levels recorded during an episode of HYPP without any associated clinical signs,[105] which suggests that increased plasma potassium concentration alone is not responsible for the clinical signs.

9.11.1.3 Histologic and Muscular Characteristics

In human beings, periodic paralysis conditions sometimes are referred to as vacuolar myopathies. The vacuoles, visible on light microscopy, arise from coalescence of dilated components of sarcoplasmic reticulum, fusionof T-system networks, or focal fiber destruction.[239] In the horse, no abnormalities are found on light microscopy.[227,231]

Affected animals have a lower intracellular potassium concentration and a higher intracellular water volume than normal horses. Using whole-fiber intercostal muscle biopsies, the mean resting membrane potential of five affected horses was significantly closer to threshold potential compared with unaffected horses.[231] This may indicate a defect in membrane transport.

No difference is apparent between muscle fiber type distribution and fiber diameter between horses with HYPP and those without. However, the genetic defect may alter other less measurable muscle traits such as tone, which is why it appears that breeders and judges prefer affected horses over unaffected ones.

9.11.1.4 Electromyography

Numerous EMG abnormalities are apparent in HYPP horses at rest and during an episode.[231–233] Complex repetitive discharges are the most consistent abnormal finding, although one may observe myotonic potentials, fibrillation potentials, and positive sharp waves as well. The amount of abnormal spontaneous EMG activity can fluctuate substantially on repeated examinations.

9.11.1.5 Diagnosis

The most accurate way to confirm a diagnosis of HYPP is a genetic test using DNA extracted from equine hairor whole blood samples. The American Quarter Horse Association recognizes results of HYPP genetic tests that are performed through a licensed laboratory. The association can be contacted through their Web site at *http://www.aqha.com*. Tests are reported as homozygous normal (N/N), heterozygous (N/H), and homozygous affected (H/H). Before the development of the genetic test, a potassium challenge test was recommended to identify affected horses. This test has substantial potentially adverse affects and is no longer recommended. If a Quarter Horse dies acutely and one suspects HYPP, one may collect hair samples at postmortem for DNA testing. Increased potassium concentrations in aqueous humor samples support a diagnosis of hyperkalemia at the time of death.

Differential diagnoses for HYPP include other causes of collapse such as syncope, narcolepsy or cataplexy, and seizures, as well as other electrolyte disorders, neurologic dysfunction, and vitamin E– and selenium-responsive myopathy.[188]

9.11.1.6 Treatment

Recommended treatment for horses with acute episodes of HYPP varies depending on the severity of clinical signs. Mildly affected horses (i.e., nonrecumbent but with muscle fasciculations) may be exercised lightly. Caution is advised, because collapse is a potential risk in these horses. One may feed the horse a readily absorbable source of carbohydrate (oats or light Karo syrup) to promote insulin-induced reuptake of potassium. Acetazolamide at 3 mg/kg orally also may be beneficial. Such procedures often are undertaken routinely by responsible and experienced owners of affected animals.

During more severe episodes, especially when recumbency occurs, one may administer 5% dextrose with sodium bicarbonate (with or without insulin) to decrease serum potassium concentration. Slow intravenous administration of 23% calcium gluconate at 0.2 to 0.4 ml/kg diluted in 1 to 2 L of 5% dextrose (5% dextrose at 4.4 to 6.6 ml/kg) or bicarbonate at 1 to 2 mEq/kg have been recommended for treatment of acute, severe episodes of HYPP.[226,227,231,239] If required, one may administer potassium-free isotonic fluids.

Glucocorticoids may be contraindicated in susceptible horses because they induce episodes in human beings with similar disorders.[226] Inhalation of β-adrenergic agents has aborted acute attacks in human beings, but the use of such drugs has not been reported in HYPP-affected horses.[239,240]

9.11.1.7 Prognosis

In human beings with HYPP the prognosis for normal activity is considered good, although a permanent myopathy and weakness may occur. This also seems to be the case in the horse. In at least one horse the condition progressed, necessitating euthanasia.[226] A recent report suggested that “the chances of a paralytic episode occurring while the horse is being ridden appear unlikely. However, because episodes of paralysis are unpredictable, we recommend that only persons experienced with the symptomatology handle and ride affected horses and to use caution if any abnormal clinical signs are observed.”[229] Thousands of carrier horses are handled, ridden, and shown daily throughout the United States and elsewhere with little to no apparent danger to human beings. 496

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9.11.1.8 Prevention

In horses, acetazolamide has been recommended as a daily medication to decrease the frequency and the severity of HYPP episodes. The recommended dose is 2 to 2.2 mg/kg orally every 12 hours for maintenance.[226,231] Hydrochlorothiazide also has been used.[227] One should feed affected horses oats or timothy hay rather than alfalfa, to decrease total potassium intake. Feeding grain 2 or more times a day, providing access to white salt (without potassium chloride added), and regular mild exercise also may be beneficial. One should establish a regular feeding and exercise schedule and should avoid rapid changes in diet, fasting, and water deprivation. Turnout to pasture or paddock may be beneficial.

In human beings, preventive therapy consists of frequent meals with a high carbohydrate content, avoidance of fasting or of exposure to cold or overexertion, and the use of diuretics such as acetazolamide or chlorothiazide, which promote potassium wasting in the urine. Small doses of albuterol, a β-adrenergic agent, also have been recommended in human beings.[240] Albuterol is believed to work by stimulating sodium and potassium ion pumps, enhancing potassium transport across the muscle cell membrane. Tocainide, a frequency-dependent antiarrhythmic drug, may prevent the weakness caused by the myotonia.[239]

Figure 9-6 Prominent muscle groups of the hindquarters and a knot of sustained muscular contraction following mechanical stimulation in a foal suffering from clinical myotonia.

9.11.2 MYOTONIA CONGENITA AND DYSTROPHICA

Like HYPP, myotonia congenita and myotonia dystrophica are characterized by abnormal electric conduction across the muscle cell membrane.[241,242] *Myotonia* is defined as the "delayed relaxation of skeletal muscle after a voluntary contraction or a contraction induced by an electric or mechanical stimulus."[8] Two forms of myotonia congenita are described in human beings, Thomsen's disease and Becker myotonia. Both result from chloride channel mutations. Thomsen's disease is inherited as a dominant autosomal trait; Becker myotonia is inherited as a recessive trait.[8,242]

In the horse, no membrane chloride defect has been identified. As in human beings, at least one form of the condition in horses persists in the face of neuromuscular blockade.[243,244] Only a few horses with myotonia have been described. Affected foals have a severely abnormal gait at birth and usually are euthanized. Because of the small number of affected horses described, whether myotonia in this species is heritable or results from spontaneous point mutations is unknown. To date, a complete description of clinical, histopathologic, and electrophysiologic findings in horses with myotonia congenita or myotonia dystrophica is not available.

9.11.2.1 Clinical Signs and Laboratory Findings

Stiffness of gait and prolonged contraction of affected muscle after local mechanical stimulation (finger flick) are the hallmarks of clinical myotonia (Figures 9-6, 9-7, and 9-8). Clinical presentation of horses with 497
myotonia varies.[188,245–249] One horse exhibited presence of myotonic discharges on EMG with no overt 498
primary muscular problems. This presentation is observed in Quarter Horses and does not appear to be progressive.

Figure 9-7 A young horse with myotonia. The heavy muscling of the hindquarters with atrophy of the distal limb and neck musculature are notable.

Figure 9-8 Prolonged myotonic discharges present on needle electromyographic examination. These high-frequency discharges sound much like a revving motorcycle but have no true waxing and waning. Instead the amplitude and frequency of the discharges change suddenly (as shown), altering the tone heard. Under general anethesia, suxamethonium or tubocurarine do not abolish these discharges.

A second myotonic syndrome in horses is characterized by early onset of progressive deterioration in muscle function. Signs are usually apparent within the first 6 months of life. Initially, lameness associated with stiffness and hypertrophy of the affected muscles may occur. The lack of fluid, smooth movement is most pronounced after rest and diminishes with exercise. In most affected horses the abnormality is confined to the hindquarters, although all four limbs may be involved.[249,250] No other body systems are affected. In many cases the horses become progressively weaker, although the clinical course may vary. On the basis of clinical, electrophysiologic, and pathologic findings,[246] this form of myotonia in horses has been suggested most to resemble human myotonia congenita or an undefined myotonia.[213]

A third, severe, and progressive form of myotonia reported in young horses is characterized by onset of clinical signs as early as 1 month of age.[249] Initially, horses exhibit generalized myotonia with hypertonicity of the larger proximal limb muscles, progressing rapidly to muscle stiffness, atrophy, and weakness. In one report, the authors also observed testicular hypoplasia, early cataract formation, and mild glucose intolerance, suggesting multisystemic involvement. This myotonic dystrophy–like disorder is associated with specific histologic changes.[213,249]

Serum CK and AST activities may be elevated in horses with myotonia, but these increases often are not sustained throughout the course of the disease and tend to be unpredictable.

Not all muscles that exhibit classic myotonic discharges (see the diagnosis section) show histologic changes. Fiber size variation, changes in number and size of the individual fiber types, increased numbers of central nuclei, and clustering together of fiber types with signs of necrosis and degeneration have been reported.[6,246,251] Differences in the histologic findings may reflect different forms of the condition.[246] Dystrophic changes and a normal distribution of fibers would be more likely in a diagnosis of myotonia dystrophica. In a muscle biopsy from a yearling horse with a likely diagnosis of myotonia congenita, histopathologic examination of the muscle revealed small anguloid atrophy of type 2A and 2B fibers. In addition, the muscle fibers in the biopsy were almost exclusively type 2B with fewer type 1 and 2A fibers. No evidence of central nuclei, muscle necrosis, or macrophage infiltration was apparent.

9.11.2.2 Diagnosis

The diagnosis of myotonia congenita or myotonia dystrophica is based on clinical signs, appropriate histopathologic changes in muscle, and the classic waning (dive-bomber) or waxing and waning (revving motorcycle) sounds of the EMG trains of high-frequency discharges (see Figure 9-8). These waveforms are visible on insertion of the EMG electrode and on voluntary, mechanical, or chemical stimulation of the affected muscle. Following percussion or stimulation, positive sharp waves and fibrillation potentials may be visible. These abnormal discharges ultimately subside when the muscle has been at complete rest for some time, although they can be initiated readily after the patient is given neuromuscular blockade (e.g., curarization).

9.11.2.3 Treatment

In human beings, quinine, procainamide, taurine, and phenytoin (diphenylhydantoin) have been recommended for treatment of myotonia. Most of these drugs decrease muscle excitability by blocking sodium movement.[237] The duration of EMG relaxation time after maximum voluntary effort is the only reliable indicator of muscle excitation and response to therapy in human beings[242] and is impractical in the horse.

Acepromazine, xylazine, thiopentone, pancuronium bromide, quinidine, and phenytoin have been used to treat horses with myotonia; however, significant improvement in clinical signs has not been observed.[151,252] Prolonged times to 90% muscle relaxation in vitro were observed in two horses with myotonia; these relaxation times were normalized by the administration of phenytoin to the affected horses. However, no obvious clinical improvement or significant effect on EMG was observed with the short duration of therapy. 498
[245] The long-term effectsof phenytoin on the clinical signs or progression of myotonia is unknown. The 499
prognosis for horses with any type of myotonia is poor; most affected horses are euthanized because of their inability to function as athletes.

9.11.3 EQUINE MOTOR NEURON DISEASE

Equine motor neuron disease is a recently described disorder in horses characterized by neurologic and muscular dysfunction and muscle wasting. Initially the disorder was thought to have an inherited component or a specific geographic distribution.[253,254] Equine motor neuron disease now is accepted as being caused by chronic (6 to 24

months or more) vitamin E deficiency and has been reproduced experimentally.[255,256] Chronic vitamin E deficiency leads to chronic oxidative stress and resulting death of motor neurons and myopathy. Some horses manifest equine motor neuron disease as a primarily neurogenic disorder, and others have pathologic conditions of the muscles and nerves.

Clinically, affected horses present for various signs depending on the stage of the disease. Early complaints include weight loss and muscle atrophy (Figure 9-9). As the disease progresses, gait abnormalities become evident. The muscles of posture primarily are affected. The horse stands with a base-narrow stance, frequently shifts weight between legs and may stand with the tail elevated. This progresses to tremors when the horse stands still. At the walk or trot, the gait initially appears normal despite the return of tremors when the horse stands still. Some horses stand with the head low, apparently too weak to hold it in an elevated position. The penis may be partially prolapsed. Affected horses may progress to recumbency or may stabilize without further progression. Ocular manifestations include a characteristic fundic abnormality with a mosaic pattern of dark brown to yellow brown pigment deposited in the tapetal zone and a horizontal band of pigment at the junction of the tapetum and nontapetum. This abnormal pigmentation is associated with chronic oxidative injury resulting in lipid peroxidation of the retina.[253]

Figure 9-9 A horse with equine motor neuron disease. The generalized loss of muscle mass, the base-narrow stance, and raised tail head are notable.

Horses with equine motor neuron disease may have mild elevations in CK and AST activity. Complete blood counts are usually normal. Cerebrospinal fluid may have mild increases in protein and CK activity. Muscle biopsy may reveal neurogenic and myogenic atrophy.[257] Biopsy of the ventral branch of the accessory nerve reveals degeneration of the myelinated axon of the nerve and is present in acute and arrested cases. Muscle and

nerve biopsies may yield important information. Vitamin E levels in affected horses are significantly decreased (too low to read to 100 μg/dl; normal 400 μg/dl). Often multiple animals housed under identical conditions have similarly low vitamin E levels, but only one or a small number of horses may be demonstrating clinical signs. All horses should receive oral supplementation of vitamin E at 2000 to 4000 IU/day. Horses eating a vitamin E–deficient diet for many months have consistently low serum vitamin E concentrations that may take weeks to months to return to the normal range even with supplementation of the grass hay diet with commercial grains. Therefore top dressing commercial grain mixes with additional vitamin to provide 2000 to 4000 IU/day is recommended.

Horses displaying clinical signs at the time of diagnosis are unlikely to regain normal function. Once serum vitamin E levels are normal, aggressive vitamin E supplementation is no longer necessary. However, one should optimize daily intake to prevent future deficiency.

Low vitamin E intake is most common in horses eating hay and living without access to green pasture. Hay stored for long periods of time has less vitamin E than freshly cut hay. Many grain supplements have minimal quantities of vitamin E. Horses fed a grass hay diet without fresh pasture should have their vitamin E levels monitored and supplementation altered appropriately.

9.12 Disorders Characterizedby Muscle Wasting

Disorders characterized by muscle wasting or atrophy may be divided into two main categories, generalized and segmental. Generalized muscle wasting may occur in horses with equine motor neuron disease (discussed previously). Horses with streptococcal immune-mediated myositis often show a rapid onset of muscle wasting. A more insidious onset of loss of muscle mass occurs in horses with cachectic or disuse atrophy.

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Horses with segmental atrophy may have atrophy arising from disuse, denervation (neurogenic atrophy), or fibrotic myopathy. These conditions are discussed elsewhere in this text. 500

9.12.1 STREPTOCOCCAL IMMUNE-MEDIATED MYOSITIS

Purpura hemorrhagica is an immune-mediated vasculitis associated with sensitization and cross-reactivity to proteins in the cell wall of *Streptococcus equi*. Purpura hemorrhagica in its most common form may be associated with mild muscular lesions following the vasculitis.[258] Skeletal muscle necrosis with fragmentation and swelling of muscle fibers and a cellular infiltration may be visible histologically. Classically, the lesions are believed to be caused by a type III hypersensitivity vasculitis.

Some bacteria may share similar immunodeterminants with muscle so that antibodies produced against the bacteria also affect the muscle directly, causing necrosis.[259] This type of purpura has myositis as its primary clinical sign rather than vasculitis. Two forms of immune-mediated myositis have been described in horses in association with *S. equi:* immunoglobulin G– and IgA-mediated. Both are less common complications of *S. equi* infection than purpura hemorrhagica.

IgG-mediated myositis in horses is characterized by rapid onset of muscle wasting (over days to 1 to 2 weeks) (Figure 9-10). The amount of muscle mass lost often is astounding considering the time frame. Horses typically have a history of clinical *S. equi* infection or live onfarms where infection is endemic. Muscle biopsy reveals inflammatory cell infiltration of the muscle and muscle cell atrophy. Horses are otherwise bright and alert and have a normal appetite. Blood and serum profiles are within normal limits with the exception of increased CK and AST activity. Globulin fractions may increase slightly. Horses respond favorably to a 4- to 8-week course of

corticosteroid therapy (dexamethasone and/or prednisolone). In horses in which one suspects an internal nidus of infection (abscess) as the cause of long-term exposure to the antigen, one should institute concurrent antibiotic therapy.

Figure 9-10 A horse with immunoglobulin G–mediated *Streptococcus equi* myositis. The profound atrophy of the lumbar muscles is notable. The horse responded well to a course of prednisolone therapy.

The IgA-mediated form of poststreptococcal myositis of horses is known as Henoch-Schönlein purpura and is a significantly more malignant form of myositis. Horses do not present for loss of muscle mass but may have residual loss of muscle mass should they survive. Affected horses have severe depression, muscle pain, stiff gait, thrombosis, and colic and may have areas of flocculent serosanguinous fluid several centimeters in diameter over the major muscle groups. Signs are progressive over the first 24 hours. In the first hours, blood analysis may show only a stress or endotoxic leukogram and high CK and AST activities. Physical examination and blood analysis progresses to support early disseminated intravascular coagulopathy and CK activities of greater than 100,000. Postmortem examination of these horses shows large areas of hemorrhagic necrosis within the large muscle groups (Figure 9-11). Histologic examination demonstrates myositis. Early aggressive cardiovascular support combined with large doses of dexamethasone is essential to attempt to treat these horses because mortality rates are high. If major vital organs are infarcted, the horse likely will not survive. However, with aggressive support and long-term steroid therapy (1 to 2 weeks at high doses, followed by a longer-term, declining dose regimen) the horse may survive. These horses often have pockets of *S. equi* infection internally 500
that may have been the nidus initiating the autoimmune reaction. Areas to be examined include the guttural 501

pouches and thoracic and abdominal cavities. Because these horses may have a nidus of infection, treatment also should include aggressive antibiotic therapy.

Figure 9-11 Muscle taken at postmortem from a horse with immunoglobulin A–mediated *Streptococcus equi* myositis. The sharp demarcation between normal and infarcted muscle is notable.

9.12.2 MUSCLE WASTING WITH MALIGNANT DISEASE/CACHECTIC ATROPHY

Because of a combination of cachexia, malnutrition, disuse, infection, and old age, muscle weakness and occasionally muscle necrosis may develop with malignancies involving other body systems.

9.12.3 ACUTE RHABDOMYOLYSIS, RECUMBENCY, SWOLLEN PAINFUL MUSCULATURE, AND DEATH

This section covers causes of acute rhabdomyolysis that may result in prolonged recumbency, systemic complications, or acute death that were not discussed previously. Of the conditions already discussed, horses with overexertion rhabdomyolysis; horses with underlying myopathies such as RER, PSSM, and EPSM; and horses with the IgA-mediated form of *S. equi* myositis may have episodes of rhabdomyolysis severe enough to cause recumbency or death. Neonatal forms of rhabdomyolysis, which are discussed in the following section, also may cause acute life-threatening rhabdomyolysis.

This section covers postanesthetic myositis, infectious forms of myositis, aortic-iliac thrombosis and toxic causes of rhabdomyolysis.

9.12.4 POSTANESTHETIC MYOSITIS

Postanesthetic muscle damage may occur in 6% of anesthetized horses.[260] Postanesthetic myopathy may be divided into two forms: localized (the more common form) and generalized.

9.12.4.1 Pathophysiology of LocalizedPostanesthetic Myositis

The localized form of postanesthetic myositis is likely a consequence of undergoing anesthesia combined with positioning the horse during the anesthesia with minimal padding (Figure 9-12). However, in some horses with adequate padding, recumbency results in direct pressure damage to the muscle or perfusion disturbances. Damage may occur because of increases in pressure within the osteofascial compartments, and a local ischemia. In this context, *compartment* is defined as a muscle or group of muscles enclosed within a low-compliance envelope of fascia and sometimes periosteum.[261] This definition is comparable to compartmental syndrome described in human beings and down cattle. Venous occlusion tends to result in a greater inflammatory response and more extensive fibrous tissue repair than arterial occlusion. Sufficiently high intramuscular pressures have been reported during anesthesia of horses to compromise capillary blood flow and possibly affect neural transmission.[261–264] Postanesthetic compartmental syndrome per se has been reported only rarely in the horse, perhaps because compartmental pressures decrease rapidly when a horse stands.[265]

Figure 9-12 A horse with postanesthetic myositis of the right gluteal muscles. The severe swelling is notable. The horse recovered fully with supportive care.

An increase in the lactate concentration of blood draining from dependent muscle, a significant postischemic hyperemia in dependent muscles, and increases in the plasma concentrations of thromboxane and prostaglandin E_2 have been reported in anesthetized horses. Damage may result from reperfusion of ischemic muscles rather than the ischemia itself. Reperfusion generates strong oxidants, possibly initiating membrane lipid peroxidation and muscle damage.[266]

Risk factors associated with postanesthetic myositis include the weight and fitness of the horse, positioning, padding, duration of anesthesia, blood pressure, and the type of drugs or anesthetic used.

In one study, researchers measured intramuscular pressure with a catheter in the lowermost limb of 11 laterally recumbent horses and observed the effect of position and padding. They found the highest pressure readings (up to 92 mm Hg) in muscles with no padding when the limb in contact with the table was kept perpendicular to the body and the free limb was unsupported. They measured the lowest pressure (11 mm Hg) with padding when the limb in contact with the table had been pulled forward and the free limb was supported.[263] They did not correlate muscle pressures with weight in this study, but animals with greater muscle mass appeared to have higher muscle pressures. Postanesthetic myositis has been reported in the upper limb as well 501
as the dependent limb. Therefore one should provide support for the upper limb. 502

Halothane anesthesia results in a decreased blood flow to muscle (0.40 ml/min/100 g muscle) compared with that seen in unanesthetized muscle (1.20 ml/min/100 g muscle).[267] A predisposition to ischemia may occur with certain disease states associated with underlying hypotension and a decrease in muscle perfusion. However, one study found no correlation between body weight, halothane vaporizer concentration, intracompartmental muscle pressures, cardiac output, blood pressure, and the development of postanesthetic forelimb lameness, although prolonging anesthetic time appeared to increase the degree or severity of lameness.[261] Since that study, two studies have looked at the effect of maintaining horses for a long time (3½ to 4 hours) under normotensive anesthesia (with a mean arterial blood pressure around 80 to 95 mm Hg) or hypotensive anesthesia, as a result of increasing the inspired halothane concentration (with a mean arterial blood pressure around 50 to 65 mm Hg). Hypotension, resulting from a high concentration of halothane, may predispose horses to postanesthetic myositis even when protective padding is used.[268,269] No increase in the incidence of problems is apparent when repeated episodes of normotensive anesthesia are undertaken, even with halothane use.[268]

Usually one observes clinical signs in the limb that is down (compressed) when in lateral recumbency. In the forelimbs, the triceps, deltoids, and occasionally the brachiocephalic and cranial pectoral muscles tend to be affected. In the hindlimbs, usually the biceps and vastus lateralis are affected. The flank muscles and masseter muscles also may be involved. Occasionally, however, the upper limb may be affected if its circulation is compromised. One study investigated the effect of limb position on cephalic venous pressure and triceps compartmental pressure in the upper limb of anesthetized normotensive ponies.[270] When the upper limb was in the position often used to allow surgical access to the lower limb (i.e., leg parallel to the floor and pulled back with reasonable force), considerably higher venous pressures were recorded compared with the limb in the neutral position (i.e., leg raised so that it was parallel to the floor and at right angles to the spine). The intracompartmental pressures were only found to be increased significantly when the upper limb was flexed horizontally, hard forward (i.e., with the leg parallel to the floor, fully flexed at the carpus, and pushed forward with reasonable force), which is a potential alternative leg position allowing surgical access to the lower limb. This position also resulted in significantly higher venous pressures than the neutral limb position, which suggests that this position is not advisable for clinical use. Impaired venous drainage may be a contributory

factor in the development of postanesthetic myositis, at least respecting the upper limb.[180] The position that appears to provide adequate surgical access to the lower limb with low compartmental and venous pressures is the flexed horizontal forward position (i.e., leg parallel to the floor, fully flexed at the carpus, and pushed gently forward).

When a horse is anesthetized in dorsal recumbency, the gluteal and occasionally the longissimus dorsi muscles tend to be affected. In one study, the adductor, pectineus, and gracilis muscles were involved, a finding attributed to arterial hypotension coupled with partial occlusion of the artery primarily supplying these muscles (the medial circumflex femoral artery), perhaps because of the hindlimbs being flexed passively.[271]

9.12.4.2 Clinical Signs

Clinical signs of postanesthetic myositis normally are seen during the initial recovery period but occasionally may be delayed for 1 hour or more. One first may suspect the condition if recovery is prolonged or the horse has repeated unsuccessful attempts to stand. Signs attributable to damage to one muscle group, one peripheral nerve, or mixtures of muscle groups and peripheral nerve(s) may be visible. Affected muscle groups tend to be hot andmay be swollen. The horse often resents palpation and usually is reluctant to bear weight on the affected limb. One may observe signs of severe pain with sweating. Classically, with forelimb muscle involvement, the affected animal stands as if the radial nerve has been paralyzed but can use the extensor muscles. If hindlimbs are involved, knuckling of the fetlock may occur; if the quadriceps femoris is involved, the stifle and hock may buckle and the animal may not be able to rise, especially if both hindlimbs are affected. Signs may persist for several days, even in uncomplicated cases.

In some horses, the only abnormality one may see is isolated, raised painful plaques, or one may find these along with forelimb or hindlimb dysfunction. These swellings usually occur over the table contact areas (hip, rib, or facial area) and may be caused by inadequate padding, trauma from positioning devices, or the weight of the patient.[269] Serum CK and AST activities increase in affected individuals, and one may observe myoglobinuria with significant muscle damage.

On occasion, after recovery from general anesthesia, a horse demonstrates considerable discomfort with continued treading of the feet, sweating, holding its limbs (usually the hindlimbs) in abnormal positions, and kicking out. The syndromes appear nonresponsive even to large doses of opioid and other analgesics, serum CK activities are not increased within the subsequent 48 to 96 hours, and no myoglobinuria is observable. These syndromes are assumed to be a form of paresthesia (pins and needles) caused by sensory neuropraxis, 502
but a strong clinical similarity to the condition of postanesthetic neuromyopathy is apparent. 503

9.12.4.3 Pathophysiology of Generalized Postanesthetic Myositis

The generalized form of postanesthetic myositis may be caused by a condition similar to malignant hyperthermia in human beings and swine. Alternatively, signs may appear during recovery, with many muscle groups affected independent of positioning.

The cause of generalized postanesthetic myositis in horses is uncertain but has been suggested to result from local ischemia that becomes more generalized because of hypotension, sensitivity of muscle cells to anesthetic drugs, or depolarizing muscle relaxants (suxamethonium), or a combination of these factors. In the two studies that looked at the effect of hypotensive anesthesia, cases of generalized myositis occurred during recovery.[268,269] In vitro examination of muscle using an adapted human contracture test (used for the identification of

malignant hyperthermia susceptibility) has shown increased sensitivity to caffeine and halothane in samples taken from certain susceptible equidae.[180] In this study the examined horse possibly had RER.

Whether horses with inherited myopathies are more or less prone to malignant hyperthermic events or postanesthetic rhabdomyolysis compared with the general horse population is unknown. Certainly, given the common nature of these disorders, one can be confident in saying that not every horse anesthetized that has an underlying myopathy has a malignant hyperthermic or postanesthetic rhabdomyolytic event. However, in human beings many different muscle disorders have been associated with an increased chance of malignant hyperthermic event when under general anesthesia. Therefore the potential exists, and inquiring whether a horse has a history of exertional rhabdomyolysis before performing general anesthesia is prudent. If a horse is believed to be at risk, premedicating that horse with dantrolene may be of benefit.

Generalized myositis may occur at any time during anesthesia. The temperature, heart rate, and respiratory rate increase, and muscles may fasciculate and contract. The horse may resist assisted ventilation and appear rigid. Death can occur.

Postoperative cases also tend to occur regardless of the length of the anesthesia and the positioning. Signs of extreme pain are apparent, and often the affected animal is unable to rise. The muscles may be rigid, and excessive sweating usually occurs. Signs of colic with myoglobinuria may be present, as well as fluid disturbances.

One may need to differentiate the condition from postanesthetic myelopathy, in which signs can range from difficulty in standing to paraplegia with flaccid paralysis and analgesia involving the pelvic limbs. In addition, loss of sensation and spinal reflexes over several contiguous segments may occur.

9.12.4.4 Laboratory Findings of Both Forms

Plasma and serum CK, AST, and LDH activities usually increase in both forms of postanesthetic myositis of horses. Increased plasma muscle enzyme activities may help differentiate those animals with overt muscular damage from those with conditions similar to paresthesia (possibly caused by a reduced blood supply to sensory nerve endings). Fluid and electrolyte disturbances also may be present. Hypocalcemia, hypomagnesemia, hyperphosphatemia, metabolic acidosis, hyperkalemia, and hyperglycemia have been reported and should be treated if present. Myoglobinuria may occur, and renal function may be compromised, necessitating treatment with intravenous fluids, furosemide, and dopamine. Muscle biopsy histologic examination is consistent with rhabdomyolysis.

9.12.4.5 Treatment

The aim of any treatment regimen is to relieve pain, prevent further damage, correct fluid and electrolyte disturbances, and maintain renal function. The added clinical manifestation in some cases is malignant hyperthermia in which muscle contractures cause a rapid increase in body temperature. The treatment usually recommended is similar to that for exertional rhabdomyolysis. If a metabolic acidosis is present, the horse may require intravenously administered sodium bicarbonate. If one recognizes the signs while the horse is anesthetized, one should stop using the anesthetic, whenever possible, and use alternative intravenous agents. If hyperthermia is present, the horse should be cooled rapidly and one should consider administration of fluids and dantrolene intravenously. Initial fluid infusion rates of up to 10 to 20 ml/kg/hour have been recommended, slowing to 4 to 5 ml/kg/hour. Analgesics that have been used include the NSAIDs and opioids. The effect of intravenously administered lidocaine in this scenario has not been evaluated. Diazepam and glyceryl

guaiacolate to reduce muscle spasm may be of value, but one should use them with caution because of possible ataxia and therefore increased problems with standing. The horse may require sedation if it is awake and anxious because of the pain. The α_2-agonists (xylazine, detomidine, romifidine) may provide good sedation and analgesia but may exacerbate ataxia; promote sweating, hypoinsulinemia, and hyperglycemia; and increased urine output, which may further exacerbate fluid loss. The vasoconstrictive effect of these agents together with their effect on cardiac output may compromise tissue blood flow further. Their use in the more violent cases may be necessary. Acetylpromazine in combination with opioids may provide good 503
sedation with minimal ataxia, and the vasodilatory effect may help improve tissue flow, provided circulatory 504
volume is maintained and hypovolemia does not occur.

Intravenously and then orally administered dantrolene sodium has been suggested to be beneficial.[181] Dantrolene at 1 mg/kg body mass orally has been used successfully to treat postoperative cases. However, recent work on the pharmacokinetics of dantrolene[180] has suggested that higher doses are likely to be needed to establish and maintain effective blood levels. An intragastric dose of 2.5 mg/kg every hour after a loading dose of10 mg/kg has been recommended.[181] Alternatively, an intravenous loading dose of 1.9 mg/kg has been suggested to achieve a more immediate therapeutic effect.[180] Dantrolene is not licensed for use in horses, is expensive, and is potentially hepatotoxic. Transient weakness and ataxia have been associated with intravenous administration.

9.12.4.6 Prevention

Correct positioning to prevent restriction of venous drainage or arterial input and the use of appropriate padding are recommended. Foam padding, for example, has been reported to be inferior to air mattresses or waterbeds.[272] Positioning is important, and various recommendations have been made: for example, one should elevate the upper limb and reduce pressure on the lower triceps by pulling the leg forward.[263] In lateral recumbency one should keep the hindlegs parallel or above parallel to the table and sufficiently separated to promote venous drainage. In dorsally recumbent horses, rather than letting the hindlimbs position themselves passively, one should provide support for them in slight extension by a hoist. Problems with hindlimb adductor myopathies do not always develop with passive positioning. One should avoid pulling back the hindlimbs. If one must draw back the hindlimbs in full extension, for example, for certain arthroscopic examinations, one should keep the surgical time to a minimum to reduce the risk of quadriceps myopathy. The flexed horizontal forward position, with the uppermost (noncompressed) forelimb parallel to the floor, fully flexed at the carpus, and pushed gently forward, is preferable when the surgeon needs access to the medial aspect of the lower carpus and distal radius.[270]

Withholding grain feed for 48 to 72 hours before general anesthesia may be beneficial. Heavily muscled, Draft-breed, and more athletically fit horses may be at an increased risk for postanesthetic myositis, in particular those with athletic-induced sports injuries. One should maintain blood pressure at more than 80 mm Hg (mean arterial blood pressure) to maintain muscle perfusion, should minimize halothane use, and may find intraoperative fluids and inotropes valuable.

Prophylactically administered dantrolene has been recommended, especially if the horse has a history of exercise-induced rhabdomyolysis or muscle problems following previous episodes of general anesthesia. Various doses have been used. In the United Kingdom, intragastric administration at 2 to 4 mg/kg has been recommended, whereas higher doses (10 mg/kg) have been used in the United States.[181] However, no

objective data exist to confirm the efficacy of prophylactic dantrolene administration for prevention of postanesthetic myositis in horses.

9.12.4.7 Prognosis

Prognosis depends partially on the extent of the muscle damage, the treatment instituted, and the temperament of the individual. Affected horses often recover completely, especially if the condition is localized to one muscle group. Occasionally, the horse may be left with residual muscle atrophy, fibrosis, and scarring. Death can occur with massive areas of ischemic myonecrosis and intrafascicular nerve fiber degeneration postmortem. Euthanasia may be required on humane grounds in horses that remain recumbent, have severe rhabdomyolysis, or that have uncontrollable pain. Further discussion of the pathophysiology, treatment, and prevention of anesthetic myoneuropathy is available.[*]

* References 68, 109, 126, 180, 181, 188, 271.

9.12.5 INFECTIOUS MYOSITIS

Infectious agents (bacteria, viruses, and parasites) can affect skeletal muscles. Some produce inflammatory reactions (myositis), whereas others give rise to often mild degenerative changes (i.e., myopathy). The differentiation can be difficult, especially in subacute and chronic cases, because an inflammatory reaction often induces secondary degenerative changes and vice versa.

9.12.5.1 Bacterial Gas Gangrene and Malignant Edema

Gas gangrene, malignant edema, and *clostridial myonecrosis* are terms used to describe infection of skeletal muscles with any of several *Clostridium* spp. organisms (*C. septicum, C. perfringens, C. novyi, C. sordellii*, and *C. chauvoei*). Germination of spores and vegetative growth occur when suitable local anaerobic conditions (alkaline pH, low oxidative reduction potential, etc.) exist following castration, parturition injuries, puncture wounds, and in particular, intramuscular injections.[273] Toxin production results in the destruction of the cellular defense mechanisms and significant tissue necrosis.

Affected animals may be found recumbent or dead. In less acute cases, lameness often occurs, which can be severe, or the horse is depressed. Painful muscular swellings may be present, and one may feel flocculation or 504
gas crepitation. The overlying skin initially may appear hot and be discolored but progress to become cool to 505
the touch, firm, and insensitive. Affected animals are often extremely depressed with systemic signs of profound toxemia. The prognosis is poor because the condition often progresses rapidly with ataxia, recumbency, coma, and death.

Diagnosis usually is made from the clinical signs and history. The finding of a nonclotting, malodorous fluid on needle aspiration (with or without gas) can be indicative of clostridial infection. Anaerobic culture, cytologic examination, and fluorescent antibody identification of the organism in tissue specimens have been recommended for a definitive diagnosis.[274]

Clinicopathologic changes tend to be nonspecificand similar to those seen in other septic-toxic conditions. Although increased serum muscle enzyme activities may be observable, they often do not appear to be in proportion to the degree of muscle damage.[274] In gas gangrene, extensive disintegration of muscle tissue tends

tooccur, with serosanguinous exudate and bubbles of gas. Malignant edema presents as a cellulitis with sparing of muscle fibers, although this may sometimes progress to gangrene.[37,274]

Treatment must be aggressive and typically includes initial administration of high doses of potassium penicillin G (44,000 IU/kg intravenously every 6 to8 hours) and surgical debridement. One may administer penicillin with metronidazole (20 to 25 mg/kg orally every 8 hours or 20 mg/kg intravenously every 8 to12 hours). Surgical debridement, fenestration, or both are essential to remove necrotic tissue and disrupt the anaerobic environment.[274] Long-term antibiotic and nursing care of the resulting wound usually are required. One should continue antibiotic therapy until infection has resolved and for a minimum of 7 days thereafter. In many instances, diffuse cellulitis extending down fascial planes occurs, necessitating therapy for at least several weeks. Supportive fluid therapy and analgesics are often necessary in the initial stages while signs of systemic toxemia are evident. One should use corticosteroidswith caution, although initial short-term therapy may be beneficial in horses with evidence of shock. Prognosis in most horses is poor.[273] Survival appears to be most frequent with *C. perfringens* infections, although extensive skin and muscle sloughing may occur, which in turnmay necessitate euthanasia.

9.12.5.2 *Sarcocystis*

Sarcocystis spp. are protozoal parasites that have an obligatory two-host life cycle. *Sarcocystis* spp. are found in horse muscle as part of the intermediate host infection. The horse consumes sporocysts with herbage that has been contaminated by carnivore feces (most commonly dog). Sporozoites are released and migrate to various sites. Second- or third-generation schizonts develop within muscle fibers as thin-walled cysts. Entry into muscle fibers can result in extensive fiber degeneration and significant enzyme release if a heavy infestation occurs. Enlargement of the cysts over the next 100 days orso can result in further muscle damage and lameness. Although three *Sarcocystis* species (*S. bertrami, S. equicanis,* and *S. fayeri*) have been recognized in horses, some controversy exists as to whether the three are distinct species.[37] In addition, whether the causative agent of equine protozoal myelitis, *S. neurona*, may affect skeletal muscle in the horse is unknown. A high postmortem prevalence of sarcocysts in muscle sections, occasional presence of sarcocysts in muscle biopsies, and evidence of transplacental infection have been reported,[275,276] suggesting that inapparent infection is probably common. A light and electron microscope study of sarcocysts in the horse has been described.[277]

Some dispute exists as to how heavy an infestation is necessary to cause clinical muscle disease in the horse. Twelve of 91 horses with a history of chronic muscle problems were positive for sarcocysts on muscle biopsy.[276] Separating the group of animals with sarcocysts from others on the basis of laboratory findings, history, or clinical signs was not possible. Weight loss, lethargy, difficulty in chewing and swallowing, generalized muscle weakness, and fasciculations have been reported in one horse with widely distributed sarcocysts, and clinical signs were attributed to the sarcocyst infestation.[278] Chronic illness in an experimentally infected pony also has been reported.[279] A heavy infestation is likely necessary to cause clinical disease.[280]

9.12.5.3 *Trichinella spiralis*

The nematode *Trichinella spiralis* may be found as an encysted larva in a bulging glassy segment of a muscle fiber in horses given feed containing contaminated porcine muscle tissue. Trichinosis therefore is rare. Usually one cyst per fiber is present, and the larva may be up to100 μm long. Degeneration (plus regeneration) may occur in neighboring muscle fibers. The larva can live for many years, but if the parasitized segment

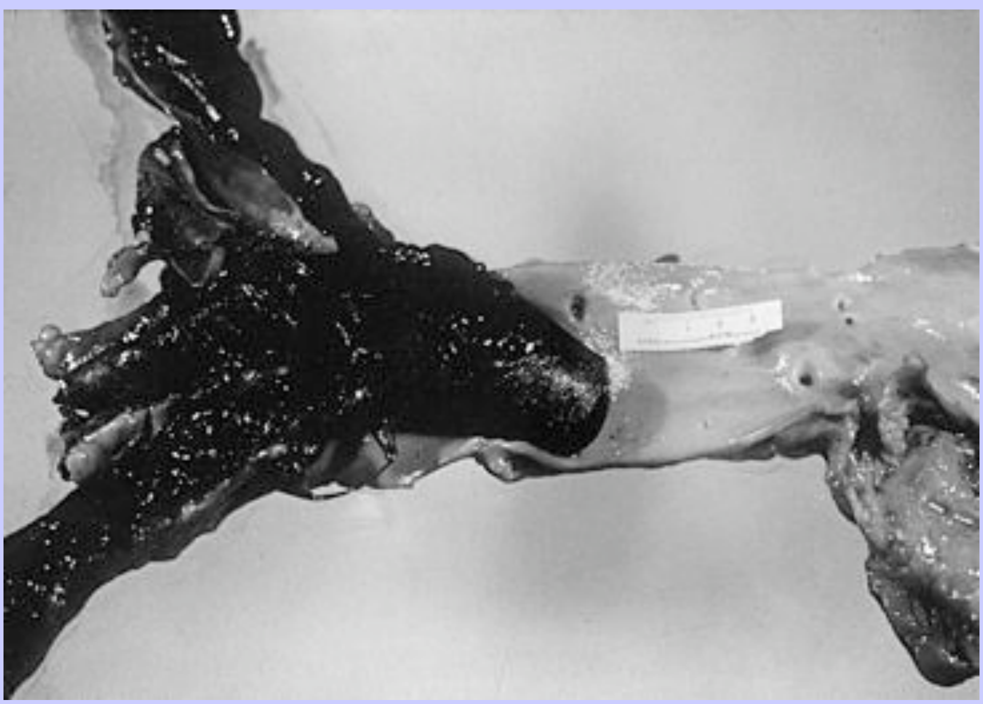
degenerates, the larva is exposed and soon dies. This in turn results in an acute, predominantly eosinophilic inflammation.[37,124] The parasitic infection appears to be asymptomatic.

9.12.6 AORTIC-ILIAC THROMBOSIS

9.12.6.1 Pathophysiology

Predisposing factors and causes of aortic-iliac thrombosis remain unclear.[281,282] Thrombi may result from damage to the vessel intima by migrating *Strongylus vulgaris* larvae. Signs of verminous arteritis in affected vessels (e.g., larvae, eosinophils, other inflammatory cells) have not been reported, however.[282] Larvae do not 505
tend to migrate as far caudally as the thrombi are found. The condition is apparently more common in 506
racehorses, which tend to be dewormed regularly. However, in horses not asked to perform maximally, the condition may not be clinically apparent and therefore may be underdiagnosed. An alternative suggestion is that *S. vulgaris* larvae could be an indirect cause of the condition via the formation of thromboemboli, which become organized and result in arterial occlusion and further thrombosis (Figure 9-13).[281,282] Hormonal, nutritional, and mechanical factors, as wellas prior infection with *Streptococcus equi* or equine influenza virus, have been proposed as predisposing factors to thrombus formation.[282–285]

Figure 9-13 Aortic-iliac thrombosis.



The internal iliac arteries are bound more closely to fascia than many other arteries. This, coupled with the large forces generated during movement, could increase the risk of injury to these vessels. In affected horses, plaques ascribed to intimal *S. vulgaris* migration have been reported in various branches of the abdominal aorta. However, similar thickenings also have been reportedin brachial, carotid, and cerebral arteries (sites in

which strongylus migrations are unlikely) in control and affected horses. Repair of spontaneous damage occurring to the arterial endothelium at areas of turbulence or arterial branching may result in plaque formation and eventually thrombus formation. One postulation therefore is that aortic-iliac thrombosis is similar to the human condition arteriosclerosis obliterans, a form of arteriosclerosis causing intermittent claudication.[282] Individual variation in the aortic quadrification or vascular repair mechanisms could explain why certain animals are predisposed to the development of this condition.

9.12.6.2 Clinical Signs and Laboratory Findings

Following exercise in affected animals, ischemia of the muscle usually occurs because of circulatory interference. The ischemia is usually reversible because the blood flow may be adequate when at rest or at light work. The clinical signs vary in severity according to the degree ofvascular occlusion, the vessels affected, and the extent of collateral circulation. The lameness tends to become more severe as exercise continues. Mild cases may be missed, however, because the horse appears normalwhen pulled up after a disappointing performance. Clinical signs may include poor performance, intermittent hindlimb lameness, transient lameness or weakness with exercise, a tendency to drag the toe and occasionally knuckling over, and gradual shortening of the stride leading to a transient inability to move. A peracute condition with paraplegia, shock, and death also has been recognized.

Hindlimbs may be affected, although usually one more than the other. After exercise the superficial veins on the more affected limb may appear collapsed (because of delayed filling) compared with the distended vessels on the more normal limb. The superficial veins of the affected limb(s) may take up to 90 seconds to fill after intense exercise compared with about10 seconds in normal horses. The affected limb often feels cooler than expected, especially around the gaskin, and may not sweat. One may feel a reduced digitalpulse. The animal may sweat profusely on the body, head, and neck and sometimes cow-kicks or shakes the affected limb (perhaps because of paraesthesias following reduced blood supply to sensory nerve endings). Full recovery tends to occur within 20 to 30 minutes. On palpation of the peripheral pulses at rest, one may detect abnormalities, including a flattened, weak, and prolonged contour to the pulse distal to the site of obstruction, whereas proximally the pulse may be normal or increased in strength. Alternatively, certain pulses may be absent.

Aortic-iliac thrombosis has been reported in males and females, although a greater incidence of clinical problems has been reported in males. One of the reasons for this difference in incidence may be a more efficient collateral circulation in females. The mean age of affected animals was 5.2 years in one study. Plasma and serum muscle enzyme activities are usually within normal limits before and after exercise. In severe cases with significant muscle damage, however, increases in CK have been reported.[282]

On postmortem examination, affected vessels tend to be enlarged and a large thrombotic mass usually is present at the aortic quadrification (see Figure 9-13) that may be attached to, and therefore possibly originate from, organized masses in the internal and external iliac arteries. These may extend to the bifurcation of the popliteal artery, but rarely do they extend far into the tibial arteries or the muscular branches of the femoral 506
artery. Histologically, affected muscles show signs of ischemia. Damaged fibers and supporting structures 507
tend to be removed with little sign of inflammation or fibrosis.[281]

9.12.6.3 Diagnosis

Diagnosis of aortic-iliac thrombosis is based on clinical signs and palpation of the thrombus per rectum or ultrasonic demonstration of the thrombus. In affected horses rectal examination often reveals no abnormality, with one easily missing signs such as a decreased arterial pulse or an unusual firmness of a vessel. The obstruction possibly may be fairly peripheral, which may in fact result in an increase in the pulse proximally. Ultrasonography is therefore the definitive method for diagnosis. A linear array ultrasound probe with a frequency of 5.0 or 7.5 MHz has been recommended.[285,286] Differential diagnoses include exertional rhabdomyolysis, cervical vertebral malformation, and degenerative joint disease of the tarsometatarsal joints. Perineural and intraarticular anesthesia help to eliminate lameness originating from the distal limb as a cause of rear limb pain.

9.12.6.4 Treatment

Treatment for aortic-iliac thrombosis aims toward elimination of the thrombus or development of collateral circulation. At present, an effective drug, selectively able to break down mature thrombi, is not available, and surgical intervention in the horse is difficult.

Sodium gluconate at 450 mg/kg body mass by slow intravenous infusion has been recommended to treat horses with aortic-iliac thrombosis,[287] but no evidence exists that the drug has any effect on a mature thrombus.[282] Prednisolone sodium succinate (100 mg 30 minutes before administration of sodium gluconate) mayhelp eliminate the systemic reactions frequently associated with this drug.[281,282] Other suggested protocols include monthly administration of ivermectin (Eqvalan, MSD-AGVET) and twice-daily oral doses of phenylbutazoneat 2.2 mg/kg body mass for 3 months.[285] However, improvement in treated horses may be due to the development over time of effective collateral circulation. Prognosis for horses severely affected with this condition is poor, and a hereditary predisposition has been suggested,[281] although little supporting evidence exists. Alternatively, horses may be treated conservatively with rest and administration of 60 grains of aspirin orally once daily.

9.13 Toxicoses

Only a few available and palatable toxic compounds cause muscle fiber degeneration in the horse. White snakeroot and *Cassia occidentalis* are the most common plants that may cause rhabdomyolysis. Ionophore contamination of feed also may lead to life-threatening rhabdomyolysis. For additional information, see Chapter 20.

9.13.1 WHITE SNAKEROOT

White snakeroot *(Eupatorium rugosum)* is a common plant in the central midwestern and northeastern United States. White snakeroot is a shade-loving plant growing well in damp, wooded areas, shaded river banks, and in steep canyons.[288] The toxic principle is tremetol, a fat-soluble high-molecular-weight alcohol, and plants may be toxic in fresh and dried forms. Clinical signs include progressive muscle tremors, weakness, choke, constipation, recumbency, arrhythmias, and death. Serum muscle enzyme activities increase significantly. At postmortem, one observes skeletal muscle necrosis and subepicardial and myocardial hemorrhages with grayish streaks in the myocardium.[289]

9.13.2 *CASSIA OCCIDENTALIS*

Cassia occidentalis (coffeeweed, senna) is toxic to horses and may cause ataxia, incoordination, recumbency, and death with liver and muscle damage. However, natural ingestion of this plant is uncommon.[290]

9.13.3 MISCELLANEOUS TOXICOSES

Selenium, iron, thallium, and perhaps sulfur and cobalt may cause muscle disease when fed at toxic levels. Metals such as iron may act by affecting vitamin E and selenium status or lipid peroxidation.[37] If ingested, bracken *(Pteridium aquilinum)*, horsetail *(Equisetum arvense)*, and rock fern *(Cheilanthes sieberi)* may induce a thiamine deficiency with signs of anorexia, gait disturbances, staggering, lack of coordination, lethargy, a weak and irregular pulse, and muscular tremors.[291]

9.13.4 IONOPHORE TOXICITY

9.13.4.1 Pathophysiology

Monensin, rumensin, and lasalocid are ionophores commonly fed as coccidiostats in food animals. Monensin is a carboxylic ionophorous antibiotic fermentation product derived from *Streptomyces cinnamonensis* and is used as a coccidiostat for poultry and as a feed additive for cattle because it increases feed utilization by altering rumen fermentation. Access to ruminant feed or accidental contamination of horse feed in a mill producing cattle and horse feed are the most common causes for intoxication.

The lipid-soluble monensin-sodium complex releases sodium in exchange for a proton after it crosses the cell membrane. The protonated monensin leaves the cell to pick up more sodium, and the cycle repeats. The increase in intracellular sodium concentration stimulates the sodium-potassium ATPase pump and indirectly results in an increase in calcium. This increase in intracellular calcium then may result in the release of certain factors such as catecholamines, which in turn may be responsible for some of the clinical signs seen, 507
especially in relation to the heart. When mitochondria become saturated with calcium, the process of oxidative 508
phosphorylation is disturbed and supplies of ATP decrease. Swelling and disruption of mitochondria follows, resulting in release of stored calcium, which may potentiate or precipitate catecholamine-induced cardiac arrhythmias. Increased intracellular calcium concentrations also may result in a brief period of extreme contraction of muscle (because of effects on actin-myosin binding) as well as release of various cellular lytic enzymes. The rapid onset of cardiac or skeletal muscle necrosis may result from energy deficiency resulting from these lytic processes. Small concentrations of monensin also may result in an increase in intracellular potassium concentration; large concentrations of monensin tend to decrease intracellular potassium concentration. Other tissues less dependent on ATP may not show such severe signs.[124,292,293] In muscle, swelling and disintegration of mitochondria are the first visible lesions, although monensin does not cause mitochondrial structural defects in cultured muscle cells.[294] Further work is needed to determine if other substances or metabolites of monensin are involved in pathophysiology of toxicity in vivo.[292]

9.13.4.2
Clinical Signs

Horses are sensitive to monensin. Signs of toxicity may be apparent after ingestion of 2 to 3 mg/kg body mass of crystalline monensin (compared with 20 to 34 mg/kg for cattle). (The median lethal dose for mycelial monensin is estimated to be 1.38 ± 0.19 mg/kg body mass.) The increased susceptibility of horses to monensin toxicity may be because horses do not clear monensin from the bloodstream as rapidly as cattle. In addition, equine heart muscle is sensitive to the effects of catecholamines.[292] Following ingestion of a single toxic dose, signs of lethargy, muscular weakness, and stiffness, often with recumbency, occur within 24 hours. In the early stages, progressive hypokalemia resulting in cardiac conduction disturbances occurs.[293] Cardiovascular signs may include tachycardia with possible arrhythmias, prominent jugular pulse, congested or pale mucous membranes, cold extremities, weak pulse, and profuse sweating. Tachypnea, hyperpnea, or dyspnea may be apparent. Early on, affected animals may show signs of colic, including sweating, increased pulse, and increased or absent borborygmus. Amounts of feces may be reduced, or no feces may be passed. Animals may be anorectic. Myoglobinuria and muscle tremor also may be apparent, together with progressive ataxia and signs of central nervous system malfunction. Depending on the dose ingested (and the individual), death may occur within 24 hours. Hindlimb muscles tend to be involved most severely.[37,124,292] Progressive cardiac insufficiency, weight loss, and sometimes renal failure are more common with chronic toxicity in the weeks following a single, lower dose exposure. In such cases, clinical signs related to skeletal muscle involvement may disappear, although poor performance and muscular weakness may be apparent if the horse is asked to perform. Signs of chronic toxicity may not be noticed for weeks after ingestion of the compound has stopped.[295] Monensin-containing feeds are often unpalatable, and owners may recollect a "bad" bag or shipment of feed that some horses refused to eat in the recent past.

9.13.4.3
Laboratory Findings

In peracute cases, in which the horse dies within 24 hours, a progressive hemoconcentration associated with increased urine output and decreased urine specific gravity occurs, together with increased blood urea nitrogen and creatinine concentrations.[293]

Serum CK and AST activities may be elevated moderately to significantly, primarily because of skeletal muscle damage. The increase in total LDH activity appears (at least initially) to be caused by LDH_1 and LDH_2 isoenzymes and may be related to increased erythrocyte fragility and hemolysis. Increases in alkaline phosphatase activity also have been observed, apparently from an increase in the bone isoenzyme.[293] Hemoglobinuria, myoglobinuria, or both may occur.

Sodium concentrations do not change greatly. Serum calcium concentration tends to decrease initially, within the first 12 to 24 hours, by about 10% to 15% and then recovers. Hypokalemia tends to occur in the first 24 hours with a decrease of 1 to 2 mmol/L followed by a return to normal by 36 hours. In chronic toxicity, clinical findings are often nonspecific and reflect the various organs involved. In one study of 32 horses with a history of unthriftiness and poor performance following prior ingestion of monensin-contaminated feed, the authors observed decreased serum bilirubin concentration and increased alkaline phosphatase activity. In four horses, they also observed increased LDH_5 activity.[295]

In horses that die peracutely, one may not observe gross lesions and may rely on feed analysis for diagnosis. In less acute cases, the gross changes are not pathognomonic for monensin toxicosis and can include edema,

hydropericardium, hydrothorax, ascites, hemorrhages, and pale areas in the heart or diaphragm.[292,294,295] Differentiating this condition histologically from vitamin E and selenium deficiency or exertional rhabdomyolysis may be difficult.[37] However, history of feed problems, signs in multiple horses, and evidence of heart failure in chronic cases aids in diagnosis.

9.13.4.4 Diagnosis

The clinical signs and history of access to monensinare important in the diagnosis. The finding of high monensin concentrations in feed and stomach contents may confirm the diagnosis. The ingestion of blister 508
beetles (*Epicauta* spp.) can cause cardiomyopathy, and one should exclude this from the differential diagnosis 509
along with white snakeroot toxicosis.

9.13.4.5 Treatment and Prognosis

In acute monensin toxicosis, intensive isotonic polyionic fluid therapy with additional potassium has been recommended for treatment. Although this may support the horse during the initial crisis, prognosis is guarded because longer-term actions of monensin, particularly on the heart, still may cause death.[293,295] Activated charcoal or mineral oil administered orally may help to decrease further monensin absorption. One should avoid purgatives that act by stimulating the vagal system because of the risk of causing arrhythmias in an already damaged heart. Similarly, intravenously administered calcium may not be advisable. One should avoid cardiac glycosides because they may work synergistically with monensin, resulting in extensive cardiac muscle damage. Horses that have clinical signs of heart failure after previous exposure have a poor prognosis for long-term survival.

9.14 Muscle Disorders of the Neonate

9.14.1 NUTRITIONAL MYODEGENERATION/WHITE MUSCLE DISEASE

Vitamin E or selenium deficiency and the resulting muscle disorder often are described in the literature as a muscular dystrophy. The term is inappropriate considering that *muscular dystrophy* is defined in human medicine as a group of progressive genetically determined primary myopathies. They usually are not present at birth, and neural and vascular components are not involved initially. Regeneration of muscle cells is absent or inadequate, and replacement of muscle cells with fat and fibrous tissue occurs. This differs from the nutritionally associated disease in horses, which therefore should not be called a muscular dystrophy.

9.14.1.1 Clinical Signs and Laboratory Findings

Clinical signs and laboratory findings in horses with nutritional myodegeneration vary depending on distribution of the muscular lesions and the extent of damage. Signs are seen most commonly in animals less than2 months of age and can occur peracutely and progress rapidly. Box 9-6 lists differential diagnoses for nutritional myodegeneration. Death may occur immediately from a fatal arrhythmia, or after a few hours from exhaustion and circulatory collapse. The myocardium, diaphragm, and respiratory muscles usually are involved, leading to heart failure, dyspnea, and pulmonary edema. Occasionally, one may find painful subcutaneous swellings in particular over the rump, ventral abdominal wall, and nuchal crest. These swellings

are more common in older foals. In older animals, sudden postexercise recumbency may occur with death within a few hours caused by pulmonary edema and myocardial failure.[188]

9.14.1.1.1

BOX 9-6 DIFFERENTIAL DIAGNOSIS OF NUTRITIONALLY RELATED MYODEGENERATION

Atypical myoglobinuria

Botulism

Cerebellar diseases

Colic

Equine motor neuron disease

Equine rhabdomyolysis syndrome

Medullary diseases such as herpesvirus type 1 and myelitis

Muscular dystrophy

Polymyositis

Purpura hemorrhagica

Rabies

Septic polyarthritis

Spinal cord diseases

Suppurative meningitis

Tetanus

Tick paralysis

Trauma

Various causes of dysphagia (e.g., cleft palate, ulcers, and peripheral damage to certain cranial nerves)

Various causes of dyspnea (e.g., pneumonia and cardiac disease)

In less acute cases, weakness, stiffness, and lethargy are common findings. The body temperature can vary from subnormal to supranormal. The affected horse may become recumbent and unable to rise. Hindlimb muscles often appear to be sore on palpation. Dysphagia is often the first sign noted because of involvement of the tongue and pharyngeal and sometimes masticatory muscles and often contributes to the development of aspiration pneumonia. Regurgitation, starvation, and ptyalism are other possible complications. If the heart is involved, one also may find tachycardia, arrhythmias, systolic murmurs, and respiratory distress. Myoglobinuria has been reported in a number of cases. A swollen tongue and tachypnea are unusual

presenting signs. A 10-month-old aborted fetus with histologic lesions typical of this condition has been reported.[152]

The condition appears to occur in any breed and does not appear to have a sex predilection. Recovery can occur within days or weeks of the presenting signs.[152] Signs may occur in an individual animal or several animals within a group.

In the acute form, AST and CK activities are elevated. In the recovery stages the values may be increased only 509
slightly or within the normal range. Increased CK and AST activities also have been found in about 25% of 510
the mares of affected foals and in 20% of clinically normal foals in the same stables.[296] This finding was suggested to reflect the existence of subclinical lesions in these animals.

Because selenium is incorporated into erythrocytes only during erythropoiesis, the GSHPx activity of red blood cells may be a better indicator of long-term selenium status than serum selenium values (although GSHPx values plateau at high selenium intakes, i.e., when blood selenium levels of approximately 0.12 μg/ml serum or 0.16 μg/ml whole blood are reached). Low GSHPx, selenium, and vitamin E values have been reported in affected animals. In the author's laboratory, for example, GSHPx values of greater than 30 IU/ml of red blood cells would be expected in stabled animals fed a balanced diet. Values greater than 25 IU/ml may be expected in grazing animals on an adequate intake; values less than 20 IU/ml are associated with animals with deficient dietary intake. Serum vitamin E concentrations of at least 400 μg/dl are considered normal. Concentrations between 200 and 400 μg/dl are considered marginal, and below 200 μg/dl suggests deficiency. Reference values vary according to the laboratory. One should take care with collection of samples, especially for vitamin E determination, because contact with rubber may interfere with analysis. One cannot use single-serum sample assays as an indicator of vitamin E status in individual horses because of high variability in values between animals and within the same animal.[297] Normal vitamin E concentrations have been reported in some affected foals, possibly reflecting recent ingestion of colostrums. Many of the dams of these foals had low serum vitamin E concentrations (<200 μg/dl), and possibly the selenium levels of affected foald were low before suckling. The liver plays a role in selenium storage, and liver selenium concentrations of three foals (119 ± 5 μg/kg) were statistically lower than those of foals with other diseases (162 ± 20 μg/kg).[296] Liver selenium concentrations on a wet weight basis have been reported to be adequate at 0.300 to 1.000 ppm, marginal at 0.161 to 0.299 ppm, and deficient at 0.160 ppm or less.[298]

Acetate electrophoresis confirmed myoglobinuria in one horse.[152] In other horses the diagnosis of myoglobinuria was made visually or via a positive reaction to orthotoluidine agent in a test strip. Hyperlipemia has been reported in some horses with and without signs of steatitis post mortem.[37,150]

Electrolyte disturbances include hyperkalemia, hyponatremia, and hypochloremia. Such findings may be associated with a poorer prognosis. Metabolic acidosis may be present, and the concentration of blood urea nitrogen often is increased; this may be due at least in part to prerenal factors. One may observe alterations in the hemogram depending on the presence or absence of complications such as aspiration pneumonia.

In foals with evidence of nutritional myodegeneration, a full hematologic and clinical chemistry profile with a urinalysis is advisable. In very young foals, especially those with dysphagia or poor sucking reflex, checking IgG concentrations also is advisable.

9.14.1.2 Postmortem Findings

Postmortem examination of neonatal foals with peracute white muscle disease often reveals few macroscopic lesions. In acute cases, muscle may appear pale and streaks may be present representing areas of coagulative necrosis next to less affected tissue. All muscle groups of the pelvicand thoracic limbs commonly are affected. The muscles of mastication, diaphragm, tongue, pharynx, and cervical musculature also commonly are involved. Distribution of lesions is usually bilateral and symmetric. In subacute cases, one may observe yellow-white streaks in the heart and muscle, representing calcification.[157] Steatitis, yellow fat deposits, and fat necrosis also have been described in some but not all cases.[152]

9.14.1.3 Histologic Findings

Typical histologic findings observed at various stages of nutritional myodegeneration have been described.[157] During the first few days, extensive floccular, granular, and severe hyaline degeneration of myofibers occurs. After about a week, phagocytosis of necrotic tissue occurs with endomysial thickening caused by edema, mononuclear infiltration, and proliferation of fibroblasts. By 2 weeks, epimyseal connective tissue has proliferated. Early regenerating fibers coexist with signs of myodegeneration.

9.14.1.4 Diagnosis

At present, diagnosis of nutritional myodegeneration is based on the following:

1. Appropriate clinical signs
2. Increased serum AST and CK activities, especially in the acute stages
3. Decreased blood selenium and GSHPx values
4. Response to vitamin E and selenium administration

The list of differential diagnoses (see Box 9-6) may be extensive, depending on the signs in the affected animal. However, few of the conditions listed in Box 9-6 result in significant increases in muscle enzyme activity or myoglobinuria. Differentiating this condition from colic is important.[150] Foals with botulism usually have decreased muscle tone.

9.14.1.5 Treatment

Reduction of physical activity and prompt administration of vitamin E and selenium usually are 510
recommended. Injections of vitamin E and selenium usually are required, but because of the potential for 511
anaphylactoid reactions, one should avoid the intravenous route. One should give deep intramuscular injections, although one should note that intramuscular injections may give rise to muscle soreness and have been associated with abscess formation. Therefore one should take care regarding possible toxicity.[295] Aggressive daily oral supplementation of vitamin E coupled with a single intramuscular injection of selenium may be the best route of therapy, because many commercially available selenium/vitamin E combination injectable preparations actually contain little vitamin E. The selenium injection may be repeated in 3 to 7 days if necessary. Nasogastric feeding is preferable in dysphagic, anorectic, or recumbent foals.[298] The foal often

needs fluid therapy to combat dehydration and electrolyte disturbances and to maintain urine output and prevent myoglobin-associated renal damage. One should not use fluids with high potassium content when hyperkalemia is present, although one should take care after the animal has been rehydrated and its acid-base status restored to ensure that hypokalemia does not occur, especially if the horse is dysphagic.

Antiinflammatory agents may be helpful to reduce pain and swelling but should be used with caution in foals because of their ulcerogenic properties. Antibiotic therapy may be advisable if the animal is dysphagic or shows signs of respiratory distress or pneumonia. Selenium deficiency by itself may result in immunosuppression,[298] which may be compounded by complete or partial failure of passive transfer.

9.14.1.6 Prevention

Feeding pregnant mares an adequate level of nutrition including adequate amounts of vitamin E and selenium is imperative. Although whether the status of the mareat delivery has a significant influence on the reserves of these compounds in foals is unproven, nutritionalstatus of the mare is likely at least one importantfactor. Certainly the vitamin E status of the mare has an influence on the likelihood that the foal may develop equine degenerative myelopathy, a neurologic disorder. Therefore vitamin E status may influence the development of white muscle disease as well. In herds or areas where horses are known to be deficient in either compound, supplementation of the foal is recommended. Feeding a constant daily level is preferrable because injectable preparations have been associated with fatalities.

Recommendations for supplementation of vitamin E range from 1.5 to 4.4 mg/kg α-tocopherol acetate daily for adult Standardbred horses on a low vitamin E diet.[299] A least eight different tocopherols with vitamin E activity exist. α-Tocopherol has the greatest activity andaccounts for 70% to 90% of total biologic activity. The supplementary form of vitamin E is usually α-tocopherol acetate, which is not an antioxidant and is therefore much more stable in moisture, heat, and oxygen. α-Tocopherol acetate is converted to active tocopherol during digestion and absorption from the gut. Adding 5 mg of additional vitamin E for every 1% of blended fat has been recommended and may be important for endurance horses. The value of vitamin E injections has been questioned, especially because the amounts within the injectable preparations are low.[152] Feeding good-quality, properly stored hay and grain and allowing access to good-quality green forage may help to maintain vitamin E intake.

Feeding 1 mg/day of selenium maintains blood selenium values above the level associated with myodegeneration in horses and foals.[300] Only limited amounts of selenium cross the placenta, which may explain why clinical cases may occur in offspring of supplemented mares.[301] The milk from mares that have been supplemented appears to contain more selenium than milk from unsupplemented animals, but the amount present is small because selenium is not concentrated in milk. Organic selenium supplementation appears to be more beneficial than inorganic. Therefore although many recommend, especially in deficient areas, that mares be supplemented from late gestation through lactation, others prefer to supplement the foals at birth, 2 weeks, and 6 weeks later,[152,300] although this may not prevent the cases seen at birth or in the first few days of life. Others recommend that, in problem areas, a selenium injection be given at birth and then every 2 to 3 months during the first6 months of life.[126] Still others recommend injections of selenium every 5 to 10 days.[298]

In pigs, selenium has been suggested possibly to be teratogenic. Teratogenesis has not been observed in mares, but one recommendation is that selenium injections not be given early in a pregnancy. The levels recommended for oral supplementation of mares during pregnancy have varied from allowing free access to a trace mineralized salt containing 15 to 30 ppm selenium or feeding a ration containing 0.5 ppm selenium (on a

dry matter basis). Alternatively, feeding at a higher level (0.1 to 0.2 ppm), equivalent to the 1 mg/day mentioned earlier, has been recommended.[298] One must take special care with selenium administration because toxicity can occur. The single minimum lethal dose of oral sodium selenite is 3.3 mg/kg in the adult horse and results in a variety of clinical signs including severe dyspnea, incoordination, diarrhea, recumbency, and death within a few hours. One should not expect increases in GSHPx activity in the blood until 2 to 3 weeks after selenium supplementation. GSHPx values tend to plateau before toxic levels of selenium are reached, and several months may pass before levels decrease following cessation of supplementation. 511

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9.14.1.7 Prognosis

The prognosis for foals with nutritional myodegeneration is guarded, especially if the foal is recumbent or dysphagic or if treatment has been delayed. Prognosis also seems to be less favorable in animals with acid-base and electrolyte disturbances.

9.14.2 RHABDOMYOLYSIS

Acute rhabdomyolysis in neonates hospitalized for prematurity, sepsis, and failure of passive transfer has been reported.[302] Rhabdomyolysis developed after 1 to 6 days of hospitalization and was accompanied by profound hyperkalemia, hyponatremia, hypocalcemia, and hyperphosphatemia. Analysis confirmed that affected foals had low selenium and/or vitamin E status at the time rhabdomyolysis was noted. Whether selenium and vitamin E status was normal or marginal at birth and oxidant stress related to sepsis led to rhabdomyolysis or if animals were born with deficient reserves of selenium or vitamin E is unknown. Rhabdomyolysis also was accompanied by rapid weight gain of 7% to 15% of the body weight of the foal within the 24 to 48 hours preceding the clinical recognition of rhabdomyolysis and was attributed to fluid accumulation (edema) within the affected musculature. Affected foals may show cardiac and renal dysfunction as well. Foals with severe rhabdomyolysis had a poor prognosis. Because of the severity of this complication in foals presented for sepsis, the recommendation is thatall foals presented for sepsis, prematurity, and failure of passive transfer that have mild elevations in CK activity, low GSHPx activity, or low serum vitamin E concentrations be treated with selenium or vitamin E in addition to the therapies directed at their other underlying problems.

9.14.3 GLYCOGEN BRANCHING ENZYME DEFICIENCY

Glycogen branching enzyme deficiency is a recently described disorder in the horse and has been documented as a heritable disorder in human beings and cats.[303–305] Affected foals have been presented to referral hospitals for sepsis, prematurity, and failure of passive transfer. At presentation, foals often have concurrent sepsis, which necessitates treatment. These foals typically have elevated liver enzymes and persistent problems with hypoglycemia during hospitalization. Increases in serum CK activity may be present. Foals may die acutely during hospitalization or respond well to therapy and be discharged. Shortly after discharge the foal is discovered dead.

Muscle biopsy from affected horses shows a complete lack of normal glycogen staining. Normal glycogen is lacking in skeletal muscle, heart muscle, and liver. The disorder has been diagnosed only in Quarter Horses and is likely a heritable trait, although the genetic defect in the horse has not been identified to date. Quarter Horse neonatal foals presented for sepsis with persistent hypoglycemia, elevated liver enzymes, and elevated CK activity should be considered to have this emerging disorder.

9.14.4 ARTHROGRYPOSIS

Arthrogryposis is a congenital developmental defect characterized by muscle contracture and fixation of various joints in extension and flexion (Figure 9-14). The primary defect in most cases is believed to be in the skeletal or nervous system. Occasionally, the defect may be in the muscles. In such cases the muscles may show degenerative changes or atrophy and an increase in adipose and fibrous tissue. These changes also may occur following neurogenic defects. Difficulty ensues in distinguishing primary neuropathic from primary myopathic forms of the disease and results in patients being referred to as suffering from neuromuscular arthrogryposis. In one case, for example, a foal was born following an uncomplicated delivery with a single hindlimb fixed in a 512
flexed position. This foal showed loss of the lower motor neuron cell bodies in the ventral horn of the spinal cord 513
in segments L3 to S4, as well as lesions in the peripheral nerves and muscles of the affected limb. In human beings, most cases are thought to be neurogenic arising from undefined disturbances of the anterior horn cells. Skeletal malformations and connective tissue disorders are rare causes. The infrequent primary myopathic forms are associated with various muscular dystrophies or myotonia and strong hereditary factors.[306]

Figure 9-14 Arthrogryposis in a foal.

The underlying problem resulting in arthrogryposisin foals is unknown, although genetic and environmental factors such as unfavorable intrauterine conditions, uterine malpositioning, and maternal ingestion of locoweed or hybrid Sudan pasture have been suggested.[37,124,306–309] In the horse, detailed examination of neuromuscular tissues rarely has been performed; most cases are suggested to be skeletal in origin. Therefore the relative importance of primary neuropathic or myopathic conditions is unknown.

The extent of the condition varies with involvement of one to four limbs and may include the axial skeleton. Often more severely affected animals are born dead, and dystocia is common. Milder cases such as foals being born with an apparent inability to straighten one fetlock may occur. Affected muscles appear to have lost sarcomeres from the end of the myofibers, possibly because of prolonged flexion of the joint(s) in late gestation. Constant tension seems to restore these sarcomeres.[37]

The recommended symptomatic treatment of arthrogryposis of any cause includes the use of splints, casts, and physiotherapy, as well as surgery[310]; the latter is successful in human infants and calves.

9.15 Miscellaneous Disorders

9.15.1 ATYPICAL MYOGLOBINURIA

Sudden death from exertional rhabdomyolysis is rareand usually occurs in an animal that has undergone some degree of exertion before the attack. Severe and often fatal attacks of a condition with similarities to exertional rhabdomyolysis have been reported in the United Kingdom in groups of animals out at pasture with no history of sudden exertion.[311] The condition has been referred to as atypical myoglobinuria.

9.15.1.1 Pathophysiology

Factors common to many atypical myoglobinuria cases include adverse climatic conditions before the outbreak and the availability of tree bark, often on dead wood. This atypical syndrome characteristically affects a group of horses over a short period of time, and thereforethe possible predisposing trigger or etiologic factor is thought to be environmental or toxic. No consistent abnormality in selenium or GSHPx values has been seen. One affected horse appeared to have a low liver vitamin E concentration, although another animal affected in the same outbreak had an apparently normal value.[311] In some but not all cases *Trichoderma* spp. fungi were isolated from grass and wood taken from the fields grazed by the affected horses. Mycotoxins therefore have been suggested as being of possible significance.

No access to toxins such as monensin or salinomycin was demonstrated in any case. Oak trees with acorns were not always present, and gastrointestinal signs associated with acorn poisoning were not observed.[70] Spraying with an atrazine herbicide occurred before one outbreak.[312] Simazine residues were not thought to be at toxic levels and clinical signs were not those seen with triazene herbicide toxicity (i.e., severe colic, cessation of eating and drinking, and a dog-sitting posture).[311]

The cause of atypical myoglobinuria therefore hasnot been identified. In horses that died, the precise causeof death was uncertain, but damage to the heart and diaphragm in association with biochemical alterations such as hypocalcemia may have been important. Renal failure may be a contributory cause but is unlikely to be the primary cause of death.

9.15.1.2 Clinical Signs

Atypical myoglobinuria occurs most frequently in horses and ponies grazing pasture of low quality. One or more animals in a group may be affected. A sudden onset of stiffness unrelated to exertion occurs, soon followed by severe myoglobinuria. Temperature, heart rate, and respiration rate are generally within the normal range. No age, sex, or breed predilection has been observed, but few cases have been investigated. Of cases that have been well documented, progression of clinical signs is rapid and mortality is high. Despite profound weakness and recumbency, many affected horses do not appear to be in any distress or pain, and they eat and drink well, even when recumbent. Serum CK activities are greatly increased, and advanced myodegeneration of all muscles, including the heart, may be present.[311]

Myodegeneration indistinguishable from that seen in exertional rhabdomyolysis under the light microscope has been found (although fiber preference was not determined).[306] Large hyperchromatic hepatocyte nuclei were observed in some liver sections together with patchy vacuolation and infiltration of neutrophils into the portal areas. Pink proteinaceous granular material tended to be present in the kidney tubules and within Bowman's capsule.[311] Skeletal abnormalities may not be obvious on gross postmortem examination.

High CK activity (often >5000 IU/dl) caused by myodegeneration is observable. AST activity also tends to be 513
increased greatly. High sorbitol dehydrogenase values (up to 28.8 IU/dl; laboratory reference range, 4 to 15 514
IU/dl) were found in several cases in one investigation.[306] Hypocalcemia may occur, especially in the terminal stages. Myoglobin usually is present in a sufficiently high concentration in the urine to be detectable by electrophoresis on cellulose acetate.

9.15.1.3 Treatment

Because the cause of the condition is unknown, symptomatic treatment is recommended, including intensive fluid therapy, which should be based on biochemical monitoring. One should monitor the calcium status in particular, because horses may become hypocalcemic. Feeding a readily available source of calcium may be of benefit in some cases.

9.15.2 POSTANESTHETIC MYASTHENIA

Postanesthetic myasthenia has been reported in three horses.[313] The condition could be a form of botulism or a drug-induced myasthenia. The characteristic signs were a difficult recovery from anesthesia, lack of any facial expression, flaccid tongue, mydriasis, an inability to raise the head, and dysphagia. All three patients recovered totally with supportive care.

9.15.3 POLYMYOPATHY

An aged pony mare developed a slowly progressive stiffness of gait that varied in severity. She was reluctant to move or eat and exhibited general loss of muscle bulk. The condition responded temporarily to NSAIDs. Needle EMG revealed a diffuse polymyopathy. Muscle histopathologic study revealed shrinkage, fragmentation, loss of muscle fibers, replacement of muscle bundles with adipose tissue, and some evidence of myofiber regeneration. The cause of the condition was unknown. Other cases of polymyopathy of unknown cause have been suspected in horses and await documentation and further categorization.

9.15.4 SUPPURATIVE MYOSITIS: ABSCESSATION

Suppurative myositis may be hematogenous in origin or result from penetrating wounds, intramuscular injections, or an extension of an infective focus in an adjacent or distant structure. *Streptococcus equi* is a frequent cause, because injections of a variety of intramuscular vaccinations lead to abscessation. Affected horses often initially have an ill-defined cellulitis, which may heal, progressto a classic organized abscess, or in the case of certain staphylococci, may extend, resulting in extensive muscle damage. Abscesses may heal slowly, expand, or fistulate to the surface. Once fistulated, abscesses may collapse and heal, usually with scar tissue, or persist as chronic granulomata (especially *Staphylococcus aureus* in the neck and pectoral region).[37] *Corynebacterium pseudotuberculosis* also may be isolated from large abscesses in various muscles, in particular, the pectorals.

A significant geographical variation appears in the clinical signs with *C. pseudotuberculosis* infection. Worldwide, ulcerative lymphangitis—usually with sores, abscesses, fever, lameness, anorexia, and lethargy, which may progress to chronic lameness and weight loss—is the most common condition. In the western United States, especially in the more arid parts, the organism tends to be associated with ventral midline, inguinal, and pectoral abscesses, although internal abscesses may occur. The condition has been referred to as pigeon fever (after the swollen pectorals giving the appearance of a pigeon breast), or alternatively as dryland distemper or Colorado strangles (after its geographical distribution).

Suppurative myositis can occur at any time of the year but is more common in late summer, fall, and early winter. One or more horses within a group may be affected. Outbreaks in a number of horses at a single establishment have been reported. The organism can survive in the soil and enters the body via lesions in the skin or mucous membranes and spreads via the lymphatics. Insect vectors may be involved. Clinical signs varywith the stage of the condition and the site of the abscess(es).[298,314] The affected horse may be pyrexic and anorectic during the maturation phase of the abscess. Ventral pitting edema, lameness, weight loss, and depression also may occur. If the abscesses are located in the axillary or inguinal regions, the affected animal can be very lame and is more likely to be intermittently febrile. Such cases can be difficult to diagnose because the abscesses may take weeks or even months to develop fully. Increased white blood cell counts and plasma fibrinogen concentration may be observed, although in the more chronic stages few clinicopathologic changes are seen. *C. pseudotuberculosis* infections are less likely than staphylococcal or streptococcal abscesses to result in an increased white blood cell count or plasma fibrinogen concentration. Abscesses typically form deep in muscles and can be large with thick capsular walls filled with a nonodorous, light tan pus. The differential diagnosis includes seromata, tumors, and other bacterial abscesses. One can confirm the diagnosis by ultrasound or culture of any aspirated fluid. Abscesses in the axillary region, in particular, can be difficult to locate, even with ultrasound. A synergistic hemolysis inhibition test detects antibodies to the organism, but the intensity of the antibody response depends on a number of factors, including thickness of the capsule surrounding the abscess and chronicity of the infection. For example, horses with chronic thick-walled abscesses that have been lanced recently may have low or undetectable circulating antibody concentrations, perhaps because antibodies have been used in combating the massive toxin release. The test can be helpful in determining whether one should 514
include internal *C. pseudotuberculosis* abscessation in the differential diagnosis, however. 515

Recommended treatments include encouraging the maturation process via hot poultices, lancing, flushing, and draining. In some cases surgical intervention may be required to expose the abscess adequately. Some controversy exists over the use of antibiotics, especially respecting the timing of administration, which may depend on the stage of abscessation. Antibiotics commonly recommended include procaine penicillin G at 20,000 IU/kg intramuscularly every 12 hours or potassium penicillin G at 40,000 IU/kg intravenously every 6

hours, sulfadiazine-trimethoprim, and erythromycin. Rifampin at 2.5 to5.0 mg/kg orally every 12 hours often is recommended in combination with penicillin. Antibiotic therapy ideally should continue for several weeks.

Prognosis is guarded; some abscesses do not resolve completely with treatment. Some recur when antibiotic therapy is discontinued; others may recur months later. In a few horses, internal abscesses may develop, resulting in chronic weight loss and sometimes ventral edema, ascites, dyspnea, recurrent colic, exercise intolerance, recurrent pyrexia, etc. Abortion also can be a sequela.[298] Avoiding contamination of paddocks via a draining lesion and dealing with contaminated bedding appropriately has been recommended. Good fly control also can be beneficial.

9.15.5 MUSCLE TUMORS

Primary tumors of skeletal muscle are rare, malignant tumors being twice as frequent as benign ones. Rhabdomyosarcomata of the limbs, head, or neck appear as hard, spherical masses deep in the muscle. A significant proportion appears to occur in sites of earlier muscle fiber destruction and repair. Frequently, animals less than 2 years old are affected. Other benign and malignant primary tumors, including lipomata, liposarcomata, fibromata, fibrosarcomata, myxomata, and hemangiosarcomata, can occur in muscles. Metastatic spread to the muscles, especially of malignant melanomas, angiosarcomata, and tumors of the lymphoreticular system, also may occur.[37]

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9.16.2 Uncited references

106. Reference deleted in proofs.

133. Reference deleted in proofs.

234. Reference deleted in proofs.

9.17 9.1—Pathophysiology and Treatment of Acute Laminitis

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Acute laminitis (founder) is a severely debilitating, excruciatingly painful, and potentially career-ending and life-threatening disease of the soft tissues (sensitive and insensitive laminae) of the equine digit. Laminitis is important to all horse owners and trainers and horse enthusiasts because it can occur in adult horses and ponies of any breed or use (athletes or companions/pets). Laminitis usually occurs following other diseases such as acute gastrointestinal tract disease (colic), particularly strangulating obstruction and inflammatory bowel disease (anterior enteritis and enterocolitis); grain overload; retained fetal membranes and subsequent metritis; pleuropneumonia; and other diseases accompanied by endotoxemia. Gastrointestinal tract disease was the most common primary disease in 54% of horses that developed acute laminitis in a study conducted in seven private practices and at a university veterinary hospital.[1] Additionally, support limb laminitis occurs commonly in the contralateral limb because of overload or excessive weight bearing in horses that have a severe non–weight-bearing lameness (fractures, bone or joint sepsis) in the opposite limb.

Laminitis is frustrating for veterinarians because current knowledge and understanding of the pathophysiology and progression of the disease are incomplete, limiting efforts to prevent and treat this devastating disease successfully. Laminitis causes profound emotional stress and economic loss to horse owners and trainers because of the agonizing pain experienced by the horses. Laminitis often leads to poor body condition and prolonged periods of

recumbency with secondary pressure sores. Many affected horses only rise for short periods of time and demonstrate a characteristic stance of rocking or shifting their weight onto their rear feet, which is accompanied by anxiety, muscle fasiculations, and sweating. Approximately 75% of laminitic horses treated at a university hospital did not return to athletic soundness; most of these horses ultimately were euthanatized because of the severe pain associated with separation of the sensitive and insensitive laminae resulting in rotation or distal displacement of the third phalanx.[2]

Fifteen percent of horses in the United States are estimated to be afflicted with laminitis over the course of their lifetimes, and 75% of these horses develop severeor chronic lameness and debilitation that necessitates euthanasia. The percentage represents a substantial number of horses in the United States and worldwide that suffer from this devastating disease and ultimately are destroyed. From an economic perspective the diagnosis and treatment of laminitis is estimated to cost$8 million annually, and the monetary loss of animals euthanized each year following complications of laminitis is approximately $5 million.

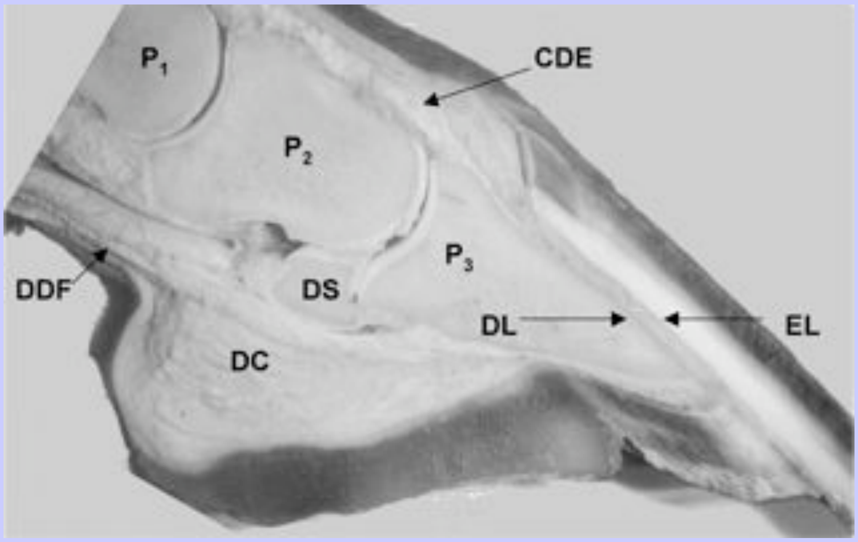
Scientific investigations have shed light on the pathophysiologic events involved with laminitis; however, additional studies are needed to unravel the remaining mysteries regarding the initiation and propagation of laminitis. Currently, numerous and varied therapies are used to prevent and treat laminitis; however, clinicians' preferences and impressions regarding the most effective treatments are based on an incomplete understanding of the initiating events in this disease. Because of the gaps in knowledge of the pathophysiology of laminitis, the effectiveness of the currently used treatments is inconsistent at best. Therefore developing a more thorough understanding of the cascade of events involved with the onset and propagation of acute laminitis should help veterinarians develop more rational, effective, and cost-efficient therapies to prevent and treat a disease with profound humane, emotional, and economic effects on horses, horse owners/trainers, and veterinarians. This section provides a comprehensive review of the current knowledge of the pathophysiology of acute laminitis and presents experimental findings that should contribute to a better understanding of this pathogenic cascade and ultimately may help improve the prevention and treatment of laminitis in horses.

9.17.1 ANATOMY AND PHYSIOLOGY OF THE DIGIT

The normal laminar tissue and its vasculature are unique in numerous aspects. The ability of the equine athleteto 522
walk depends on the integrity of the interdigitating primary and secondary laminae, which structurally unite the 523
hoof wall, distal phalanx, and the sole of the foot into a single unit.[3] The bulk of the hoof is composed of the stratum medium, which is composed of avascular, highly keratinized stratified squamous epithelium. This layer blends with the stratum internum, which comprises the primary and secondary epidermal laminae. Approximately 600 primary laminae form longitudinal grooves for interdigitation with the vascular laminae of the laminar dermis (corium). The laminar corium unites with the subcutis and periosteum of the distal phalanx.[4]

The bones of the digit are the proximal, middle, and distal phalanx and the distal sesamoid bone (navicular bone) (Figure 9-15). The primary joint of the digit is the distal interphalangeal joint composed of the middle and distal phalanx and the distal sesamoid bone. The short collateral ligaments join the distal end of the middle phalanx and the proximal edges of the distal phalanx. The collateral sesamoidean ligaments extend from the distal aspect of the proximal phalanx and insert on the edges of the distal sesamoid bone. A branch of this ligament also inserts on the palmar process of the distal phalanx. The distal sesamoid impar ligament arises from the distal aspect of the distal sesamoid bone and extends to the palmar surface of the distal phalanx. A fibrous connection between the palmar surface of the middle phalanx and the deep digital flexor tendon forms a T-ligament. The deep digital flexor tendon inserts on the palmar aspect of the distal phalanx, and the common digital extensor tendon inserts on the extensor process of the distal phalanx.[4,5]

Figure 9-15 Cross section of the equine digit. P_1, Proximal phalanx; P_2, middle phalanx; P_3, distal phalanx; *DS*, distal sesamoid bone; *DC*, digital cushion; *DDF*, deep digital flexor tendon; *CDE*, common digital extensor tendon; *DL*, dermal laminae; and *EL*, epidermal laminae.



The distal interphalangeal joint capsule joins with the common digital extensor tendon, the collateral ligaments of the distal interphalangeal joint, the distal sesamoid impar ligament, and the T-ligament. The joint capsule has two main pouches, the dorsal pouch and the palmar pouch, and the palmar pouch is divided further into proximal and distal pouches. The digital cushion is a large soft tissue structure located between the base of the cartilages, structures located palmar to the collateral ligaments composed of hyaline cartilage that progress to become predominately fibrocartilage. The digital cushion is composed of fibroelastic tissue, adipose tissue, and a small percentage of fibrocartilage. A venous plexus is also located within the digital cushion.[4]

The nutrients to maintain the integrity of the corium come from the laminar arteries branching from the circumflex artery as it curves around the toe. These laminar arteries course in a distal to proximal direction. The laminar veins that course distally into the circumflexvein remove metabolic wastes and drain into the bulbar vein and digital veins. Arteriovenous shunts betweenthe circumflex artery and vein have been demonstrated in acute laminitis.[4] These shunts could alter hoof temperature by providing a path of rapid flow fromthe laminar arteries to the veins, thus bypassing the laminar capillaries; this cooling effect could decrease the metabolic requirements of the laminae. However, this flow pattern robs the laminae of nutrient flow via its capillary bed. 523

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The digital arteries and veins supplying the hoofhave unique characteristics. The digital veins are highly muscular compared with veins in other tissues and other species.[6] This muscular wall probably is needed to withstand the high vascular pressures exerted in these dependent tissues. The highly muscular wall is likely responsible for the low compliance of the veins.[7] In exercising horses the pressure in the venous circulation may reach 200 mm Hg.[8] The equine digital arteries and veins are highly sensitive to vasoconstrictive substances, most notably norepinephrine and endothelin. Furthermore, the digital veins are most sensitive in vitro to

vasoconstrictive substances.[9] For example, contraction induced by angiotensin, thromboxane, norepinephrine, serotonin, and endothelin is twice as strong in veins as arteries. The culminating effects of low compliance and high sensitivity to vasoconstrictive substances predispose the equine digit to high venous pressures, thereby increasing hydrostatic pressure and thus the likelihood of edema formation.

The microcirculation of the equine foot is adapted poorly to handling edema. In normal tissues, three safety factors counteract edema formation, including capillary permeability, pre- to postcapillary resistance, and lymphatic drainage. An impermeability of the capillary endothelium serves as a barrier to fluid and protein transudation, which results in a higher gradient between the capillary and tissue oncotic pressure, favoring movement of fluid into the capillary lumen. Paradoxically, the equine digital capillary bed is highly permeable to fluid and macromolecules and is more permeable than the vasculature of the dog and rat paw.[7] This permeability results in a higher concentration of interstitial protein, favoring edema formation. A high precapillary (arteriolar) resistance and low postcapillary (venous) resistance reduce the capillary pressure, thereby reducing the hydrostatic pressurefor transcapillary fluid filtration. The precapillary-to-postcapillary resistance ratio in healthy horses is comparable to that in other musculoskeletal beds in other species. However, during the prodromal stages of experimentally induced laminitis (either the black walnut extract [BWE] or the carbohydrate overload [CHO] models), the contribution by the postcapillary portion increases, thus favoring edema formation. Lymphatic drainage provides the third edema safety factor. The small diameter and number of metacarpal lymphatics and the hydrostatic gradient to lymph flow reduce the likelihood that lymphatic circulation effectively can protect the foot against edema when the hydrostatic forces in the capillary favor edema formation.[10]

9.17.2 CLINICAL SIGNS AND DIAGNOSIS

Laminitis is a disease that can affect all four feet; however, laminitis most commonly affects the forelimbs because they bear approximately 60% of the mass of the horse.[3] The increased load of the forelimbs compared with the hindlimbs is thought to account for the increased occurrence of laminitis in the forelimbs. To define better the severity of clinical signs exhibited by horses, Obel established a grading system in 1948. Grade 1 is the least severe and states that the horse alternately and incessantly lifts the feet and that lameness is not evident at a walk but is evident at a trot as a short, stilted gait. Horses that walk with a stilted gait but still have a foot lifted are classified as grade 2. Horses with grade 3 laminitis move reluctantly and vigorously resist lifting of a foot. The most severe classification is grade 4, noted by the horse refusing to move unless forced.[11] Other clinical signs characteristic of laminitis are heat present over the dorsal surface of the hoof wall, bounding of the digital pulse (increase in the difference between the systolic and diastolic digital arterial pressure), sensitivity to hoof testers, swelling of the coronary band, and alteration of stance to redistribute weight to the hindlimbs (sawhorse stance or rocking of weight to the hindlimbs) if laminitis is principally affecting the front limbs (Figure 9-16). More severe signs are a dropped sole or palpation of a depression located at the level of the coronary band, both indications of rotation or distal displacement (sinking) of the distal phalanx within the hoof wall.[5,12] Lateral radiographs of the digit are indicated for detection of rotation and distal displacement of the distal phalanx within the hoof capsule (Figures 9-17, *A* and *B*). The placement of a radiopaque marker 1 cm below the coronary band is helpful when determining the presence of distal displacement of the distal phalanx. The presence of a linear radiopaque marker in the block on which the horse stands is helpful to assess the angle and degree of rotation.

Figure 9-16 A few of the clinical signs commonly associated with laminitis include weight redistribution, pressure sores, and poor body condition as demonstrated in this horse with forelimb laminitis.

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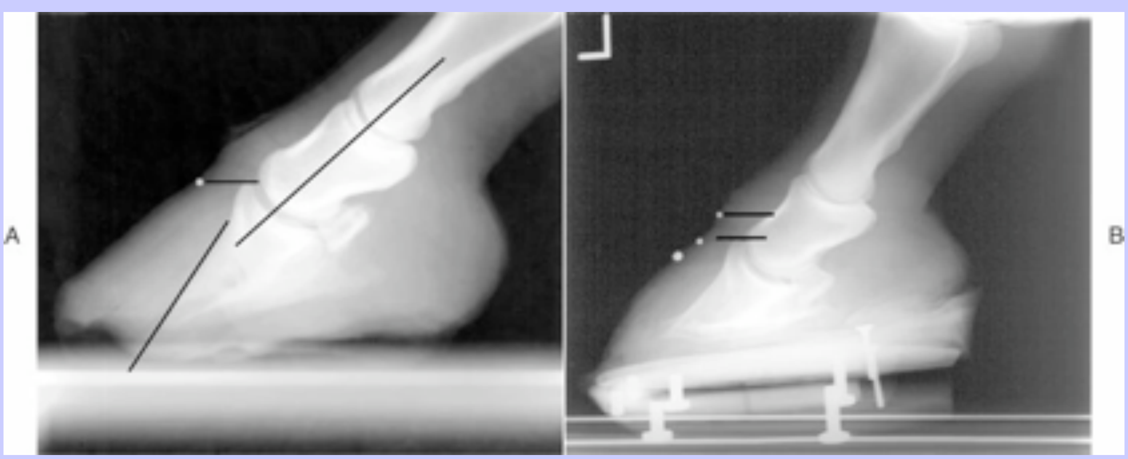
Figure 9-17 A & B **A**, Lateral radiograph of the equine digit demonstrating rotation of the distal phalanx within the hoof capsule. Note the black line drawn parallel to the dorsal surface of the phalanx and the angle this forms with the radiopaque marker in the block, which is used to aid in the assessment of the magnitude of rotation. The line drawn axially through the proximal and distal interphalanageal joints should be approximately parallel to the dorsal surface of the distal phalanx in the normal horse, and deviation from this angle represents distal phalanx rotation. Also, note the placement of the round radiopaque marker in the dorsal hoof wall 1-cm distal to the coronary band, which is used to evaluate distal displacement of the phalanx. This marker should be approximately at the level of the extensor process in normal horses. The vertical distance between this line and the line drawn at the level of the extensor process represents the degree of displacement. **B**, Lateral radiograph of the digit demonstrating marked distal displacement of the phalanx. Note the three radiopaque markers that were used for serial radiographic evaluation. These can also be helpful in assessing dorsal hoof wall growth. (Courtesy Dr. Ralph E. Beadle.)



9.17.3
HISTOPATHOLOGIC FINDINGS

Histologic study of laminar changes during laminitis has been performed 48 to 96 hours after induction of laminitis with cornstarch or wheat flour gruel. Lameness begins approximately 30 hours after administration of the induction ration.[10] Evaluation of a progression of lesions by these studies is difficult because the onset and severity of lameness varies substantially from horse to horse. Studies using these models are confounded further by the fact that approximately 10% of horses appear to be resistant to the ration.

Following the onset of lameness, the initial histologic alteration occurs in the digital vasculature, including swelling of the endothelial cells and mild edema formation.[13] Laminar capillaries become obstructed with erythrocytes within 8 hours. Within 6 to 12 hours, a perivascular leukocyte infiltration occurs that then dissipates as the inflammatory cells migrate into the epidermal layer. Arteriolar endothelial cells become deformed because of cytoplasmic processes that extend into the lumen. Microvascular thrombi and accompanying severe edema formation occur within 24 hours, and hemorrhage occurs within the primary dermal laminae by 72 hours.

Primary histologic alterations of the laminae occur within 8 hours after lameness develops.[13] Initially, thinning and lengthening of the lamellar structures is accompanied by reduction, flattening, and displacement of epithelial cells. The secondary laminae become redirected such that laminae nearer the base of the dermal lamina are directed toward the coffin bone and those nearer the laminar tips are directed toward the hoof wall. Morphologic alterations following epithelial cell damage include swelling, vacuolization, nuclear swelling and pyknosis, and leukocytic infiltration of the secondary epidermal lamina, which is observable as early as 24 hours after the onset of lameness.

9.17.4
PATHOGENESIS

Development of acute laminitis often follows other primary diseases; therefore the mechanisms involved in the pathogenesis of laminitis are most likely numerousand interrelated. Currently, three main theories have been 525
526 proposed regarding the pathogenesis of acute laminitis in horses: the ischemic/vascular, mechanical/traumatic, and metabolic/enzymatic theories. However, almost certainly multiple factors from each theory are part of the pathogenesis of the disease.

The ischemic/vascular theory poses altered digital perfusion as the initiating factor in the cascade of events that leads to metabolic dysfunction and structural failure of the laminae.[13] Although the pathogenesis of laminitis is not understood fully, the initial vascular mechanisms are characterized by hypoperfusion caused by venoconstriction; laminar edema formation; opening of arteriovenous shunts, allowing blood to bypass laminar tissues and leading to tissue ischemia; necrosis of the interdigitating laminae; and ultimately mechanical failure with rotation or sinking of the distal phalanx away fromthe hoof wall.[10,14–16] Venoconstriction is considered the initiating factor causing decreased laminar perfusion.[17] Increased venoconstriction results in increased vascular resistance and capillary hydrostatic pressure. Increased capillary hydrostatic pressure forces fluid out of the capillaries and into the interstitium, thereby increasing laminar interstitial pressure. When tissue pressure exceeds the capillary critical closing pressure, the capillaries collapse, leading to tissue ischemia. Increased pressure in a confined anatomic space can affect blood flow of those tissuesand can lead to ischemia of those tissues; this condition is referred to as compartment syndrome. Allen, Clark, Moore, et al. hypothesized that horses develop compartment syndrome within the digit during the developmental stages of laminitis, leading to laminar ischemia.[10] Formation of arteriovenous shunts at the level of the coronary band is hypothesized to

reduce blood flow futher.[17,18] The digital laminae undergo necrosis after prolonged ischemia. Separation of the interdigitating sensitive and insensitive laminae develops, and distal phalanx rotation, distal displacement, or both subsequently occur.[19]

Garner, Coffman, Hahn, et al. introduced the hypothesis that the predominant cause of laminitis after CHO overload was a disturbance in digital blood flow, which occurred during the onset of the syndrome after CHO overload of the gastrointestinal tract.[15] Using contrast radiography, researchers demonstrated reduced perfusion in the terminal vasculature of the foot.[20] Garner, Coffman, Hahn, et al. also determined that the changes in digital perfusion are associated with significant systemic hemodynamic changes, including a decline in right atrial pressure, diastolic systemic arterial pressure, and systolic systemic arterial pressure, which reaches a maximum about 16 hours after administration of starch via nasogastric tube.[21] This pressure drop is followed by a steady increase in right atrial pressure, diastolic arterial pressure, and systolic arterial pressure. These results suggest that appreciable cardiovascular changes occur in horses with laminitis, and increased release or activation of vasoactive mediators occurs.

The mechanisms responsible for digital hypoperfusion were evaluated first by tracing radioactive albumin particles through the foot during the development of laminitis. A reduction in laminar capillary perfusion and shunting of blood at the level of the coronary band suggests the presence of arteriovenous anastomoses (arteriovenous shunts) that open during the development of laminitis, resulting in hypoperfusion of the digital microcirculation.[14] Although Allen, Clark, Moore, et al. demonstrated a reduction in digital blood flow and perfusion at 16 hours after experimentally induced CHO overload,[10] Pollitt and Davies recently demonstrated increases in hoof temperature, suggesting an increase in blood flow to the tissues encased within the hoof capsule.[22] Conversely, Hood, Wagner, Brumbaugh, et al. demonstrated a decrease in hoof temperature after CHO overload.[23] Hinckley, Fearn, Howard, et al. recently used infrared techniques to demonstrate stasis of blood within the hoof during early laminitis.[24] Studies suggest that arteriovenous shunts open between the laminar arteries and veins,[25] diverting blood flow from the capillaries providing nutrients to laminar tissues while increasing hoof wall temperature. Collectively, these studies suggest an increase in resistance in the digital circulation (possibly in the venous circulation, causing stasis) diverting blood to low resistance shunts. These results illustrate the difficulty of evaluating capillary perfusion by use of temperature data alone.

In subsequent studies using the isolated perfused digit, the specific hemodynamic forces acting on the laminar microcirculation in healthy and experimentally induced laminitic horses have been defined extensively.[10,16,26] Several alterations in the digital vascular system of horses with experimentally induced Obel grade I laminitis (CHO overload and BWE models) have been identified.[10] Of particular importance is the finding that the precapillary-to-postcapillary resistance ratio is decreased in the prodromal stages of laminitis. This imbalance increases the hydrostatic force in the capillary, promoting the fluxof fluid across the capillary bed within the foot and resulting in laminar edema while capillary permeability remains normal. These findings support the hypothesis that increased venomotor tone initiates laminitis.

Although laminitis induced by CHO overload or BWE are accompanied by increases in capillary pressure and tissue pressure, differences between the two models have been observed.[10,26] The severity of the venoconstriction accompanying laminitis induced with BWE was less than that associated with CHO overload. These less severe changes with BWE may be caused by a difference in the pathophysiology of the disease or may be because the Starling's forces accompanying laminitis caused by BWE were evaluated at a different stage 526
of the disease. Adair, Gole, Schmidhammer, et al. recently determined that laminar microvascular blood flow 527

decreases in the first 1 to 2 hours after administration of BWE.[27] This initial decrease then is followed by a return of laminar microvascular blood flow to near baseline values. Then at about 8 hours into the disease, laminar blood flow again decreases corresponding to the development of clinical signs of laminitis. The time course of blood flow changes associated with CHO-induced laminitis has not been studied for comparison. More information is needed to determine the relationship between CHO overload andBWE-induced laminitis.

Weiss, Evanson, McClenahan, et al. demonstrated a significant increase in platelet-neutrophil aggregates, but not platelet aggregates or clotting times, in ponies administered CHO overload.[28] Furthermore, pretreatment with a platelet aggregation inhibitor prevented laminitis in ponies administered CHO overload, highlightingthe importance of these activated cell aggregates in the pathogenesis of laminitis.[29] Local production of platelet-activating factor within the enterohepatic circulation may result in formation of platelet-platelet and platelet-neutrophil aggregates that lodge in the microvasculature of the digit.

The ischemic hypothesis has focused on digital hemodynamic alterations; however, the initiating mediator or mediators that trigger these vascular alterations have yet to be determined. Katwa, Johnson, Ganjam, et al. recently demonstrated that the concentration of endothelin-1, a potent endothelial-derived vasoconstrictor, in laminar connective tissues obtained from experimentally induced acutely laminitic horses and naturally occurring chronically laminitic horses was increased compared with a control group.[30] Another study examining the potent vasoconstrictor endothelin-1 found that in vitro, contractile responses of equine palmar digital veins were more than 3 times greater than responses of arteries to endothelin-1 administration.[9,31–33] Previous research has demonstrated that nitric oxide donor administration improves digital perfusion and reduces the bounding digital pulses associated with acute laminitis in ponies.[34,35] Nitric oxide is an endothelial-derived vasodilator that plays a role in regulating endothelin-1 release.[36] Based on these studies, an imbalance in endogenous endothelial-derived substances, such as endothelin-1 and nitric oxide, possibly may play a role in the vascular alterations that occur during the development of laminitis in horses.

The mechanical or traumatic theory is based on causes of laminitis resulting from direct trauma to the digit and not a primary systemic disease leading to the development of laminitis.[12] Common examples of traumatically linked laminitis are road founder, laminitis following unilateral lameness of the opposite foot (support limb laminitis), and development of laminitis after long trailer rides.[12,37] The exact mechanisms that lead to structural failure of the laminae are unknown, but several hypotheses have been suggested. Excessive force applied to the dermal and epidermal laminar interdigitations may initiate an inflammatory response with vasospasm, thereby increasing capillary hydrostatic pressure, leading to edema formation and ultimately resulting in a compartment-like syndrome much like that of the ischemic/vascular theory. Another hypothesis is that application of excessive force results in tearing of the dermal and epidermal laminar interdigitations; the inflammatory response or vasospasm or both then ensue, leading to ischemic damage of the laminar interdigitations.[12]

The toxic or enzymatic theory states that the fundamental event leading to the failure of the laminar interdigitations is delivery of blood-borne toxins to the epidermal laminae resulting in weakening and loss of cellular attachments.[38] Based on this theory, the loss of these cellular attachments precedes the vascular and inflammatory alterations described within the ischemic theory. Pollitt and Davies state that instead of the hypoperfusion demonstrated by ischemic theory proponents, hyperperfusion of the digit is responsible for delivery of these toxins to the laminar tissues.[22] Pollitt and Daradka state that the targets of the blood-borne toxins are the mediators of enzymatic remodeling that are a part of the normal processes involved in the movement of the continually proliferating hoof wall past the distal phalanx. Laminin and type IV and type VII collagen are components of the laminar basement membrane. The enzymes metalloproteinase-2 and

metalloproteinase-9 are believed to dissolve these substances, and under normal physiologic states, controlled dissolution allows the movement of epidermal laminae past the dermal laminae as growth occurs.[38,39] Excessive activation of these enzymes leadsto uncontrolled dissolution of the basement membrane components, resulting in separation of the epidermal laminae from the dermal laminae. Laminar samples from horses 48 hours after induction of laminitis using the CHO model demonstrated loss of basement membrane attachments.[40] In horses with naturally acquired acute and chronic laminitis, zymography of laminar connective tissues found increased activation of extracellular metalloproteinases compared with nonlaminitic horses.[41] Activation of the metalloproteinases is hypothesized to be induced by the exotoxin(s) from *Streptococcus* species, especially *Streptococcus bovis*, a gram-positive bacteria found in the normal cecal flora.[38,42,43] Using the CHO model, researchers have identified changes in the bacterial population of the cecum with fermentation of the CHO, resulting in excessive lactate production, rapid decline in intracecal pH, and death of cecal bacteria including *Streptococcus* species.[43,44] Based on this theory and its supporting data, prevention of laminitis should aim 527 toward abolishing activation of the enzymes responsible for the dissolution of the basement membrane. 528

Obviously, numerous mediators are present in acutely laminitic horses; therefore the authors believe that a single unifying mechanism is unlikely to cause the laminar damage. Most likely, vascular derangement and direct mediator damage coincide.

9.17.5 CURRENT TREATMENT

Treatment of laminitis remains empiric and often based on the experience and preference of the clinician. Effective treatment of laminitis requires aggressive and appropriate treatment of the primary disease process. Additionally, the cornerstones of treatment of horses with acute laminitis are directed at different components of the pathophysiologic process. One should consider acute laminitis a medical emergency and should institute treatment immediately after clinical signs develop or, preferably, before the onset of clinical signs. Effective prevention and treatment of acute laminitis involves a multitherapeutic approach. The authors believe the goals of treatment are to eliminate or minimize any predisposing factors; reduce the pain/hypertension cycle; reduce or prevent the magnitude of permanent laminar damage; improve or reverse deleterious digital or laminar hemodynamics, including normalizing digital Starling's forces; and prevent further movement of the distal phalanx within the hoof capsule. Considerable controversy exists regarding the treatment of laminitis because of the lack of understanding of the pathophysiology of this disease. The authors developeda therapeutic plan by concentrating on the cornerstones of treatment of acute laminitis, which include treating the primary disease, giving analgesic and antiinflammatory therapy, restoring normal digital Starling's forces, normalizing digital blood flow and hemodynamics, providing mechanical support to the third phalanx via frog support, and limiting mechanical forces on the interdigitating attachments of the dermal and epidermal laminae.

To institute preventive treatment for laminitis, one must identify those horses at risk. Many of the primary diseases thought to predispose horses to develop laminitis are associated with circulating endotoxin. One of the most important preventive measures is combating the effects of endotoxemia and sepsis effectively by decreasing the severity of the primary illness. Recommended treatments include administration of mineral oil (if the horse engorged on grain), intravenous fluids, parenteral antibiotics, nonsteroidal antiinflammatory drugs, and hyperimmune serum or plasma. Other preventive treatments include heparin, aspirin, vasodilators, corrective hoof trimming and shoeing, placement of the horse in a deeply bedded stall, and frog support. Many of these preventive measures also are instituted therapeutically.

The authors believe one of the most important considerations in developing a preventive and therapeutic plan is to attempt to normalize digital Starling's forces. Because of the anatomy of the normal lamina and hoof and the

microvascular alterations that develop with the onset of laminitis, horses are predisposed to developing significant laminar edema, which leads to compressionof the nutrient capillaries, resulting in laminar ischemia, reduction of metabolic waste removal and ultimately laminar necrosis and degeneration. Therefore one should take steps to normalize these Starling's forces. The best approach is to make sure plasma oncotic pressure is sufficient by supplementation with plasma or another colloidal solution such as hydroxyethyl starch or plasma. Additionally, one should take care when administering intravenous fluid therapy to horses with acute laminitis or those predisposed to develop it, because excessive intravascular volume caused by overzealous fluid administration could tend to perpetuate development of laminar edema in horses with abnormal digital hemodynamics (i.e., increased capillary hydrostatic pressure). Therefore the authors suggest that one monitor fluid therapy carefully so as not to administer fluids in excess of the volume needed to maintain normal hydration.

Because many clinicians believe, and substantial scientific data suggest, that the initiating event in laminitis is a vasoconstrictive event, treatments directed at improving digital blood flow and laminar perfusion often are suggested. The authors believe that an ischemic episode caused predominantly by venoconstriction is the initiating event and recommend administration of drugs to cause vasodilation and subsequently improve digital blood flow, Starling's forces, and laminar perfusion. The drugs most commonly used to improve digital blood flow are acepromazine (0.03 to 0.06 mg/kg intramuscularly every6 to 8 hours for a minimum of 3 days), isoxsuprine hydrochloride (1.2 mg/kg orally every 12 hours), and topically applied glyceryl trinitrate (2 to 4 mg/hr). These drugs have been shown not to alter blood flow or laminar perfusion in normal, nonlaminitic horses; however, their effectiveness in laminitic horses has not been reported.

With the recent suggestion that vasoconstriction early in the onset of laminitis may have a protective effect by limiting the delivery of gut-derived toxic substances that have direct cellular damaging effects to the laminae, some investigators and clinicians suggest that vasodilation may not be the most appropriate vascular effect in the developmental stages. Based on this information, some investigators and clinicians suggest preventing the vasodilatory events during the developmental phases of laminitis to reduce delivery of these substances to the laminae. Additionally, exercise of an intensity that increases core hoof temperature or local anesthesia of the 528
palmar or plantar nerves results in hoof wall warming and, by inference, vasodilation; therefore intense exercise 529
is believed to be contraindicated during the early phases of laminitis. One method reported to be effective in preventing the vasodilatory phase that has been recommended is to soak the feet in crushed ice or cold water. This is a time-honored treatment; however, one would need to bathe the hooves continuously and during the developmental phase before the onset of lamellar damage. Otherwise, vasodilation and a rebound hyperemia would occur once the feet were removed from the cold therapy. The ability to keep the feet cold enough to alter blood flow is difficult practically speaking, and the normal regulation of blood flow (based on tissue needs) may overcome cold-induced vasoconstriction. As many times is the case, the developmental phase (preclinical signs) goes unnoticed and lamellar damage has occurred by the time treatment is initiated. At this stage, blocking delivery of cytotoxic substances would be too late, and the authors think that reestablishment of flow is important for oxygen and nutrient supply to the tissues and to remove potential cytotoxic substances and cellular wastes. Although the understanding of the pathophysiology of laminitis is incomplete, based on the current, collective scientific literature, the authors do not believe that reducing digital blood flow is justified and actually may cause exacerbation of the disease, depending on when in the courseof the disease the cold-induced digital vasoconstriction occurs.

Antiinflammatory medications are indicated to decrease inflammation, edema, and pain associated with laminitis. Pain relief helps interrupt the pain-catecholamine–induced vasoconstriction cycle. Phenylbutazone appears to have the best antiinflammatory and analgesic effect of any of the nonsteroidal antiinflammatory drugs commonly used in horses. One can administer a dose of 2.2 to 4.4 mg/kg of phenylbutazone intravenously or

orally every 12 hours. Alternatively, one can administer flunixin meglumineat 0.5 to 1.1 mg/kg intravenously or orally every 8 to12 hours. A dose of 0.25 mg/kg flunixin meglumine administered intravenously every 8 hours interrupts eicosanoid production associated with endotoxemia. One can use phenylbutazone concurrently with flunixin meglumine to control foot pain and endotoxemia if each is given at the lowest dose rate and the horse is hydrated adequately. One can administer ketoprofen at 2.2 mg/kg intravenously every 12 hours. Dimethyl sulfoxide (DMSO) is an antiinflammatory drug that scavenges hydroxyl radicals, decreases edema, and therefore is used to counteract the effects of ischemia-reperfusion injury. Although the involvement of ischemia-reperfusion and oxygen free radicals in the pathogenesis of laminitis is unclear, the fact that a biphasic decrease in laminar perfusion normalizes or increases during the intervening period suggests that hyperemia and subsequent ischemia-reperfusion injury may play a role. The reason DMSO has not been shown to be particularly effective in preventing or treating laminitis could be that ischemia-reperfusion injury does not occur or the dose or timing of DMSO is not appropriate. One should administer DMSO, if used, at a dose of 0.1 to1.0 g/kg intravenously diluted in a polyionic fluid with dextrose to a concentration of 10% to 20% every 8 to12 hours. Some clinicians prefer to place DMSO topically on the coronary bands.

Because microthrombi and platelet-platelet or platelet-neturophil aggregates have been shown to form during laminitis, some clinicians prefer to administer heparin or aspirin to horses as a preventive or therapeutic agent. One can administer heparin using several regimens, but administration often is subcutaneous at a dose of approximately 20,000 to 40,000 units per 450-kg horse. Heparin leads to microagglutination and a subsequent decrease in packed cell volume. No evidence indicates that administration of heparin prevents the onset of laminitis. Aspirin oftenis administered at a dose of 10 to 20 mg/kg orallyonce every 48 hours. Aspirin irreversibly inhibits platelet cyclooxygenase and therefore production of thromboxane, which should decrease platelet aggregation and vasoconstriction.

Efforts to reduce mechanical forces and stabilize the distal phalanx are imperative to effective treatment of acute laminitis. Horses should not be exercised during the acute stages because this can lead to increased mechanical forces that could lead to shearing of laminae. Owners should bed the stall deeply with sand or other material that provides support to the frog and provides some cushion if the horse spends long periods recumbent. Providing early and effective mechanical support of the distal phalanx can spare weakened, separating lamellae and improve the outcome. Ideally, one should institute mechanical support before or at the onset of foot pain. Frog support is one of the more effective methods of providing support to the distal phalanx and can be achieved by using roll gauze taped to the frog in the shape of a triangle. One also can use a commercially available triangular, rubber frog pad. Using a moldable material such as dental putty or a thermoplastic material, one can conform a frog support to the shape and sulci of the frog and sole, allowing for a more effective distribution of the mechanical support to the frog and subsequently the distal phalanx. One must take care to support the frog fully but not allow excessive pressure on the sole because this may increase pain.

Another method to decrease mechanical forces on the distal phalanx is to transect the deep digital flexor tendon to reduce caudal pull on the coffin bone. A deep digital flexor tenotomy has been performed in several horses in
acute and chronic stages of laminitis to prevent or reduce coffin bone rotation. Although short-term outcome was 529
promising, the long-term survival and soundness proved to be less successful. The most appropriate use of a 530
deep digital flexor tenotomy may be to perform it in association with corrective trimming and shoeing during the chronic stages of laminitis to help reverse the amount of rotation.

Each clinician undoubtedly will develop a therapeutic plan based on current literature and on past experiences with the effectiveness of these treatments. The information presented simply represents some of the currently used methods. Obviously, others methods have not been discussed that also may have merit. The effectivenessof preventive and therapeutic measures needs to improve significantly to help manage this devastating disease.

Improvement will become a reality only as researchers collectively work to unravel the remaining mysteries of the pathophysiology of acute laminitis.

9.17.6 PROGNOSIS

Many horses that demonstrate clinical signs of acute laminitis that receive prompt, appropriate medical treatment and mechanical foot support may recover completely. However, some horses, even with mild laminitis, should be withheld from exercise sufficiently until all signs have subsided and only cautiously returned to athletic function. If radiographs demonstrate signs of coffin bone rotation, the prognosis for soundness and even survival must be more guarded. The primary disease that initiates the onset of laminitis also plays an important role in the prognosis and outcome. In general, the greater the degree of coffin bone rotation, the worse the prognosis. Horses with greater than 15 degrees of rotation accompanied by distal displacement into the hoof capsule within 4 to 6 weeks of the onset of laminitis have a poor prognosis. Prolapse of the distal phalanx through the sole often is accompanied by subsolar abscessation. These horses often require extensive, long-term treatment, and the prognosis is grave because of the recurrent, crippling pain and recumbency, and they often require euthanasia for humane reasons. In one study of horses with acute laminitis admitted to a university veterinary hospital, 75% did not return to athletic function and most were destroyed humanely within 1 year because of a lack of response to therapy or development of severe complications.

9.17.7 SUMMARY

Numerous vascular alterations have been identified in acute laminitis. Vascular derangement may lead to impairment of nutrient delivery to laminar tissue, resulting in necrosis. The balance of microvascular pressure in healthy horses favors edema formation. Therefore an imbalance in the forces caused by increased venomotor tone could lead to sufficient increases in tissue pressure to collapse capillaries, open arteriovenous shunts, and lead to ischemic necrosis. Further studies are needed to confirm the relationship of the vascular changes to the damage of the laminar tissue so that appropriate therapies can be developed. Current therapies are varied and inconsistent in their effectiveness and typically result in a guarded prognosis; newer, more effective treatments must be developed as understanding of the pathophysiology of this devastating disease increases.

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10 CHAPTER 10 DISORDERS OF THE NEUROLOGIC SYSTEM

Stephen M. Reed

Frank M. Andrews

10.1 10.1—Neurologic Examination

Stephen M. Reed

Assessment of the central nervous system in horses may seem a difficult task; however, with careful examination and by following a craniocaudal approach, assessment is not difficult. A craniocaudal approach is the most logical and efficient. The examination focuses on the neuroanatomic localization of the lesion or lesions and should be completed as part of the physical examination. Subtle neurologic deficits may be hidden by musculoskeletal disease or missed because of lack of knowledge or understanding of these disorders. To accomplish a complete and accurate neurologic examination, the veterinarian must feel comfortable with the format chosen for evaluating the nervous system and must have knowledge of which musculoskeletal disorders commonly are associated with neurologic disease. Problems such as osteochondrosis of the stifle, hock, and shoulder joints often occur concurrently in horses with cervical vertebral stenotic myelopathy. Examples of typical histories in horses presented for neurologic examination are previous medial patellar desmotomy or bilateral bog spavin in early life. Osteochondrosis of the distal tibia or femur and contracted tendons are examples of conditions that may occur simultaneously. Bilateral bog spavin often is associated with osteochondrosis of the distal tibia or other sites in the tibiotarsal joint. Patellar desmotomy to correct upward fixation of the patella may be necessary because of a conformational problem of the stifle joint or because of abnormal joint proprioception or quadriceps weakness following neurologic disease. The foregoing problem is more commonly associated with neurologic disease than many veterinarians realize, and the gait deficits caused by these lamenesses often mimic neurologic disease.

The goals of a neurologic examination are to establish whether a neurologic problem is present and to determine the anatomic localization of the problem. Being able to account for all clinical signs with a single lesion is ideal; however, if this is not possible, one should consider the presence of multifocal disease or multiple diseases. Following anatomic localization, one must decide what additional testing is necessary to determine the underlying cause of the clinical signs. Cervical radiography, cerebrospinal fluid analysis, and electrodiagnostic testing may be useful in locating and determining the cause of the lesions.

The author always begins the examination at the head and proceeds to the tail, although some segments of the neurologic examination are part of the physical examination. The examiner should proceed in a consistent fashion and should record findings in an orderly manner so as to avoid any part of the examination being omitted. Figure 10.1-1 shows a sample format. One may use the craniocaudal approach for all animals, whether ambulatory or recumbent.

The examination procedure is described elsewhere.[1–12] The author follows the format developed by Mayhew,[1] which divides the examination into five categories: the head and mental status, gait and posture, neck and forelimbs, trunk and hindlimbs, and tail and anus. The functional divisions of the nervous system include the sensory, integration, and motor systems. 533

Figure 10.1-1 Example of a neurologic examination form. *PLR,* Pupillary light response.

NEUROLOGIC EXAMINATION

Date: ______
Sire ______
Dam ______
Dam's sire ______

History ______

General observations ______

CRANIAL NERVE EXAMINATION:

Menace (2, 7)	______	Facial symmetry:	
Pupil size (2, 3, sym.)	______	Temporal/masseter (5)	______
Pupil symmetry (3, sym.)	______	Expressive muscles (7)	______
PLR (2, 3)	______	Palpebral reflex (5, 7)	______
Doll's eye (8, 3, 4, 6)	______	Retractor oculi (5, 6)	______
Ocular position (8, 3, 4, 6)	______	Gag reflex (9, 10)	______
Pathologic nystagmus	______	Tongue (12)	______

Symmetry of neck/body (muscle mass, scoliosis, etc.) ______

Manipulation of the neck L/R ______
up/down ______

Spontaneous involuntary movements (tremor, myoclonus, myotonia, etc.) ______

Description of gait (at walk and trot) ______

Circling	large L	______		
	R	______		
	small L	______		
	R	______		
Backing		______		
Up/down an incline		______		
Elevation of head		______		
Proprioception	LF	______	RF	______
	LR	______	RR	______
Sway reaction	fore	______		
	rear	______		
	tail pull	______		

Grading System—write in grading according to deLahunta
0 = No gait deficits
1 = Deficits barely perceptible—worsened with head elevation
2 = Deficits noted at a walk
3 = Deficits noted at rest, walking; nearly falls with head elevation
4 = Falls or nearly falls at normal gaits
5 = Recumbent patient

Gait and posture (graded 0 to +4)

	Motor		Sensory
	Weakness	Spasticity	Ataxia
LF	____	____	____
RF	____	____	____
LR	____	____	____
RR	____	____	____

Reflexes Anal ____
Patellar ____
Triceps ____
Other ____

Nociceptive (withdrawal) ____

Tail tone ____

Autonomic Urinary ____
Rectal ____
Sweating ____

Cutaneous sensation ____

Assessment/comments ____

Lesion localization ____

Tentative DX ____

CSF	Cells	Protein	Culture	Cytology
L/S	____	____	____	____
A/O	____	____	____	____

Radiographs ____

Myelogram ____

Comments ____

535

536

Before starting the examination, the veterinarian should know the age, sex, breed, and use of the horse, although these are not essential. This information is useful because horses of various breeds behave differently. One should ask the owner about any unusual behavior the horse has exhibited and the date of onset of the behavior. Age is helpful because problems such as cervical vertebral stenotic myelopathy and cerebellar abiotrophy begin at a young age, usually less than 1 year. These problems occur more often in certain breeds. For example, cervical vertebral stenotic myelopathy is most common in Thoroughbreds, and cerebellar abiotrophy most often is observed in Arabians.

10.1.1 Examination

The evaluation of the head should include observation of the horse at rest and during motion; palpation, postural reactions, cranial nerve function, and cervicofacial reflexes; and evaluation of sensation. Evaluation of the head includes observation of the behavior and mental status of the horse. One can complete this portion of the examination partially before handling the horse. A close and careful examination is necessary to evaluate the head and neck posture and its coordination and to identify abnormalities of the cranial nerves. Initial consideration includes the environmental awareness of the horse. A normal horse appears bright and alert and responds appropriately to external stimuli. While the horse is being caught, the examiner can look for unusual behaviors such as yawning, abnormal or aimless wandering, seizures, or circling or head tilt and can begin to assess the vision and hearing of the horse. If the horse shows behavior abnormalities, such as head pressing or aggressiveness, one should note these. If the examiner suspects rabies, then the examiner should take precautions to avoid unnecessary and potentially dangerous exposure.

Lesions of the reticular activating system or the cerebral hemispheres could result in severe signs such as coma or obtundation of consciousness. Horses that have a serious systemic illness also may appear depressed or stuporous. One records the level of consciousness as alert, depressed, stuporous, comatose, or semicomatose. An animal that is depressed may react to its environment in an inappropriate or unresponsive fashion. Stuporous horses may appear to be asleep unless stimulated with pain, light, or noise and often demonstrate impaired reactivity.

Head posture and coordination are controlled by the cerebellar and vestibular regions of the brain and brainstem in response to sensory input from receptors in the head, limbs, and body. Examining the head and neck posture with the horse at rest, while eating, and while moving is helpful. Careful examination of the vestibular region is important because many horses develop a head tilt resulting from head trauma, an inner ear infection, or a guttural pouch infection. A head tilt is characterized by the poll deviated about the muzzle and must be distinguished from abnormal or unusual turning of the head as may occur with damage to the forebrain or injury to the cervical vertebrae. Additional signs that may accompany vestibular disease or injury include nystagmus, ipsilateral weakness, and facial nerve paralysis.

Postural abnormalities of the head and neck sometimes can be difficult to distinguish from head tilt. Horses with torticollis of the head and neck may have a congenital abnormality of the vertebrae or may have injured the muscles of the neck region. Careful examination, including palpation, should help identify fractures of the cervical vertebrae or painful muscles caused by trauma or an injection reaction. In some horses, radiographs of the cervical vertebrae may be useful to confirm a fracture or osteomyelitis. Blindness sometimes can lead to an abnormal head or neck posture.

The cerebellum helps regulate rate and range of motion. With damage to this area a horse often shows fine resting tremors of the head that worsen with intentional movements.[3] One may observe this tremorous movement of the head and neck in the newborn or young foal, but it is not normal in older foals and adult horses. In young Arabians and a few other breeds, a condition of cerebellar abiotrophy has been reported.[1–3] Horses with this condition show a hypermetric gait, failure to blink when exposed to bright light, and absence of a menace response.

After evaluating the alertness, mental attitude, head and neck posture, and coordination of the horse, the examiner should examine the cranial nerves closely. Examination from first to twelfth cervical vertebrae helps to ensure that a subtle lesion along the brainstem is less likely to be missed, although in fact the examiner assesses

many of the cranial nerves simultaneously on approach to the horse. The examiner evaluates facial symmetry, facial sensation (including sensation inside the nares), head posture, eyes, nose, mouth, jaw tone, pharynx, and larynx. To determine if an animal can smell is usually not as important, but determining if it can see, hear, breathe, and swallow is critical.

Examination of the eyes should include evaluation of the blink or menace response, the ability of the horse to negotiate in a strange environment, pupillary light response, and in some cases a funduscopic examination. If a horse has a lesion of the eye or optic nerve, the lesion will result in complete or partial ipsilateral blindness. To develop contralateral blindness the horse would have to have a lesion in the optic tract or the lateral geniculate nucleus.[1]

Examination of the face should include evaluation of pupil size and symmetry, which are under control of the autonomic nervous system and are affected by environmental light and level of fear or excitement. To test the 536
pupillary response, one should direct a light in each eye and note the constriction of the pupil. Identifying a 537
consensual response in the horse is often difficult when one works alone. A swinging light test has been described.[1] Moving the light from side to side takes advantage of the ipsilateral light response being stronger than the consensual response. The examiner can perform this procedure alone. These reflexes are in the brainstem and are not affected by lesions in the visual cortex.

One possible cause of asymmetric pupils in a horse is Horner's syndrome. This syndrome includes ptosis of the upper eyelid, miosis of the pupil, and protrusion of the third eyelid (nictitating membrane). In addition, the horse has unilateral facial sweating, increased facial temperature, and hyperemia of the nasal and conjunctival membranes. These signs should alert the examiner to the possibility of a previous perivascular injection, a guttural pouch infection, or damage to the sympathetic nerves in the vagosympathetic trunk, which courses from the cranial thoracic spinal cord through the thoracic inlet and upward to the orbit.[5] Loss of sympathetic innervation to the head results in the triad of clinical signs described, with the most prominent initial sign being increased sweating, which is not what one would expect and the exact cause of which is not well understood.[6,7]

Loss of symmetric positioning of the eyes or the presence of abnormal deviation (i.e., strabismus) occurs when a horse has injured the third (oculomotor), fourth (trochlear), or sixth (abducens) cranial nerve or the connections between the eighth (vestibulocochlear) cranial nerve and these nuclei in the brainstem along the medial longitudinal fasciculus. Deviations of the eyes may occur with head trauma or midbrain lesions or can be normal variations in newborn foals.

The nuclei along the fifth (trigeminal) cranial nerve are among the largest nuclei along the brainstem of the horse. The fifth cranial nerve contains motor and sensory branches that supply innervation to the muscles of mastication and sensation to the skin and mucous membranes of the head. Injury to this nerve leads to dropped jaw and ipsilateral loss of, or decreased sensation to, the side of the face and the inside of the nares.

Damage to the seventh (facial) and eighth cranial nerves is common in horses. Injury to the seventh cranial nerve results in unilateral facial paralysis. This nerve contains branches that supply the ears, eyelids, and nares, and so injury to this nerve may affect all or only part of these structures. The most easily recognized sign is deviation of the nares toward the unaffected side coupled with drooping of the eyelid and ear on the affected side. Because this nerve also innervates the salivary and lacrimal glands, loss of or damage to this nerve may cause dry eye and decreased salivation.

Eighth cranial nerve deficits are easy to recognize, because unilateral injury to this nerve results in a head tilt toward the affected side. The eighth cranial nerve is important to hearing and control of balance. Projections

from this nerve pass to the medulla and cerebellum. A horse that has damage to this nerve often appears disoriented and has a head tilt toward the side of the lesion along with abnormal position of the limbs and body and horizontal nystagmus. If the lesion involves the peripheral portion of the vestibular system, then the fast phase of the nystagmus is directed away from the side of the lesion. If the lesion involves the central portion of the vestibular system, the nystagmus may appear vertical, rotary, or horizontal and may not always appear the same, depending on head position.

Horses that have peripheral damage to the vestibular system usually compensate for the deficits in a short time by use of visual and proprioceptive input. Therefore avoiding the use of a blindfold in cases of suspected vestibular disease is judicious. Using a blindfold hampers the ability of the horse to compensate, and the horse may become dangerous. However, blindfolding a horse with a suspected vestibular disease, but no longer showing an obvious head tilt, may be helpful to localize the lesion.

Within the medial compartment of the guttural pouch along the caudodorsal and lateral walls are the ninth (glossopharyngeal) cranial nerve and a branch of the vagus nerve. When a horse develops a guttural pouch infection, these nerves may be damaged, resulting in loss of innervation to the pharyngeal muscles. The clinical signs include dysphagia on the same side as the damaged nerve. If the infection is severe enough to involve the internal carotid nerve, which contains postganglionic sympathetic fibers to the structures of the head and eye, Horner's syndrome results. As mentioned previously, the signs include ptosis of the upper eyelid, enophthalmos resulting in prolapse of the third eyelid, miosis of the pupil, and sweating along the side of the face.

Other diseases one should consider when Horner's syndrome is evident during a neurologic examination include injury or infarction to the cranial thoracic spinal cord, avulsion of the brachial plexus, a hematoma, or tumor invading the sympathetic trunk in the region of the caudal, cervical, or cranial thoracic sympathetic trunk. Mycosis of the guttural pouch can cause damage to the internal carotid nerve or the cranial cervical ganglion along the caudodorsal wall of the guttural pouch. Finally, an injury to or neoplasia of the structures within or just behind the orbit also may cause Horner's syndrome.

Focal sweating in a horse indicates involvement of the peripheral pre- or postganglionic sympathetic neurons. Identification of this problem also can aid in the anatomic localization of a neurologic lesion.[1]

Intact innervation to the larynx and pharynx is important, especially if the horse is to be used as an athlete. The 537
easiest means to evaluate this region is by endoscopic examination of the pharyngeal and laryngeal regions. One 538
can perform throat latch palpation and a laryngeal adductory slap test. Endoscopic examination is helpful and important to a complete evaluation. The innervation of the pharynx and larynx is via the ninth, tenth (vagus), and eleventh (accessory) cranial nerves and the connections of these nerves in the caudal medulla oblongata.

Following careful examination of the mental status and behavior of the horse and of the head, neck, and cranial nerves, the examiner should look for asymmetry of the muscles of the trunk, pelvic region, tail, and anus. Identification of focal sweating, focal muscle atrophy, or increased or decreased pain responses is helpful in localizing signs. In addition, the horse has two cervical reflexes. The cervicofacial reflexes result in a local twitch and drawing back of the lips (smile reflex) when one pricks the skin along the side of the neck down to the region of the second cervical vertebra. Below the region of the second cervical vertebra one should observe a local response.[13]

To evaluate the tail and anus, one should begin by observing the tail carriage at rest and with the horse in motion. The normal tail carriage is straight down but with free movement in all directions. Some normal horses allow the tail to be lifted, giving little resistance, whereas other horses strongly resist and clamp the tail. The usual

response to anal stimulation is to clamp the tail and squat down, although with prolonged stimulation the horse may relax and eventually raise its tail.

10.1.1.1 EVALUATION OF GAIT

Evaluation of the gait of the horse should include examination of postural reactions and may include evaluation of spinal reflexes in young or small horses. Although they may appear weak and ataxic, foals are ambulatory within hours after birth, making it possible to evaluate gait. Because postural reactions are sometimes difficult to interpret in horses, using gait abnormalities to help localize a lesion is essential. The author nearly always places the feet and limbs in an unusual position to observe the response of the horse. Gait abnormalities that are observed commonly in horses with neurologic disease include ataxia, spasticity, and weakness or paresis.[1–3,4,15]

Evaluation of gait is critical because subtle neurologic gait deficits often go unrecognized or sometimes, incorrectly, may be considered insignificant. One should conduct the examination so as to observe the horse at a walk and trot in a straight line and while turning. In some horses, observing the horse negotiate over small obstacles such as a curb is helpful. When possible, the examiner should observe the horse turned free and walking up and down an incline. Elevation of the head and walking on a slope may exaggerate a subtle deficit and make it more noticeable.

One should conduct the examination with the animal at rest to observe its posture, while walking, while trotting, and sometimes while the horse is being ridden. The examiner should pay attention to which limbs show abnormal posture or abnormal movements and must be able to determine whether the horse has a painful, mechanical musculoskeletal problem or a neurologic gait deficit. The examiner must identify the presence of weakness, ataxia, and spasticity in each limb.

The important centers for posture and coordination in the brainstem and spinal cord have been described briefly. These centers are located in the regions of the sixth cervical to second thoracic (T2) and fourth lumbar to second sacral (S2) vertebrae in the spinal cord, along with coordination centers in the brainstem. Horses that demonstrate a wide base stance at rest often have a lesion of the cerebellum or vestibular system or may have conscious proprioceptive abnormalities.

To evaluate the gait of an animal, one begins by observing the horse at a walk and trot. At a walk the examiner can walk alongside, in step with first the rearlimbs and then the forelimbs. This allows the examiner more easily to determine the stride length and foot placement. A weak limb often has a low arc and longer stride length.

One also should observe the horse walking in a circle, on a slope, and with its head elevated. These procedures help to demonstrate if the horse is showing persistent irregular movements with its limbs. Horses with a musculoskeletal problem have been described as being regularly irregular, whereas a horse with neurologic gait deficits shows fewer consistent mistakes when positioning its limbs. Although observing the horse running free in a paddock or round pen and while being ridden is helpful, this is not always possible. The veterinarian must consider certain legal ramifications before asking a person to ride a horse during the examination.

Additional tests that may be helpful during a neurologic examination include blindfolding the horse, walking it over a curb or other obstacle, and walking it with its head and neck extended. One can evaluate the strength and ability of the horse to correct body positions by performing a sway test by applying lateral pressure at the

shoulder, hip, and tail while the horse is standing and walking. One should apply pressure several times while the horse is walking to catch the limb in various stages of weight-bearing. Observing a horse while it is backing is important. When a normal horse is backing, it should lift each leg and place it in a coordinated and appropriate location. Horses with neurologic abnormalities often place the limbs in wide-based positions or lean back and are reluctant or refuse to move. The horse also may step on the rear feet with the front feet.

One should observe the horse closely while being turned in a tight circle. One may identify abnormal wide 538
outward excursions of the rearlimb (circumduction) during this procedure. Additional tests to assess 539
proprioceptive function include a standing sway test in which one applies pressure to the the shoulder. The horse initially should press into the examiner and then lean away, and finally it should step away with the offside limb. The author also crosses the limbs over the opposite fore- or rearlimb to determine if the horse recognizes and tolerates these unusual limb positions. Following this, the author lifts one front limb and force the horse to hop on the opposite front limb in a modified postural reaction.

The examiner should observe the movements of each limb carefully to determine if a deficit is present and should assign a grade to the deficit. The author uses a system modified from the grades described by deLahunta[3] and Mayhew.[1] The severity is graded between 0 and 5. Grade 0 means no gait deficits. A grade 1 deficit requires careful observation to be certain the gait abnormality is caused by a neurologic dysfunction. Grade 2 deficits are moderate but obvious to most observers as soon as the horse begins to move but are still mild to moderate in severity. Grade 3 deficits are obvious and are exaggerated during the negotiation of a slope or with head elevation. Grade 4 gait deficits may cause a horse to fall or nearly fall. When attempting to walk, an animal with these severe deficits often displays abnormal positioning while standing in its stall. Grade 5 horses are recumbent.

Weakness is used to describe knuckling, stumbling, or buckling and sometimes can be characterized by toe-dragging while walking. Weakness may be associated with an upper motor neuron or lower motor neuron lesion. In the case of a lower motor neuron or peripheral nerve injury or illness, the horse shows muscle atrophy and sensory loss. Ataxia is typified by abnormal foot placement and wide swaying of the foot and limb, especially while turning.

The gait abnormalities along with the findings from other parts of the neurologic examination allow the examiner to determine the neuroanatomic site of the problem. The severity of the clinical signs also helps one evaluate the extent or severity of the problem.

One should reexamine horses with an obscure or unusual gait that might be lameness after using local anesthetics to block selected peripheral nerves or following treatment with intraarticular medications. The use of nonsteroidal antiinflammatory drugs for a period of 1 to 2 days also may alleviate pain and help one distinguish between a lameness and a neurologic gait deficit.

One must realize that many horses that have minimal (grade 1 or mild grade 2) deficits often can race or perform other athletic activities.[12] The examining veterinarian has the responsibility of separating a neurologic from a musculoskeletal gait deficit and helping the owner determine the usefulness of the horse. For example, a horse with gait deficits up to grade 3 may be useful as a broodmare or breeding stallion if the horse is handled by careful, knowledgeable persons who understand the risks and have the facilities to accommodate a horse in need of special management. Stallions with this degree of impairment may require assistance when mounting and dismounting mares. If the breed association allows artificial insemination, the stallion may be easier to manage.

10.1.1.2 LOCALIZATION OF THE LESION

At the conclusion of the examination the veterinarian needs to determine if a neurologic abnormality exists and where it is located. If the horse showed no evidence of abnormal behavior, seizures, or abnormal mental status and showed no cranial nerve deficits, the lesion is most likely caudal to the foramen magnum.

The most difficult lesions to localize are in the brainstem, unless the signs include cranial nerve deficits or depression. Horses with brainstem lesions often show signs of weakness and ataxia similar to horses with a lesion in the cervical spinal cord. Two of the most common brainstem lesions in horses involve the seventh and eighth cranial nerves. Vestibular disease or injury may be a sequela of head trauma or an inner ear infection. Facial nerve paralysis may result from trauma to the nerve root origin in the brainstem or where the nerves course along the neck and face to the ears, eyelids, and nares. These problems may occur in horses infected with *Sarcocystis* parasites.

Specific cranial nerve involvement such as head tilt, facial nerve paralysis, or loss of facial sensation can result from trauma, guttural pouch infection, osteomyelitis of the stylohyoid bone, or equine protozoal encephalomyelitis. In addition, cranial nerve deficits may occur with polyneuritis equi and equine motor neuron disease.

Cerebellar lesions are characterized by a failure to blink to bright light, lack of a menace response, and a head tremor that worsens with intentional movements. This disease is an abiotrophy and occurs most frequently in Arabian horses.

Cervical spinal cord disease includes gait and proprioceptive deficits in all four limbs with no signs of brain, brainstem, or cranial nerve deficits. One should note that horses with cervical vertebral stenotic myelopathy may have mild pelvic limb deficits with minimal or barely detectable signs in the thoracic limbs.[15]

Horses with signs of neurologic gait deficits confined to the pelvic limbs have a neuroanatomic localization caudal to T2. When examining a horse with a lesion caudal to T2, carefully checking the tail and anus for involvement of the peripheral nerves or spinal cord segments in this region is important.

Horses that have peripheral nerve injury, equine motor neuron disease, or polyneuritis equi show evidence of 539
weakness, muscle atrophy, and in some cases selected areas of sensory loss. Primary muscle diseases such as 540
exertional rhabdomyolysis, myotonia (congenita or dystrophica), and hyperkalemic periodic paralysis are covered elsewhere in this book. These conditions sometimes can mimic a neurologic problem.

10.1.1.3 DESCRIPTION OF NORMAL GAITS

The reader is reminded that a walk is a natural four-beat gait in the horse. At this gait the normal horse has three feet on the ground at all times. Therefore the walk is a stable gait. Overtracking and interference may occur if the conformation of the horse is abnormal (e.g., with long limbs and a short body) and not as a result of neurologic disease.[10] The trot is also a useful gait to observe when one performs a neurologic examination in the face of subtle gait deficits. In this two-beat symmetric movement the diagonal limbs are in contact with the ground at the same time. If one examines the horse on hard pavement, the trot is the most helpful gait to distinguish a lameness from a neurologic gait deficit. Normally the pace is a gait that results in significant truncal sway as the legs on the same side of the body strike the ground simultaneously. In horses that have neurologic disease, identifying ataxia with truncal sway, which is often accompanied by pacing, is common.

In horses with subtle ataxia, one observes pacing when horses walk with the head held in an extended position. Whenever one observes a horse pacing, the pacing may indicate an underlying neurologic disorder.

The gallop is a high-speed four-beat gait that often seems to be easier to perform than walking in a tight circle or moving at a slow trot. Therefore some horses with a neurologic gait deficit may perform better at high speed. As the horse accelerates or slows down, one may detect abnormalities, and one must observe the horse carefully at this time when an ataxic horse is most unsafe.

Abnormal gaits associated with stringhalt, upward fixation of the patella, and fibrotic myopathy deserve careful attention. Horses that show these gait abnormalities have a mechanical lameness, although the exact cause is unknown and may sometimes be associated with underlying neurologic disease.

Stringhalt often begins as an abrupt onset of excessive flexion of one or both rearlimbs. In some horses the condition may worsen and result in frequent episodes of the foot hitting the abdomen. The condition has been reported for a long time and in some areas of the world may occur as an outbreak.[16] The clinical syndrome is similar to the movement of a horse with a tibial neurectomy with unopposed flexion of the hock and extension of the digit. In the case of Australian stringhalt, the forelimbs and neck muscles may be involved. Stringhalt deserves mention in this chapter because when the examiner observes this gait, the examiner should be certain no other signs of neurologic gait deficits exist. As most readers know, the primary condition usually can be corrected by a tenectomy of the lateral digital extensor tendon, including a portion of the muscle belly. The underlying cause of the disease may be a sensory neuropathy, a myopathy, or primary spinal cord disease. As has been described, the defect likely affects the neuromuscular spindle, as well as the efferent and afferent pathways controlling muscle tone.[9]

Fibrotic myopathy results from scar tissue formation following injury to the semitendinosus and semimembranosus muscles. One may confuse the characteristic foot placement coupled with the abrupt rearward movement of the affected limb with a spastic gait caused by spinal cord injury or disease. With careful examination, a horse suffering from fibrotic myelopathy likely will not go undiagnosed.

Upward fixation of the patella in horses with neurologic disease may result from weakness in the quadriceps muscle group. This weakness is thought to occur because of a lack of use of these muscles or may be caused by abnormal transmission of proprioceptive information to and from the muscles and joint capsule because of damage to the spinal cord.

Horses with profound ataxia often pace when they walk, which often is accompanied by circumduction of the outside rearlimb while turning. These signs suggest general proprioceptive deficits. If the horse has these deficits while walking, observing the horse walking with its head elevated and on a slope is prudent, for these maneuvers often exaggerate a subtle problem.

If the horse shows signs only in the trunk and pelvic limbs, the neuroanatomic localization of the lesion is between T2 and S2 or involves the nerves and muscles of the pelvic limbs. However, the examiner needs to realize that horses with cervical spinal cord lesions often demonstrate signs in the pelvic limbs that are one grade worse than over the thoracic limbs. Therefore a mild cervical spinal cord or brainstem lesion could show minimal or no thoracic limb signs with grade 1 or subtle signs in the pelvic limbs.

Palpating the horse over its back, rump, and muscles of the rearlimbs while being careful to detect any muscle atrophy is helpful. The author routinely stimulates the horse over the side of its body and observes for any twitching of the cutaneous trunci muscles. Such twitching usually is accompanied by a cerebral response and requires a fairly severe lesion to detect areas of analgesia.

A sway reaction performed while the horse is standing and walking is also necessary to assess the pelvic limb strength and proprioceptive functions. In addition, one may evaluate strength by slow but deliberate and forceful pressure along the back and sacral muscles. A normal horse should reflexively arch its back upward, whereas a horse with rearlimb weakness may be unable to withstand this pressure and its rearlimbs may even buckle. 540 541

To complete the neurologic examination, one must examine the tail and anus to determine whether damage has occurred to the sacrococcygeal nerve and muscle segments. A normal perineal reflex results in contraction of the anus and clamping of the tail in response to light stimulation of the skin in this region. Cauda equina neuritis or polyneuritis equi, trauma, and iatrogenic injury resulting from an alcohol tail block are some disorders that may affect this area.

The evaluation of the major peripheral nerves in the horse is also an important part of the neurologic examination. The important points to remember are that damage to a peripheral nerve can result in sensory and motor deficits in the area supplied by the nerve and that focal muscle atrophy follows within a short time after damage to one of these nerves. The examiner is referred to other portions of this book for a more detailed anatomic description of these nerves in the horse; however, a dropped elbow joint with radial nerve paralysis, inability to fix the stifle with femoral nerve paralysis, and atrophy of the supraspinatus and infraspinatus muscles (sweeney) with damage to the suprascapular nerve are classic examples of what to expect with peripheral nerve injuries.[1–3]

10.1.2 Conclusion

To conclude the description of the neurologic examination, the reader is reminded that for a horse to be a good athlete, it must gather information from muscles, tendons, and nerves; process this information in the brain, brainstem, and spinal cord; and relay this information to the musculoskeletal system. The horse must accomplish all of this in a short time to perform complex maneuvers at a high rate of speed. When performing in a setting involving many other horses or when a rider or driver is involved, the horse needs to be able to control these movements in a coordinated fashion to protect itself, other horses, and especially persons. Although for the horse to have every neuron, spinal tract, and glial cell working to be athletic is not necessary, the veterinarian must be able to distinguish between a musculoskeletal lameness and a neurologic condition that may make the horse unsafe for use and dangerous to itself or the rider.

Beyond this, veterinarians must recognize which musculoskeletal and neurologic conditions occur together to assist the prospective buyer better with the decision about whether to purchase the horse. When one recognizes and understands these things, one can determine whether the horse with subtle neurologic gait deficits caused by trauma, infection, or compression may yet be a safe and useful athlete.

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10.2

10.2—Cerebrospinal Fluid Evaluation

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Cerebrospinal fluid (CSF) evaluation has diagnostic importance regarding neurologic disease in horses. Collection and analysis of CSF is indicated to make or confirm a diagnosis of neurologic disease, but this is not without limitations. CSF values may be normal in an animal with severe neurologic deficits because the lesion is extradural, collection occurred early or late in the course of disease, one collected the CSF too far away from the lesion, or the disease involves the ventral roots and peripheral nerve. Even with its limitations, CSF provides valuable information about the central nervous system (CNS). However, one must emphasize that CSF evaluation is another piece of the diagnostic puzzle and together with the history, physical examination, neurologic examination, and other ancillary procedures may help in the diagnosis and prognosis of neurologic disease in horses.

10.2.1

Formation, Flow, and Function of Cerebrospinal Fluid

CSF is an actively transported ultrafiltrate of plasma that bathes the CNS.[1] The CSF is located in the ventricles of the brain and subarachnoid space of the spinal canal (Figure 10.2-1) and originates from the choroid plexus and ependymal lining of the ventricles.[2] CSF flows from the ventricular system up over the cerebral hemispheres and through the subarachnoid space surrounding the spinal cord.[1] Pulsation of blood in the choroid plexuses forces the CSF in a caudad direction. The rate of CSF production varies from 0.017 ml/min in cats to 0.5 ml/min

in human beings[3] and is independent of the blood hydrostatic pressure. The rate of CSF production for horses has not been determined. Osmotic and hypertonic solutions such as mannitol and dimethyl sulfoxide, when added to blood, decrease CSF production and decrease CSF pressure and edema.[1]

Collections of arachnoid villi (arachnoid granulations) are located in the venous sinus or the cerebral vein and absorb CSF. CSF absorption is related directly to the pressure gradient between the CSF and venous sinus. When CSF pressure exceeds venous pressure, these villi act as one-way ball valves forcing CSF flow to the venous sinus.[4]

CSF functions to suspend the brain and spinal cord for protection, regulate intracranial pressure, and maintain the proper ionic and acid-base balance.[1]

10.2.2 Collection of Cerebrospinal Fluid

Techniques for collecting CSF in horses have been described in detail elsewhere.[1–5] One may collect CSF from the lumbosacral site in a standing horse sedated with an intravenous injection of 0.2 to 0.5 mg/kg xylazine or 0.01 to 0.02 mg/kg butorphanol or a combination of both drugs. Alternatively, one can collect CSF from the atlantooccipital site of an anesthetized horse. In foals and recumbent adult horses, one can collect CSF while the animal is restrained and heavily sedated with 1.0 mg/kg xylazine and 0.2 mg/kg butorphanol, both administered intravenously. If the lesion is localized to an area above the foramen magnum (at least cranial to the second cervical vertebra), CSF collected from the atlantooccipital site will be more diagnostic. If the lesion is localized to an area below the foramen magnum (caudal to the second cervical vertebra), CSF collected from the lumbosacral site will be more diagnostic. These differences result from the craniocaudal circulation of CSF. Collecting CSF from both sites at the same time and comparing the findings may be helpful in cases in which neuroanatomic localization of the lesion is difficult.[5]

10.2.3 Examination of Cerebrospinal Fluid

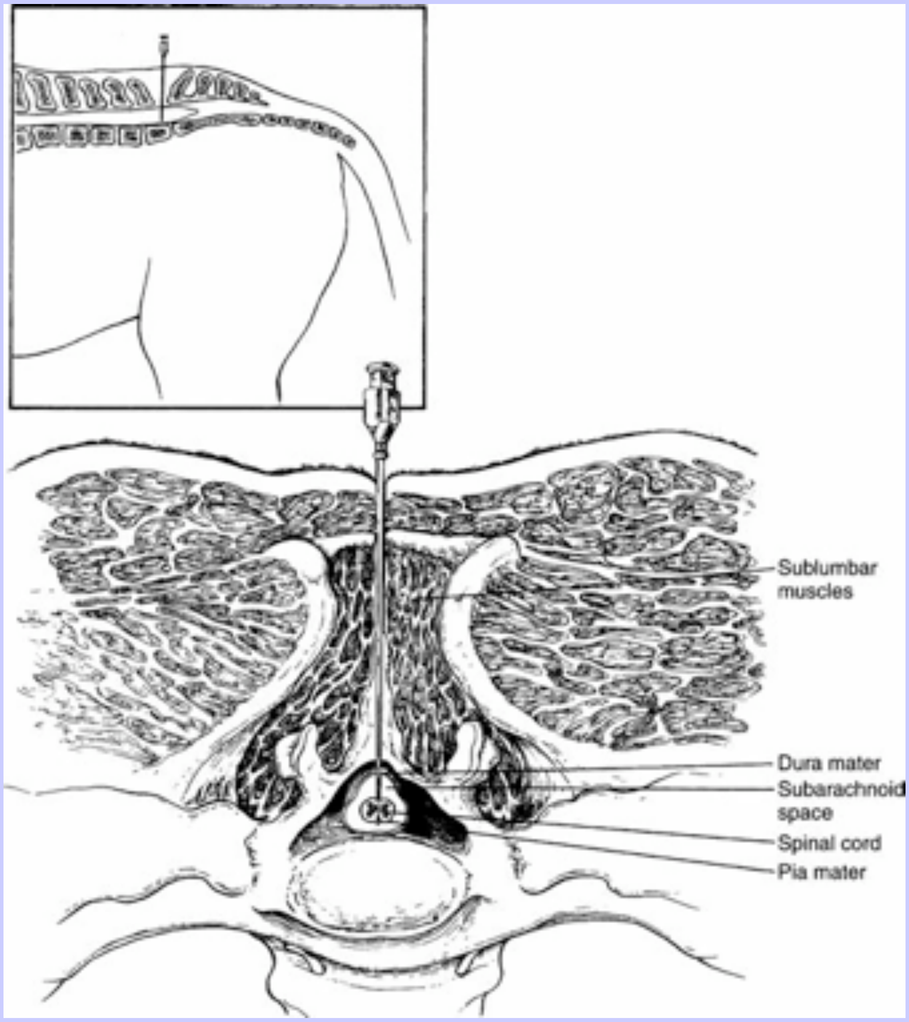
Reference values for CSF in horses have been reported,[6–11] but each laboratory should determine its own reference ranges. CSF determinations that are helpful in evaluating horses with neurologic diseases include pressure, appearance, cellular content, protein concentration (total protein, albumin, immunoglobulin G [IgG]), enzyme activity (creatinine kinase, CK; aspartate aminotransferase; lactate dehydrogenase), and lactic acid concentration.

10.2.3.1 PRESSURE

One can measure opening CSF pressure before withdrawal of CSF by attaching a manometer tube with a three-way stopcock to a properly placed spinal needle, allowing the CSF to rise within.[12] Because the cranial and vertebral cavities are enclosed in a rigid bony compartment, changes in blood pressure or volume can cause a concomitant increase in CSF pressure. Thus increased CSF pressure may occur from venous compression or jugular occlusion. Venous compression causes increased blood volume in the cranial cavity and compression of the CSF space, leading to increased CSF pressure. One can use jugular occlusion clinically to increase CSF pressure and facilitate collection of CSF fluid.[13] The jugular compression maneuver, or Queckenstedt's phenomenon, can help one diagnose compressive lesions, neoplastic lesions, or an abscess along the spinal cord. With compression and obliteration of the subarachnoid space by a 542

compressive lesion in the cervical or thoracic spinal cord, jugular vein compression does not cause a CSF pressure increase in the lumbosacral site.[1] 543

Figure 10.2-1 Lumbosacral spinal fluid collection from a horse showing the various tissue layers that the spinal needle must pass through to obtain a sample. The spinal fluid is collected ventral to the spinal cord in the subarachnoid space. *Inset,* Lateral view of spinal needle placement in the lumbosacral space for collection of cerebrospinal fluid. (From Andrews FM, Adair HS III: Anatomy and physiology of the nervous system. In Auer JA: *Equine surgery*, Philadelphia, 1992, WB Saunders; modified from deLahunta A: Veterinary neuroanatomy and clinical neurology, ed 2, Philadelphia, 1983, WB Saunders.)



Increased CSF pressure may occur following injury, following systemic changes in blood pressure, and in the presence of an intracranial space-occupying mass such as a tumor, abscess, hemorrhage, or edema. One must provide an adequate airway after injury and during surgery to prevent hypoxia-mediated cytotoxic cerebral edema and vasogenic cerebral edema.[14] Cytotoxic edema is caused by inadequate cerebral oxygenation and leads to neuronal, glial, and endothelial cell swelling. Such reactions are especially important during long surgical procedures or recumbency in which respiratory hypoxia and poor alveolar ventilation (hypercapnia) may occur. Hypercapnia increases cerebral blood flow in the cranial cavity and CSF pressure and may worsen existing cerebral edema.[1]

Increased CSF volume may occur in hydrocephalus, which is defined as an increased volume of CSF and can be classified as compensatory or obstructive.[15] Compensatory hydrocephalus is an accumulation of CSF in areas where brain tissue has been destroyed and may occur from brain injury or inflammation. 543
Hydranencephaly is destruction of brain tissue from a viral or other infectious agent and results in severe 544
accumulation of CSF.[15] CSF pressure in compensatory hydrocephalus usually does not increase.[1]

Obstructive hydrocephalus is an accumulation of CSF in the ventricles from an obstruction to CSF outflow or absorption. Cerebral aqueduct malformation may lead to obstruction of ventricular CSF outflow. Inflammatory lesions, especially of the arachnoid villi, result in decreased absorption of CSF and increased CSF pressure. The white matter is affected more severely than the gray matter in obstructive hydrocephalus, but the cerebral cortex usually is spared.[1] CSF pressure in obstructive hydrocephalus usually increases. The presence of an abnormally high opening pressure that drops by 25% to 50% after removing 1 to 2 ml of fluid suggests a space-occupying intracranial mass or spinal cord compression cranial to the site of collection. The removal of more fluid would risk causing tentorial herniation.[12]

10.2.3.2 APPEARANCE

One can evaluate the appearance of CSF immediately after collection. Normal CSF is clear and colorless and does not clot, and newsprint is visible through it.[16] CSF may be red-tinged from blood contamination after a traumatic tap or from preexisting trauma to the CNS. In the case of a traumatic tap, the CSF usually will clear if allowed to flow for several seconds (about 0.5 to 1.0 ml). With preexisting trauma and secondary hemorrhage, the supernatant of CSF after centrifugation is xanthochromic.

Other causes of CSF xanthochromia include increased protein concentration (150 mg/dl)[16] and direct bilirubin leakage from serum in horses with high serum bilirubin concentration. Also, indirect bilirubin may leak across a damaged blood-brain barrier. Clots in the CSF are abnormal and may be caused by increased amounts of fibrinogen resulting from inflammation.[1]

Turbid CSF may indicate an increased number of white blood cells (>200/μl), an increased number of red blood cells (>400/μl), epidural fat, bacteria, fungal elements, or amebic organisms.[1] Cytologic evaluation and cultures can help differentiate causes of turbidity.

10.2.3.3 CYTOLOGIC EVALUATION

One can use a standard hemocytometer to obtain a complete blood count. Also, a sedimentation chamber requiring 0.5 to 1.0 ml of CSF is a rapid method for cytologic evaluation.[17] One must perform cell counts and

cytologic evaluation within 30 minutes to avoid degeneration. If one cannot perform cell counts or cytologic evaluation immediately, then one can mix a portion of the sample with an equal volume of 50% ethanol to preserve cellular characteristics.[12] CSF from normal horses and foals usually contains fewer than 10 white blood cells per microliter. However, much variation occurs in CSF white blood cell counts in horses.[9,10]

No difference are apparent in CSF white blood cell counts in samples taken from the atlantooccipital space.[10] However, one study did show a slightly higher total white blood cell count in CSF taken from the lumbosacral site, but white blood cell counts in all of those horses were less than 10 per microliter.[6]

Small mononuclear cells (70% to 90%) and large mononuclear cells (10% to 30%) predominate in horse CSF. Rarely, one may see neutrophils in horse CSF. Increased CSF large mononuclear phagocytes are visible in horse CSF in diseases of axonal degeneration.[9] One may see increased CSF neutrophil numbers in encephalomyelitis, bacterial meningitis, parasitism, and diseases with extensive inflammation. Occasionally, in severe inflammatory diseases of the neurologic system or parasitism, eosinophils may be visible.[9,17,18] In some cases, cytologic evaluation of CSF may reveal specific etiologic agents causing neurologic disease such as fungal organisms,[16] bacteria, or tumor cells. Although CSF cytologic examination may support a diagnosis of neurologic disease, it may not yield a specific etiologic diagnosis.

10.2.3.4 PROTEIN CONCENTRATION AND COMPOSITION

Normal total protein values range from 20 to 124 mg/dl, depending on the measuring method used[1,6–10] (Table 10.2-1). Total protein concentration is higher in lumbosacral CSF compared with atlantooccipital CSF.[6] A difference of 25 mg/dl of protein between the atlantooccipital and lumbosacral spaces may suggest a lesion closer to the space with greater spinal fluid protein.[10] Proteins in the CSF are derived from the peripheral blood and include albumin, IgG, and possibly other globulins. Increased CSF albumin and IgG concentrations may occur with damage to the blood-brain barrier or increased intrathecal production of IgG. One can determine CSF albumin and IgG concentrations by electrophoresis and radial immunodiffusion, respectively, and can compare these with serum concentrations.[6] Special low-level radial immunodiffusion plates (VMRD, Inc., Pullman, Washington) are available to quantify CSF IgG concentration. One can calculate the albumin quotient ([Albc]/[Albs] × 100) and IgG index ([IgGc]/[IgGs] × [Albs]/[Albc]) to determine blood-brain barrier permeability and intrathecal IgG production.[6] Increased intrathecal IgG production (increased IgG index) may occur in inflammatory spinal cord disease such as equine protozoal myeloencephalitis (EPM), bacterial meningitis, some tumors, and equine motor neuron disease. Increased blood-brain barrier permeability (increased albumin quotient) may occur in equine herpesvirus type 1 following necrotizing vasculitis.[7] 544
Determining blood-brain barrier integrity is also important in planning therapy. If the blood-brain barrier is 545
damaged, pharmacologic agents such as penicillin that do not normally penetrate the blood-brain barrier will penetrate a disrupted blood-brain barrier and attain bactericidal CSF concentration.

TABLE 10.2-1 Cerebrospinal Fluid Values from Atlantooccipital and Lumbosacral Spaces of Normal Healthy Adult Horses[*]

	ATLANTOOCCIPITAL SPACE (MEAN ±SD [RANGE])	LUMBOSACRAL SPACE (MEAN ±SD [RANGE])
Red blood cell count (per μl)	51.0 ±160 (0–558)	36.8 ±59.7 (0–167)
White blood cell count (per μl)	0.33 ±0.49 (0–1)	0.83 ±1.11 (0–3)
Total protein (mg/dl)[*]	87.0 ±17.0 (53 ±11.6)	93.0 ±16.0 (58.0 ±11.0)
	(59–118) (35–74)	(65–124) (39–78)
Albumin (mg/dl)	35.8 ±9.7 (24–51)	37.8 ±11.2 (24–56)
Albumin quotient	1.4 ±0.4 (1.0–2.0)	1.5 ±0.4 (1.0–2.0)
Immunoglobulin G (mg/dl)	5.6 ±1.4 (3–8)	6.0 ±2.1 (3–10)
Immunoglobulin G index	0.19 ±0.046 (0.12–0.27)	0.19 ±0.5 (0.12–0.26)
Creatine kinase (IU/L)	0–8	0–8
Lactate dehydrogenase (IU/L)	0–8	0–8
Aspartate aminotransferase (IU/L)	4–16	0–16
Glucose (mg/dl)	35%-70% of blood glucose	
Lactate (mg/dl)	1.92 ±0.12	2.30 ±0.21
Sodium (mEq/L)	140–150	140–150
Potassium (mEq/L)	2.5–3.5	2.5–3.5

* Total protein concentration next to the mean ±SD in parentheses is the value expected using a total protein standard.

10.2.3.5

ENZYME DETERMINATION

CSF enzyme activity may be increased in neurologic disease. CK and aspartate aminotransferase activity may be increased in diseases with myelin degeneration and neuronal cell damage such as EPM, polyneuritis equi, equine degenerative myelopathy, and equine motor neuron disease. Increased CK activity also may occur in conditions that alter blood-brain barrier permeability, such as equine herpesvirus type 1. In diseases in which the blood-brain barrier is damaged, serum CK can leak into the CSF and increase CSF CK activity. This increased CK activity is not associated with damaged myelin.

Increased CK activity also may suggest other diseases of the CNS. In one study, CK activity (>1 IU/L) most often was associated with EPM in horses and may be helpful in differentiating compressive spinal cord disease from EPM. Furthermore, persistently increased CSF CK activity may be associated with a poor prognosis in horses with EPM.[19] Lactate dehydrogenase activity may be increased in spinal lymphosarcoma.

10.2.3.6

LACTIC ACID CONCENTRATION

CSF lactic acid concentration may be an indicator of neurologic disease (see Table 10.2-1). CSF lactic acid concentration increases in eastern equine encephalomyelitis (4.10 ± 0.6 mg/dl), head trauma (5.40 ± 0.9 mg/dl), and brain abscess (4.53 mg/dl). Lactic acid concentration may be the only CSF parameter increased in horses with brain abscess.[20]

10.2.4

Summary

Normal CSF findings do not always rule out the presence of neurologic disease. CSF values may be normal with lesions outside the CNS not bathed in the CSF, such as extradural, ventral root, and peripheral nerve lesions. Normal CSF values also may occur early or late in the disease process and in CSF samples taken away from the site of the lesion. Acute neurologic disease, especially if multifocal, may not have sufficient time to cause significant damage to the blood-brain barrier and alter CSF constituents, whereas in chronic CNS disease the blood-brain barrier may be repaired and functional but with nervous tissue replaced by fibrous tissue. Fibrosis of nervous tissue may result in significant neurologic gait deficits and normal CSF constituents. CSF taken away from the site of the lesion shows normal findings despite significant neurologic gait deficits. For example, CSF in a horse with a cervical spinal cord abscess may show a suppurative inflammation in the lumbosacral CSF and a normal atlantooccipital CSF. This discrepancy is caused by the caudad flow of CSF.

CSF may be helpful in supporting the diagnosis of neurologic disease in horses and is part of the diagnostic workup. Because CSF evaluation is an ancillary diagnostic test, one should use it with, and not as a substitute 545
for, a thorough history, physical examination, neurologic examination, and other diagnostic tests. 546

10.2.5

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10.3

10.3—Electrodiagnostic Aids and Selected Neurologic Diseases

Frank M. Andrews

Localizing lesions to and within the nervous system can be difficult in some cases using only clinical and neurologic examinations. Generally, neurologic disease is characterized by changes in cell electric activity; because electric activity has amplitude and frequency, one can measure it by electronic equipment. The observed electric activity may be helpful in defining and localizing lesions of the nervous system.

Electromyography (EMG), auditory brainstem response (ABR) testing, and electroencephalography (EEG) are electrodiagnostic aids that may be helpful in the localization, diagnosis, and prognosis of neurologic disease in horses. Needle EMG and nerve conduction studies are helpful in localizing and defining diseases of the lower motor neuron or motor unit. ABR testing is helpful in localizing lesions to cranial nerve VIII and auditory pathways along the brainstem. EEG is helpful in diagnosing focal and diffuse intracranial lesions. Use of these diagnostic aids as an extension of the neurologic examination, separately or collectively, can provide valuable information about nervous system function and help in diagnosing neurologic disease. These techniques are noninvasive and in many cases can be done on the awake horse with mild sedation. Even when these techniques do not provide the information necessary to arrive at a diagnosis, they may provide a more complete understanding of the disease process.

10.3.1 Electromyography

Needle EMG is the graphic recording of muscle cell electric activity during contraction or at rest from a recording electrode placed in the muscle. Electric activity is recorded by means of an amplifier on an oscilloscope.[1–3] Nerve conduction studies consist of stimulating a peripheral nerve with electric current and recording the resultant physiologic electric activity from other segments of the nerve or from the muscles innervated by those nerves.[4] Together, EMG and nerve conduction studies may help in the localization, diagnosis, and prognosis of diseases of the lower motor unit. The motor unit consists of the ventral horn cell bodies (located in the ventral horn of the spinal cord), its axon, the peripheral nerve, the myoneural junction, and the muscle fibers it innervates (Figure 10.3-1). 546

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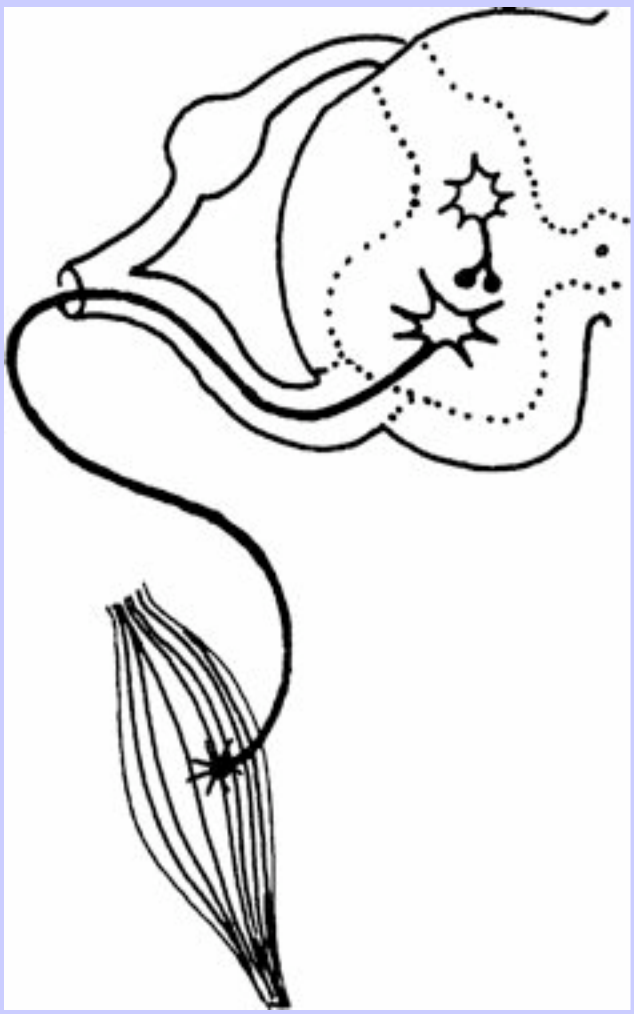
Figure 10.3-1 Illustration of the motor unit, including the ventral horn cell in the spinal cord, ventral root, peripheral nerve, neuromuscular junction, and muscle.



10.3.1.1 ELECTROMYOGRAPHIC EXAMINATION

A history and physical and neurologic examination always should precede the EMG examination; these aid in localizing the lesion, shorten the examination time, and minimize trauma to the horse. Initially, one examines the standing horse that is under mild sedation. One can tranquilize the horse with 0.2 to 0.5 mg/kg xylazine administered intravenously or 0.2 to 0.5 mg/lb xylazine and 2 to 10 mg butorphanol administered intravenously. Examination of the awake horse aids in evaluating individual motor unit action potentials (MUAPs), summated MUAPs, and interference pattern. One can evaluate normal and abnormal MUAPs and measure their amplitudes. Unfortunately, in the awake horse an interference pattern (many MUAPs) sometimes can obscure abnormal low-amplitude EMG potentials. In this case, further examination may require general anesthesia.

In the needle EMG examination, one should thrust the exploring electrode briskly into the muscle and hold it until the animal completely relaxes. To enable relaxation, one can force the animal to bear weight on the opposite limb. Once relaxation has occurred, one can evaluate the resting activity or any postinsertional activity of the muscle. One should evaluate at least four areas and depths of smaller skeletal muscles and six to eight areas and depths of larger skeletal muscles when possible. One should examine the horse systematically so as not to miss a lesion. One can perform needle EMGs in many of the major extrinsic muscles of the horse (Table 10.3-1). One also can perform needle EMGs on facial, laryngeal, esophageal, pectoral, and external anal sphincter muscles when indicated by neurologic examination. One may perform nerve conduction studies and collect muscle biopsy specimens to define suspected lesions further or confirm a diagnosis.

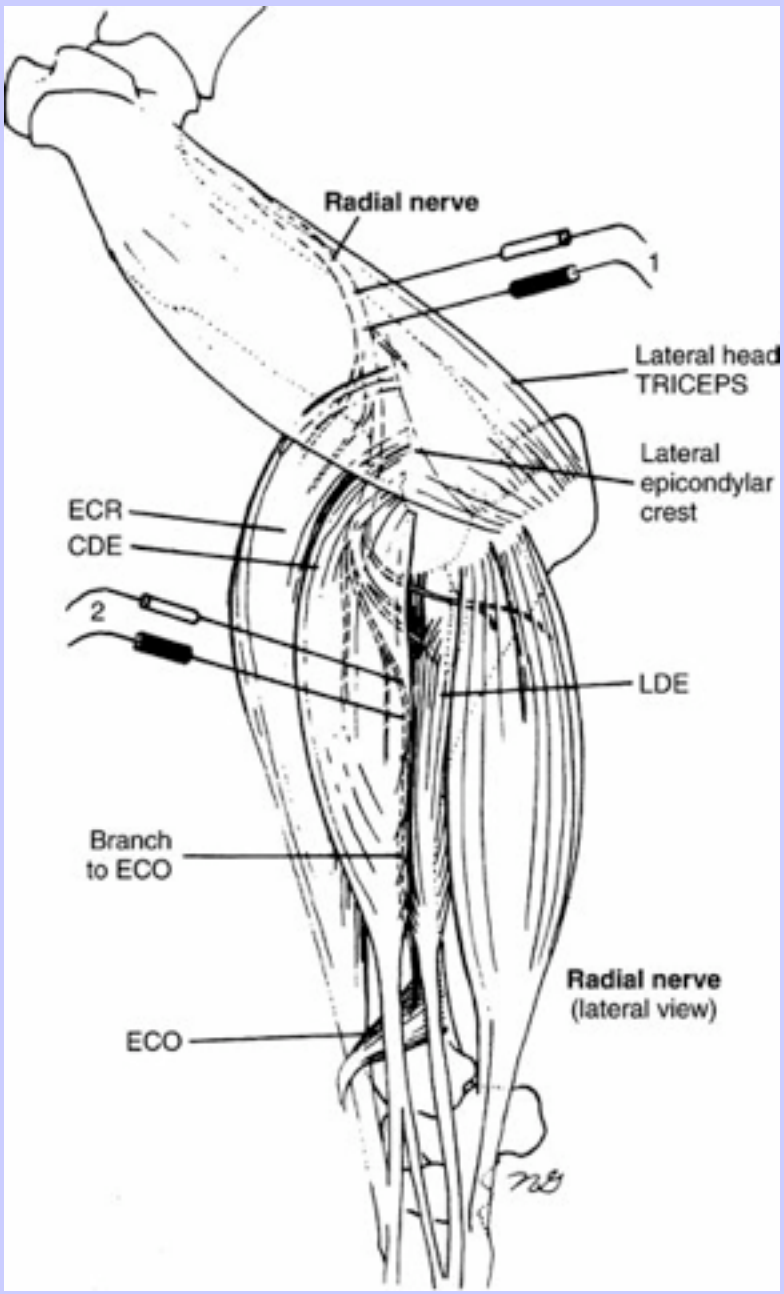
TABLE 10.3-1 Muscles, Nerves, and Nerve Roots Evaluated During Routine Electromyographic Examination of Horses

MUSCLES	PERIPHERAL NERVE	SPINAL NERVE ROOT
REAR LIMB		
Long digital extensor	Peroneal nerve	L6-S1
Gastrocnemius	Tibial nerve	S1-S2
Deep digital flexor	Tibial nerve	S1-S2
Semimembranosus	Ischiatic nerve	L5-S2
Vastus lateralis	Femoral nerve	L3-L5
Biceps femoris	Caudal gluteal, ischiatic, and peroneal nerves	L6-S2
Middle gluteal	Cranial and caudal gluteal nerves	L5-S2
PARAVERTEBRAL		
Paravertebral muscles (segmentally)	Dorsal branches of ventral spinal nerves (L6-C1)	L6-C1
THORACIC LIMB		
Subclavius	Pectoral nerve	C6-C7, T1
Supraspinatus	Suprascapular nerve	C6-C8
Infraspinatus		
Deltoideus	Axillary nerve	C6-C8
Biceps brachii	Musculocutaneous nerve	C6-C8
Triceps	Radial nerve	C7-T1
Extensor carpi radialis	Radial nerve	C7-T1
Superficial digital flexor	Ulnar nerve	C8-T2
Deep digital flexor	Ulnar and median nerve	C7-T1, T2

10.3.1.2 NERVE CONDUCTION STUDIES

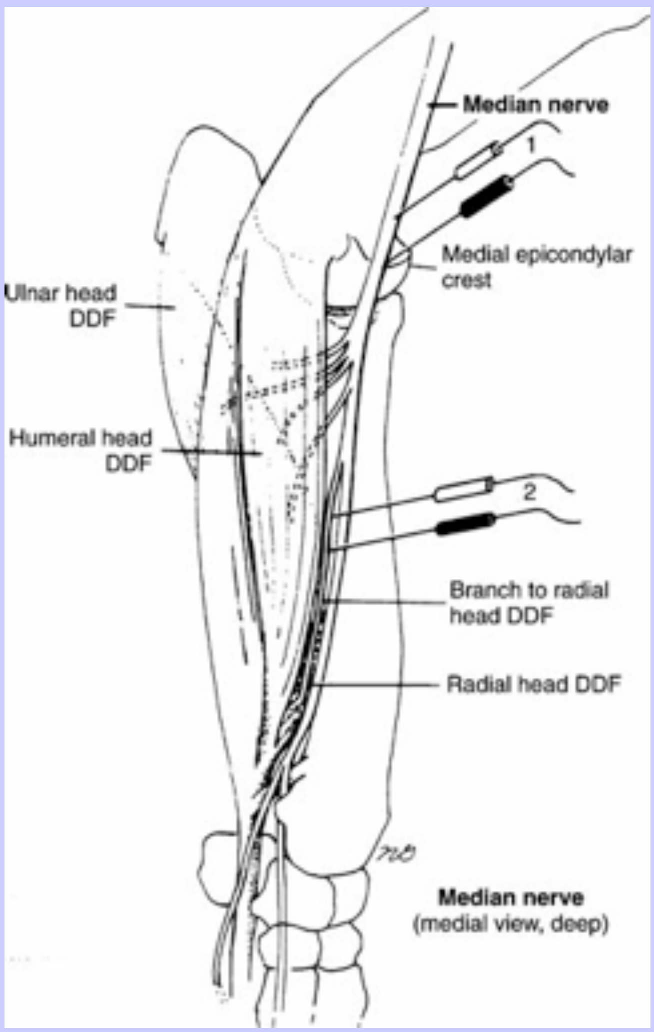
The evaluation of nerve conduction velocities requires knowledge of the topographic anatomy of nerves and muscles, plus a stimulator capable of delivering up to 150 V at durations of 0.1 to 3 ms at variable frequencies, up to 100 Hz. Most standard EMGs have built-in stimulators with adequate parameters to do nerve conduction studies. One can locate the peripheral nerve to be assessed by palpation or by anatomic landmarks and then can stimulate it. One can palpate or observe the resultant muscle contraction and can view the evoked muscle action potential, which has a thumping sound, on the oscilloscope. 547

Figure 10.3-2 Illustration of anatomy and electrode placement used in radial nerve conduction velocities. *ECR,* Extensor carpi radialis; *CDE,* common digital extensor; *LDE,* long digital extensor; *ECO,* extensor carpi obliquus. (From Henry RW, Diesem CD, Wiechers MD: Evaluation of equine radial and median nerve conduction velocities, *Am J Vet Res* 40:1406-1410, 1979.)



Nerve conduction studies are difficult to do in horses and therefore are not done routinely. However, the technique for radial and median nerve conduction studies in the horse has been reported.[5,6] One must perform nerve conduction studies with the horse under general anesthesia and may use them to evaluate the speed of conduction of large myelinated motor nerves. One should place the horse in lateral recumbency with the affected side up. One stimulates the appropriate motor nerve by monopolar needle electrodes inserted at or near the nerve and records an evoked MUAP from an innervated muscle (Figures 10.3-2 and 10.3-3). One may see or palpate the contraction of appropriate muscles and insert needle electrodes until one obtains a repeatable response. One may use the unaffected limb as a control.

Figure 10.3-3 Illustration of anatomy and electrode placement used in median nerve conduction velocities. *DDF,* Deep digital flexor. (From Henry RW, Diesem CD, Wiechers MD: Evaluation of equine radial and median nerve conduction velocities, *Am J Vet Res* 40:1406-1410, 1979.)



In horses, one can obtain radial nerve recordings from the extensor carpi radialis and abductor digiti longus (extensor carpi obliquus) muscles (see Figure 10.3-2) and can obtain median nerve recordings from the humeral and radial heads of the deep digital flexor tendon[5,6] (see Figure 10.3-3). One can obtain facial nerve recordings from the levator nasolabialis muscle by stimulating the buccal branch of the facial nerve just ventral to the facial crest.[7] Usually one can obtain a supramaximal stimulus at 70 to 90 V for 0.1-ms duration. Nerve conduction studies are helpful in diagnosing radial nerve, median nerve, and possibly facial nerve injury. 548

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10.3.2 Normal Electromyographic Potentials

Normally occurring EMG potentials and nerve stimulation studies are described next. One can examine the muscle at rest, under submaximal contraction, maximal contraction, and following direct nerve stimulation.

10.3.2.1 INSERTIONAL ACTIVITY

Insertional activity consists of short bursts of high-amplitude, moderate- to high-frequency (<200 Hz) electric activity following insertion or movement of the exploring electrode in the muscle (Figures 10.3-4 and 10.3-5). In normal skeletal muscle this activity stops a few milliseconds following cessation of needle movement. Insertional activity may be caused by mechanical stimulation, muscle fiber injury,[3,8,9] or depolarization of muscle fibers directly adjacent to the EMG needle.[1] Positive sharp waves and fibrillation potentials observed during or associated with needle insertion that stop after cessation of needle movement are considered normal. Damage to muscle fibers by needle insertion is probably the source of these potentials. However, positive sharp waves and fibrillation potentials persisting after needle insertion are considered abnormal and may suggest early muscle denervation.[3]

10.3.2.2 RESTING ACTIVITY (POSTINSERTIONAL BASELINE)

Resting activity is observed in relaxed muscle and is characterized by electric silence. A flat line appears on the oscilloscope. When the needle comes to rest near a nerve twig or end plate, the needle may irritate small intramuscular nerve terminals, which results in the production of two characteristic potentials, end-plate noise and end-plate spikes. End-plate noise produces a rippling of the baseline and a low-pitched continuous noise (Figure 10.3-6). End-plate spikes, on the other hand, are high-amplitude intermittent spikes and make a popping sound. End-plate noise and spikes can occur alone or together. The origin of end-plate noise is thought to be extracellularly recorded miniature end plate potentials,[10,11] whereas end-plate spikes are thought to be single muscle fiber contractions following needle electrode irritation of the nerve terminals.[11] In human beings these potentials are associated with dull pain,[3] and repositioning the needle often eliminates their activity.

Figure 10.3-4 Normal insertional activity in the infraspinatus muscle. Gain: 0.500 mV/division; time: 10 ms/division.

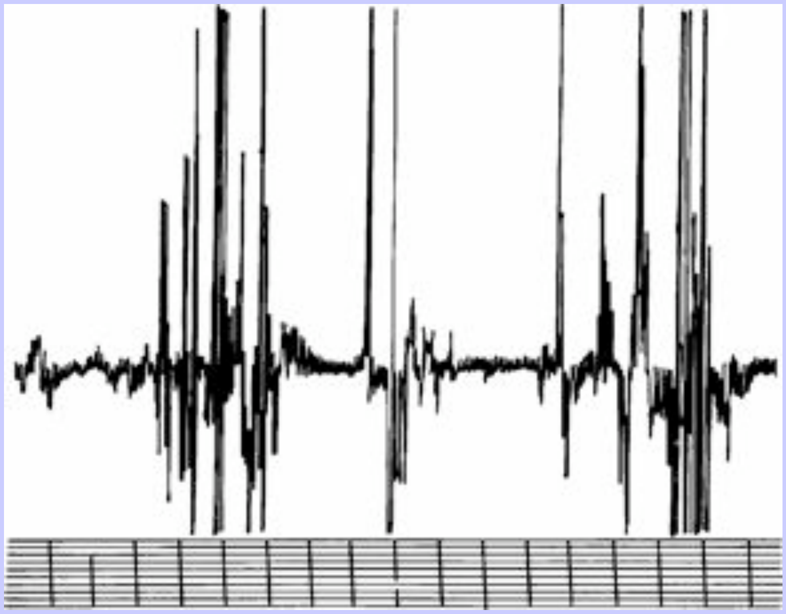


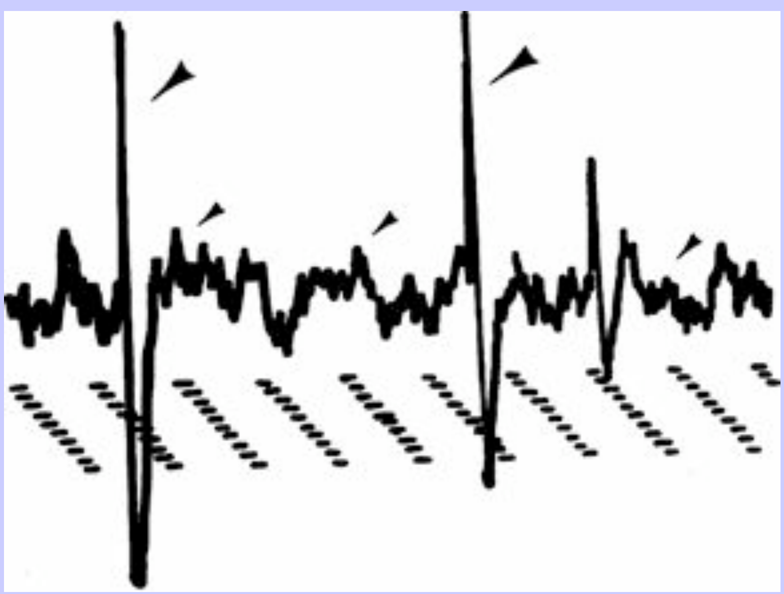
Figure 10.3-5 Electromyograph of the middle gluteal muscle showing normal resting activity *(arrowheads)*, fasciculation potentials *(A, E, G)*, fibrillation potentials *(C)*, positive sharp waves *(B, F)*, and a small motor and action potential *(D)*.

10.3.2.3 MOTOR UNIT ACTION POTENTIALS

MUAPs are voluntary or reflex muscle contractions observed after insertion of the needle electrode. They represent the sum of a number of single muscle fiber potentials belonging to the same motor unit. MUAPs are usually mono-, bi-, and triphasic. Because individual muscle fibers fire nearly synchronously, the prefixes refer to the number of phases above and below the baseline (see Figure 10.3-5). A few polyphasic potentials (greater than four phases) may occur in normal muscle but usually do not exceed 5% to 15% of the population of MUAPs observed.[3] MUAPs have an amplitude ranging from 500 to 3000 μV and a duration ranging from 1 to 15 ms. Examination of the awake horse enhances the evaluation of the amplitude and number of phases of MUAPs in the muscle.

One may see these MUAPs when one forces the animal to bear weight on or retract a limb, resulting in contraction of that explored muscle. In lightly stimulated muscle, one may see single MUAPs, as single motor
units are recruited (see Figure 10.3-5). As muscle contraction becomes more intense, more motor units are 549
recruited, and the greater frequency of MUAPs appears on the oscilloscope. Once MUAPs fill the screen, one 550
observes an interference pattern. Clinically, the number of phases and the duration of MUAPs are of greater importance than amplitude, because amplitude may be influenced by species, the muscle explored, the age of the horse, and electrode position.[9] Furthermore, MUAP duration has been shown to increase with age in human beings.[12]

Figure 10.3-6 Electromyograph from triceps brachii muscle showing end-plate spikes *(large arrowheads)* and end-plate noise *(small arrowheads)*. Gain: 0.500 mV/stairstep; time: 10 ms/division.

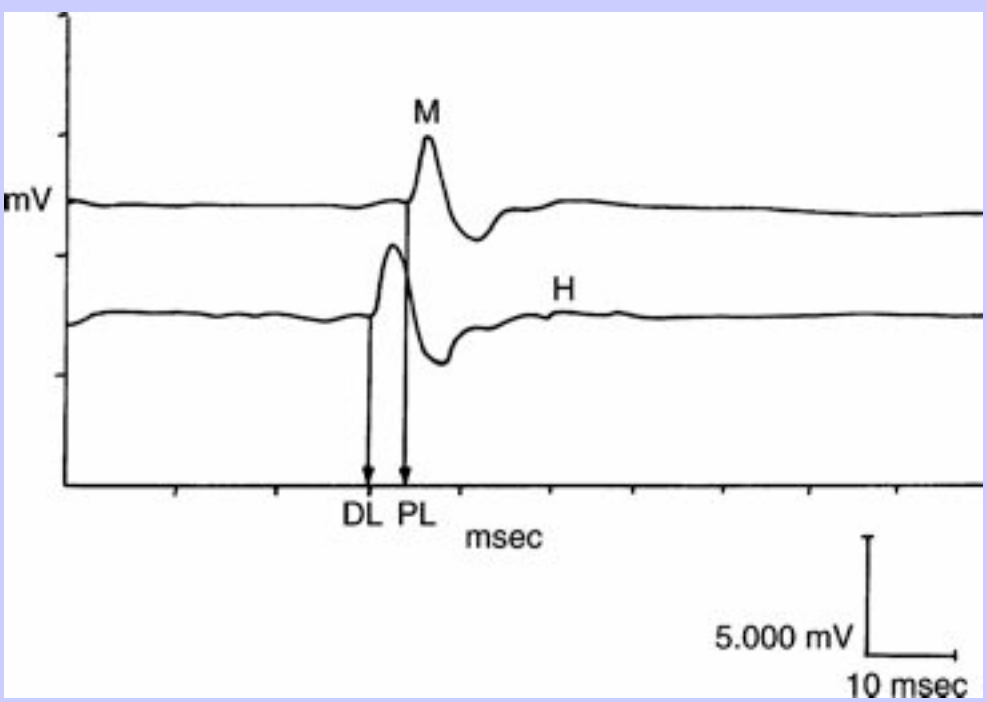


10.3.3

Evoked Muscle Potentials

Stimulation of a mixed motor and sensory nerve results in two observed potentials: the direct evoked muscle action potential (M wave) and the reflex evoked muscle action potential (H wave). The M wave is the direct muscle action potential resulting from orthodromic conduction of direct nerve stimulation (Figure 10.3-7). The M wave is the most commonly used potential in veterinary medicine. This wave is usually biphasic or triphasic and is larger than the H wave. The amplitude of these evoked potentials depends on the number of motor units activated and on the size of the muscle, predominant fiber type, and type of recording electrode used.[4] With monopolar electrodes, amplitudes observed in the horse range from 5 to 60 mV for a 2- to 10-ms duration. Normal amplitudes and durations have been reported for several muscles in the horse. The time required for the potential to travel down the motor nerve, cross the neuromuscular junction, travel down the muscle membrane, and stimulate a response in the muscle is called the latency. When two points along the motor nerve are stimulated, one can measure the distance between the stimulating electrodes (in millimeters) and divide it by the difference in latencies (in milliseconds). Normal median and radial nerve conduction velocities are 60 to 80 m/sec.[5,6] Normal facial nerve conduction velocities are 55 to 70 m/sec.[7]

Figure 10.3-7 Evoked muscle action potentials from the levator nasolabialis muscle after direct stimulation of the buccal branch of the facial nerve illustrating the M wave and H wave. Nerve conduction velocity: 66.0 m/sec. *DL,* Distal latency; *PL,* proximal latency.



10.3.4 Abnormal Electromyographic Potentials

Spontaneous activity in a relaxed muscle after cessation of needle movement may be clinically significant. Diseases affecting the motor unit can lead to altered muscle electric activity, such as prolonged or decreased insertional activity, postinsertional activity, altered waveforms, and complex repetitive discharges. Some abnormal EMG potentials are described next.

10.3.4.1 PROLONGED OR DECREASED INSERTIONAL ACTIVITY

Prolonged electric activity continuing 1 to 10 ms after needle insertion and placement in the muscle is considered abnormal and probably is caused by hyperirritability and instability of the muscle fiber membrane.[9] This activity is most prominent 4 to 5 days after denervation in dogs.[13] Increased or prolonged insertional activity usually precedes the onset of other denervation potentials (fibrillation potentials and positive sharp waves) and may suggest early denervation atrophy.[9] However, one also may see prolonged insertional activity in myotonic disorders and myositis.[13]

Decreased insertional activity (decreased amplitude, duration, or both) may be associated with a decreased number of functioning muscle fibers and may suggest a long-standing neuropathy or myopathy. Infiltration of connective tissue and fat in the muscle can lead to a decreased number of muscle fibers, which can decrease
insertional activity. Complete fibrosis of the muscle may result in loss of insertional activity. Insertional 550
activity also may be absent when muscle fibers are functionally inexcitable, as occurs during attacks of 551
familial periodic paralysis,[3,14] if one uses a faulty needle electrode, or if the EMG needle comes to rest in a normal muscle.[3]

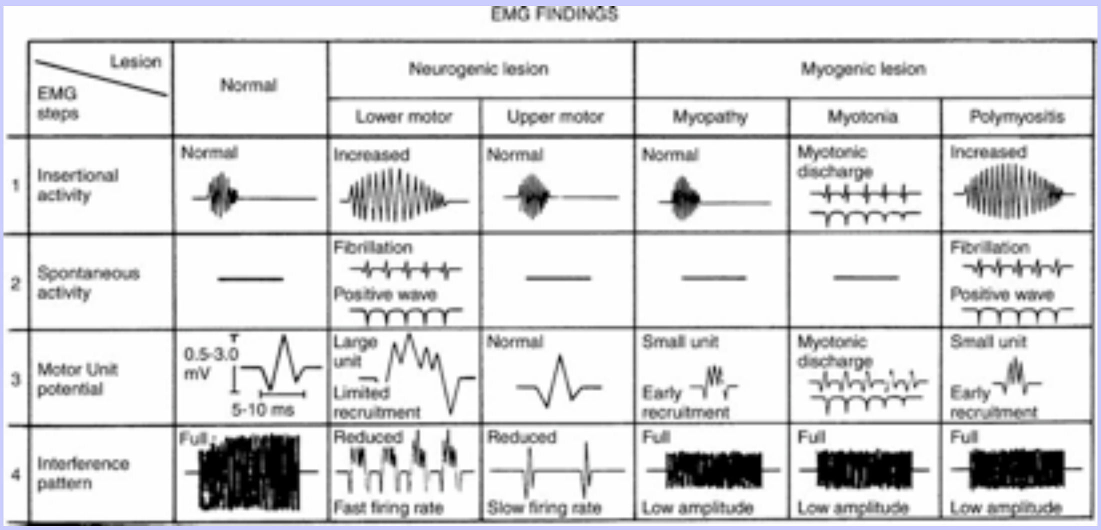
10.3.4.2 POLYPHASIC MOTOR UNIT ACTION POTENTIALS

Polyphasic MUAPs (myopathic potentials) have increased frequency (greater than four phases) and decreased amplitude and duration (Figure 10.3-8), which one observes during submaximal muscle contraction and which result from an increased number of action potentials for a given strength of contraction. Myopathic potentials result from a diffuse loss of muscle fibers[15] and indicate the need for extra motor units to perform the work normally done by fewer motor units.[16] Myopathic potentials are polyphasic and most often occur in primary myopathies such as myotonia-like syndromes, periodic paralysis, myositis, botulism, and myasthenia gravis–like syndromes. These potential also have been reported in steroid-induced myopathies, Cushing's syndrome, and membrane defect myopathies.[9]

10.3.4.3 NEUROPATHIC MOTOR UNIT ACTION POTENTIALS

Neuropathic potentials are MUAPs of decreased frequency and longer duration than myopathic potentials and may occur during minimal and maximal muscle contraction (see Figure 10.3-8). Thus one observes fewer MUAPs of increased amplitude than expected for the strength of contraction, which is more noticeable during maximal contraction and produces a sputtering or motorboat sound. Neuropathic potentials probably are caused by a decreased number of functioning axons firing during maximal muscle contraction. These potentials are most often present in primary neuropathies in which collateral reinnervation has occurred.[16]

Figure 10.3-8 Differential electromyographic findings in neurogenic and myogenic conditions. (Modified from Kimura J: *Electrodiagnosis in diseases of nerve and muscle: principles and practice,* Philadelphia, 1984, FA Davis.)



10.3.4.4 FIBRILLATION POTENTIALS

Fibrillation potentials are the most commonly observed abnormal spontaneous electropotential in EMG (see Figure 10.3-5). These spontaneous discharges sound like frying eggs, crinkling cellophane, or frying bacon and have an initial positive deflection of 100 to 300 μV in amplitude and 2 to 4 ms in duration. They are diphasic or triphasic in waveform. Fibrillation potentials strongly suggest denervation but have been observed in polymyositis, muscular dystrophy, and botulism. Their origin is uncertain, but fibrillation potentials are thought to be spontaneous discharges from acetylcholine-hypersensitive denervated muscle fibers[1,17] or may result from muscle necrosis,[18] muscle inflammation, and focal muscle degeneration. A few fibrillation potentials have been observed in normal healthy muscle, but they are usually not reproducible in other areas of the muscle.

The onset of fibrillation potentials following denervation depends on the size of the animal. The larger the 551
animal, the later the onset of fibrillation potentials[19]; they have been reported between days 5 and 16 552
postdenervation in dogs[20] and human beings.[18] The author has observed fibrillation potentials 4 to 10 days after nerve injury in horses. Fibrillation potentials often occurred along with positive sharp waves; they increase and then decrease in amplitude as the muscle atrophies, with activity ceasing on complete muscle atrophy. Fibrillation potentials occurring alone denote a more severe disease process than the presence of positive sharp wave potentials alone.[17] Fibrillation potentials are helpful in evaluating the length of time muscle denervation has been present and are important in diagnosing denervation before clinical muscle atrophy. One also can use fibrillation potentials as a prognostic indicator: by monitoring fibrillation frequency and amplitude changes with serial examinations, one can assess the extent and progress of denervation. Also, a

decrease in fibrillation potentials followed by the recording of MUAPs may indicate reinnervation and may suggest a favorable prognosis.[18]

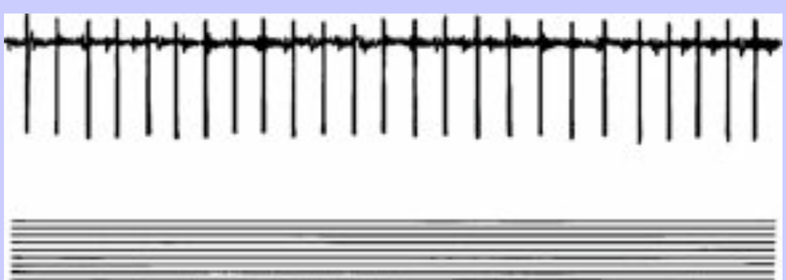
10.3.4.5 POSITIVE SHARP WAVES

Positive sharp waves are potentials in which the primary deflection is downward, followed by a lower-amplitude, longer-duration negative deflection (see Figure 10.3-5). This waveform has been described as resembling a saw tooth.[3] Positive sharp waves occur with muscle denervation and muscular diseases such as myositis, exertional rhabdomyolysis (tying-up syndrome),[21] and spinal shock.[16] Sometimes one observes positive sharp waves in association with or shortly after insertional activity that persist after electrode placement. More than two positive sharp waves occurring after insertional activity may indicate early denervation. Positive sharp waves may occur in denervated muscle following chronic exertional rhabdomyolysis, myotonia,[21] equine protozoal myeloencephalitis (EPM), laryngeal hemiplegia,[22] suprascapular nerve injury (sweeney),[23] and compressive myelopathies. Positive sharp waves often precede or appear along with fibrillation potentials in denervated muscle. One can observe these potentials singly or in trains (Figure 10.3-9), and they may sound like a machine gun. The origin of positive sharp waves is uncertain but may be associated with hyperexcitable muscle cell membranes.[17]

10.3.4.6 FASCICULATION POTENTIALS

Fasciculation potentials are spontaneous discharges from a group of muscle fibers representing the whole or part of a motor unit[3,17] (see Figure 10.3-5). The source of fasciculation potentials has not been determined yet, but evidence suggests they originate from neural discharges in the spinal cord or along the peripheral nerve.[24,25] Fasciculation potentials occur in diseases of anterior horn cells and irritative-type lesions of root or peripheral nerve, such as radiculopathies and nerve entrapments in human beings.[13] Little significance is placed on isolated fasciculation potentials in horses. However, fasciculation potentials in the presence of fibrillation potentials or positive sharp waves may indicate lower motor neuron disease and may occur in suprascapular nerve entrapment (sweeney) in horses.

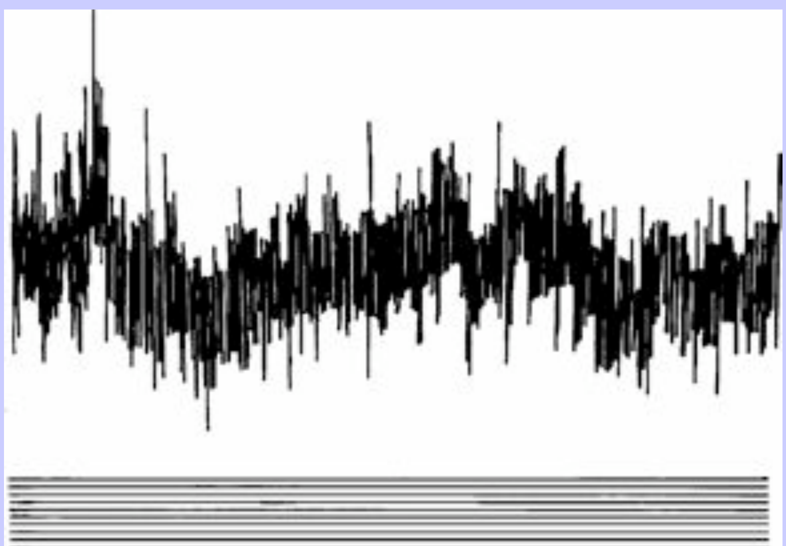
Figure 10.3-9 Electromyograph showing train of positive sharp waves. Gain: 500 V/stairstep.



10.3.4.7 COMPLEX REPETITIVE DISCHARGES VERSUS MYOTONIC POTENTIALS

Complex repetitive discharges (bizarre high-frequency potentials) and myotonic potentials occur less frequently in horses. Both of these potentials are repetitive MUAPs induced by insertion of the needle electrode or percussion of muscle. Bizarre high-frequency potentials tend to be shorter in duration and end abruptly compared with myotonic discharges. Bizarre high-frequency potentials sound like a machine gun. However, myotonic potentials often wax and wane in amplitude, last 4 to 5 seconds[2,3,9] and sound like a dive-bomber, hence the nickname "divebomber potential"[1,9] (Figure 10.3-10). Myotonic and bizarre high-frequency potentials are thought to be associated with hyperexcitability of the muscle cell membrane.[1] Bizarre high-frequency potentials may occur in diseases of the lower motor unit such as muscular dystrophy,[1,9,21] 552 steroid-induced myopathy,[9] polymyositis, chronic denervation,[26] and hyperkalemic periodic paralysis.[14] 553 Myotonic potentials may occur in myotonia congenita and myotonia dystrophica[21] and may occur in hyperkalemic periodic paralysis in human beings, which may reflect abnormal muscle chloride or potassium conductance.[3] Myotonic potentials also have been observed in horses with hyperkalemic periodic paralysis, but some were obscured by concurrent complex repetitive discharges.[14]

Figure 10.3-10 Electromyograph showing waxing and waning myotonic potentials. Gain: 500 V/stairstep.



10.3.5 Diseases Affecting the Motor Unit and Peripheral Nerves

Diseases of the motor unit and peripheral nerves can lead to changes in skeletal muscle electric activity or nerve conduction velocity or both. In these diseases EMG may be a useful diagnostic aid in localizing the lesion.

10.3.5.1 FOCAL AND MULTIFOCAL MYELOPATHIES

Compressive cervical myelopathies, cervical stenotic myelopathy (wobbler syndrome), and EPM are common causes of neurologic signs in the horse. These conditions are characterized by damage to the sensory pathways and in some instances damage to the ventral horn cells of the spinal cord, a component of the motor unit. Physical examination may reveal muscle atrophy and sweating over affected muscles. Needle EMG of the cervical axial musculature in horses presented with truncal, forelimb, and hindlimb ataxia, without cranial nerve deficits, may reveal increased insertional activity, fibrillation potentials, and positive sharp waves indicating compression of the ventral horn cells or ventral roots.[27] Abnormal postinsertional activity at the level of the spinal cord compression may allow the clinician to focus the radiographic examination.

In cases of EPM, needle EMG may reveal fibrillation potentials, positive sharp waves, and abnormal insertional activity in affected limb muscles in horses presented for obscure lameness. One also can examine horses with muscle asymmetry (Figure 10.3-11) via needle EMG to determine the extent of muscle involvement. The examination may lead to early diagnosis of EPM so that one can prescribe treatment. Serial needle EMG examination also may be helpful in monitoring the response to treatment and prognosis.

10.3.5.2 RADIAL AND SUPRASCAPULAR NERVE INJURY

Damage to the peripheral nerves leads to muscle atrophy of the innervated muscle. Damage to the radial and suprascapular nerves can occur with trauma to the cranial aspect of the shoulder. Needle EMG and nerve conduction studies are helpful in evaluating the extent of damage to these and other peripheral nerves. Needle EMG and nerve conduction studies also may be able to differentiate lost or reduced limb function caused by nerve damage from painful conditions. Muscle groups that have atrophied because of disuse (disuse atrophy) caused by a painful condition show no postinsertional activity on needle EMG examination.

Figure 10.3-11 Horse with gluteal muscle atrophy following protozoan myelitis showing distribution of abnormal electromyographic potentials.

Positive sharp waves and fibrillation potentials in the triceps brachii and extensor carpi radialis muscles may suggest radial nerve injury. Positive sharp waves and fibrillation potentials in the supraspinatus and infraspinatus muscles may suggest suprascapular nerve injury (Figure 10.3-12). Postinsertional activity in these muscle groups and the lateral head of the triceps suggests damage to the brachial plexus (Figure 10.3-13). Thus needle EMG may be helpful in differentiating suprascapular nerve injury from brachial plexus injury. Visible evidence of muscle atrophy may not be present until several weeks after injury. To confirm a radial nerve injury, one can calculate the radial nerve conduction velocity (Figure 10.3-14). If the nerve conduction velocity is less than 60 m/sec or significantly less than the opposite limb, one may suspect radial

nerve injury. Radial nerve injury also may lead to a decreased amplitude and duration of the evoked muscle action potential.

Needle EMG and nerve conduction studies may also be helpful in determining success of nerve decompression surgery. Once the nerve is decompressed surgically, serial needle EMG examinations may be helpful in determining if permanent nerve damage has occurred and the extent of return of nerve function. 553

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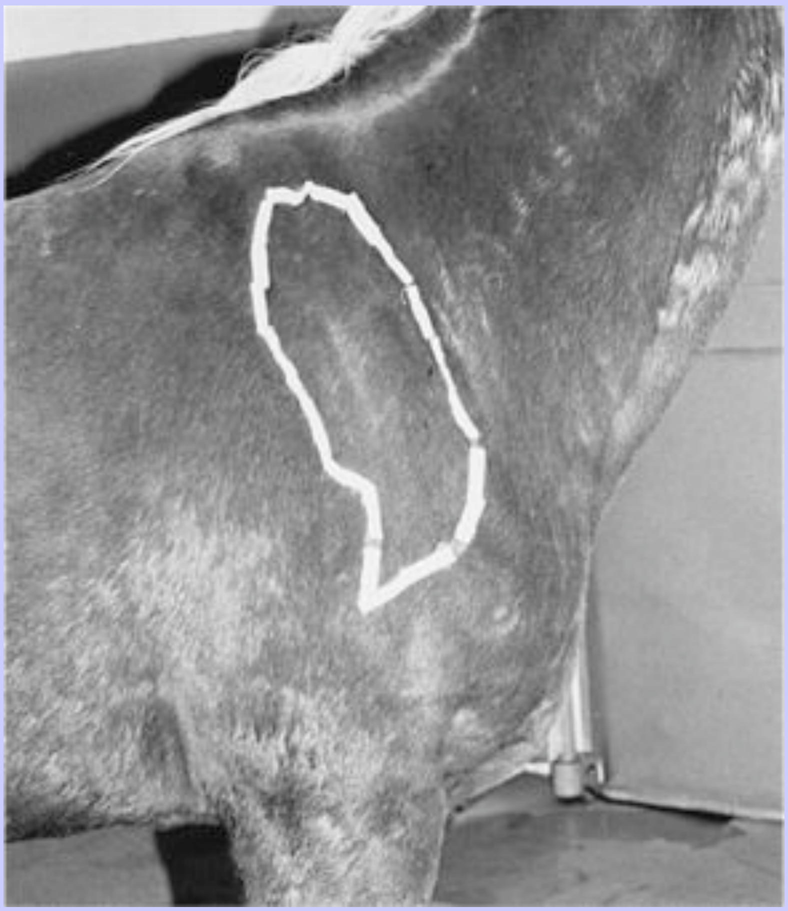
Figure 10.3-12 Horse with suprascapular nerve injury with characteristic electromyographic distribution of fibrillation potentials and positive sharp waves in supraspinatus and infraspinatus muscles.



10.3.5.3

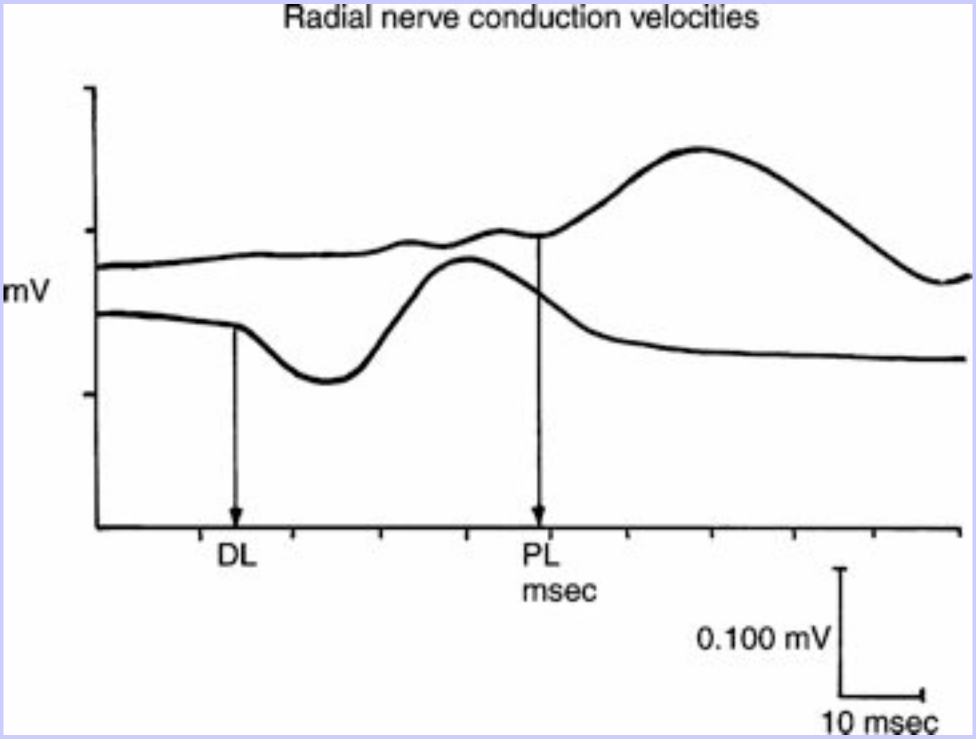
LARYNGEAL HEMIPLEGIA

Laryngeal hemiplegia has been described extensively in the literature as denervation of the intrinsic laryngeal musculature, specifically the recurrent laryngeal nerve.[5] One difficulty with this condition is that current diagnostic techniques (endoscopy, inspiratory stridor with exercise) are limited to recognizing laryngeal hemiplegia after onset of clinical signs.[28] However, fibrillation potentials and positive sharp waves in the

dorsal cricoarytenoid muscle on needle EMG examination may appear before clinical signs. Thus needle EMG may be useful in the early detection of laryngeal hemiplegia in horses. Spontaneous EMG activity, including fibrillation potentials, positive sharp waves, or bizarre high-frequency discharges, have been reported in the dorsal cricoarytenoid muscle of horses affected with laryngeal hemiplegia. Fibrillation potentials and positive sharp waves are the most common abnormal electric potential observed in horses with laryngeal hemiplegia. Decreased insertional activity and bizarre high-frequency discharges were reported at a lesser frequency.[22]

Figure 10.3-13 Horse with brachial plexus injury showing characteristic distribution of electromyographic charges, including fibrillations and positive sharp waves. The involvement of the supraspinatus, infraspinatus, triceps brachii, extensor carpi radialis, and pectoral muscles is notable.

Figure 10.3-14 Evoked muscle action potential from the extensor carpi radialis muscle showing decreased amplitude and decreased nerve conduction velocity, suggesting radial nerve injury. *DL,* Distal latency; *PL,* proximal latency.



10.3.5.4

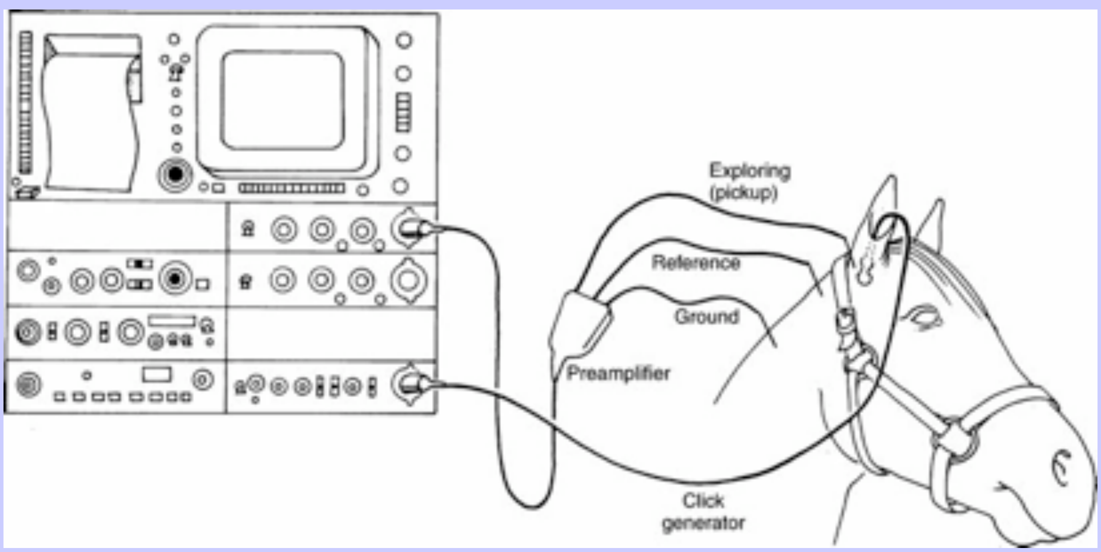
MYOPATHIES, MYOSITIS, AND MYOTONIA

Needle EMG may be helpful in evaluating horses with signs of primary muscle disease, such as muscle tremors, muscle fasciculations, muscle stiffness, weakness, and percussion dimpling. Myopathies generally are classified into inflammatory or degenerative types.[29] Degenerative myopathies are characterized by an intact motor unit, but a loss of viable muscle fibers, which results in an increased number of polyphasic MUAPs with decreased duration (see Figure 10.3-8). Inflammatory myopathies (myositis) are characterized by a subacute or acute degeneration of muscle fibers with active infiltrates of inflammatory cells. The characteristic potentials are increased insertional activity, brief low-voltage MUAPs, fibrillation potentials, and positive 554
sharp waves. Needle EMG is a valuable aid in the diagnosis and localization of focal and diffuse myopathies. 555
Primary myopathies such as exertional rhabdomyolysis and myositis can be differentiated from myotonia and myotonia-like syndromes by needle EMG examination.[21] Furthermore, fibrillation potentials and positive sharp waves have been observed on EMG examination in horses with chronic myositis, exertional rhabdomyolysis, shivers, and hyperkalemic periodic paralysis.[14,21] After completing the needle EMG examination, one may evaluate muscles with postinsertional activity further by muscle biopsy and microscopic evaluation.[21,30]

10.3.5.5 MYOTONIA

Myotonia is a disease characterized by an electrolyte conductance deficit in the skeletal muscle membrane.[3] One may observe fibrillation potentials, positive sharp waves, a train of positive sharp waves, and myotonic potentials on EMG examination in horses with myotonia congenita[21] and myotonia dystrophica.[31] Characteristic electrophysiologic findings include waxing-and-waning, high-frequency spontaneous and induced myotonic discharges (dive-bomber potentials) in many muscle groups, including the middle gluteal and semitendinosus muscles[21,31] (see Figure 10.3-10). One may use needle EMG to confirm the diagnosis of myotonia, to distinguish this condition from pseudomyotonia, and to localize areas for muscle biopsy (see Figure 10.3-8). Myotonia and myotonia-like syndromes also may present as tremors or even seizure activity. EMG along with a thorough physical and neurologic examination can help differentiate seizures from primary myopathy.

Figure 10.3-15 System used for auditory brainstem response testing in the horse. (From Rolf SL, Reed SM, Melnick W et al: Auditory brainstem response testing in anesthetized horses, *Am J Vet Res* 48:910-914, 1987.)



10.3.6 Auditory Brainstem Response Testing

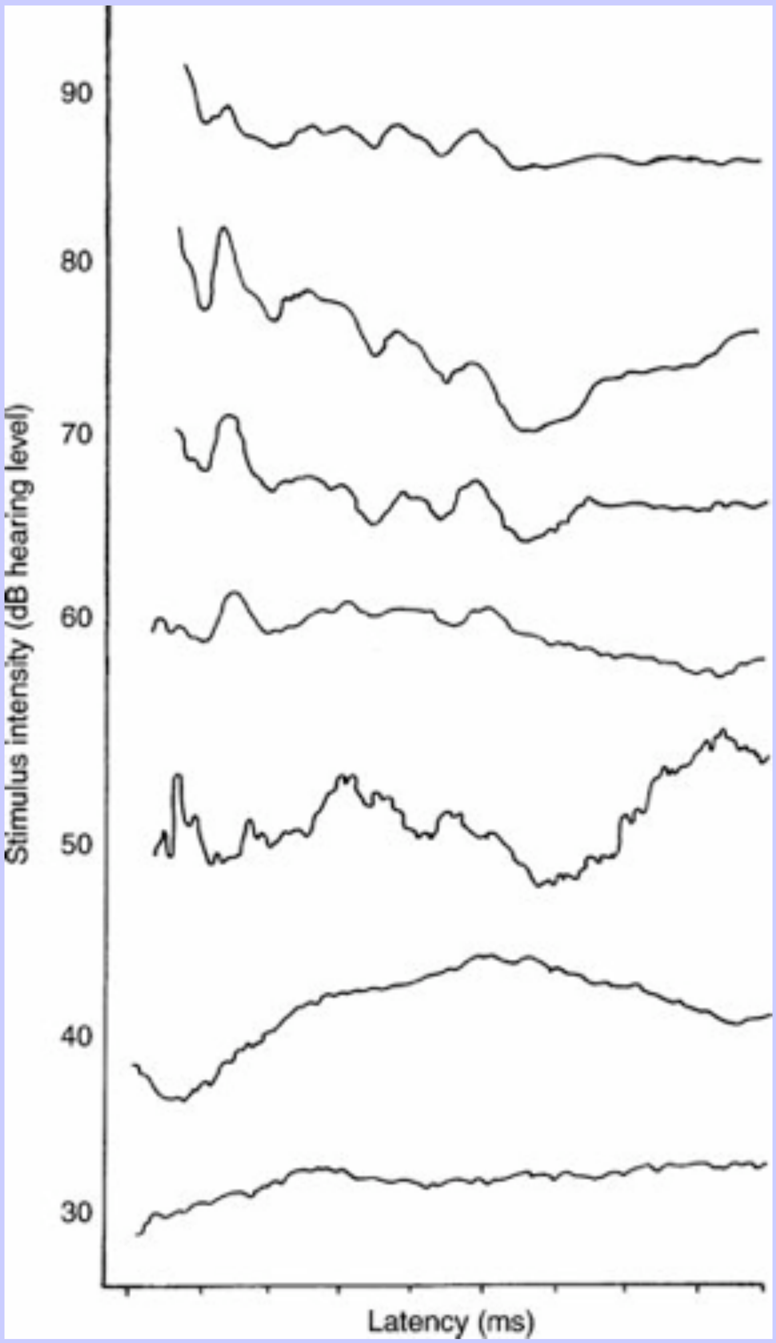
ABR testing is a method of recording potentials arising from the eighth cranial nerve and its projections in response to acoustic stimulation via surface or subcutaneously placed electrodes. The ABR is those evoked potentials, or waves, arising within the first 10 ms after delivery of an acoustic stimulus (clicks) (Figure 10.3-15). In human beings, ABR is recognized as consisting of from five to seven waves, generally designated I through VII. Of these, waves I through V are the most common. In dogs and cats fewer waves are observed.[32,33]

In human beings and animals, a correspondence exists between these waves and certain anatomic generator sites[33–39]:

1. Wave I is generated by bipolar neurons of the eighth cranial nerve.
2. Wave II also may be generated partly by the eighth cranial nerve.
3. Waves II through V probably reflect more generalized activity in the auditory system in the medulla and pons and may represent neural activity ipsilateral and contralateral to the stimulated ear.

A wide range of clinical applications of ABR in human beings has been well described.[19,34,37,39–46] However, its use in the horse has been limited.[47,48] ABR testing is a method of assessing not only auditory function but a 555
variety of neurologic disorders involving the brainstem. ABR testing is unaffected by the state of arousal of the 556
test subject and responses are not degraded by sedation or general anesthesia.[37,45]

Figure 10.3-16 Mean latency waves at 136 dB, sound pressure level (SPL) through 87 dB SPL. Five waves are present except at 40 of 80 dB SPL. (From Rolf SL, Reed SM, Melnick W et al: Auditory brainstem response testing in anesthetized horses, *Am J Vet Res* 48:910-914, 1987.)

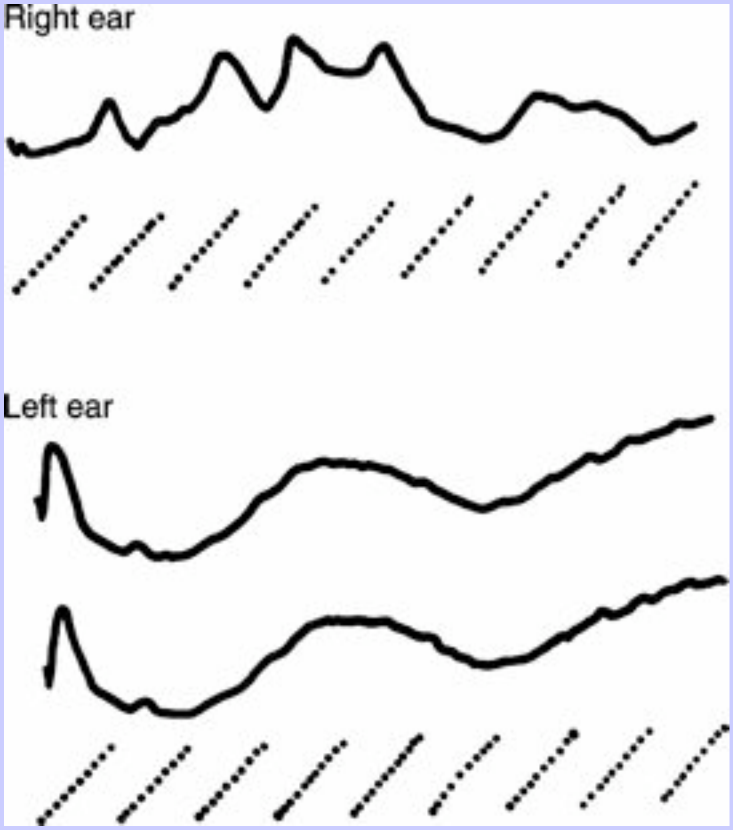


One can examine horses awake, with or without mild sedation, or anesthetized (see Electromyography). If one tests an awake horse, one stimulates each ear and records the resultant waveforms independently. If one anesthetizes the patient, one examines the uppermost ear first, turns the horse, and examines the lower ear.

One commonly observes five peaks, and these are considered analogous to waves I through V in human beings[*] (Figure 10.3-16). Mean latencies have been reported previously in horses under general anesthesia.[51] As has been observed in dogs and cats,[32,33] latencies of the waves decrease as stimulus intensity increases. One can use the ABR clinically in horses with head tilts (Figure 10.3-17) to verify the presence of hearing loss (Figure 10.3-18), middle or inner ear infections, and stylohyoid osteomyelitis. ABR testing also may be helpful in the diagnosis and prognosis of traumatic, infectious, or inflammatory brainstem lesions such as vascular infarcts or anomalies, ischemic fibrocartilaginous emboli, basisphenoid bone fracture, and protozoal encephalomyelitis. One can assess the quantitative and qualitative characteristics of the ABR-generated waveforms. Persistent prolonged latencies suggest retrocochlear or conductive abnormalities.[37] Interaural latency differences of wave V may suggest unilateral brainstem disease, except when cochlear disease is present. Qualitative ABR changes are of greater use in equine medicine because quantitative measures of normal and abnormal horses are limited. In human beings qualitative changes such as peak presence, waveform morphologic characteristics, and response 556
stability are of greater use in the diagnosis of central disorders, particularly acoustic neuromata and 557
demyelinating diseases.[37]

Figure 10.3-17 Photograph of foal with a right head tilt and dropping of the right ear. Auditory brainstem response (see Figure 10.3-18) shows a hearing loss in the left ear, the presumed cause of his head tilt. The right ear was normal.

Figure 10.3-18 Auditory brainstem response of the foal in Figure 10.3-17 showing normal right ear and abnormal left ear.



The limitations of ABR include a dependence on cochlear function; susceptibility to extraneous noise, which may affect waveform morphology; and the limits of the machinery in excluding 60-cycle interference.

* References 4, 35, 37, 39–44, 49, 50.

10.3.7 Electroencephalography

EEG is the graphic recording of electric activity arising from the cerebral cortex. The origin of this electric activity is not known but is thought to arise from pyramidal cell dendrites located within a 2-mm depth of the cerebral cortex. This electric activity may be modified by deeper structures such as the brainstem, reticular activating system, and thalamus. EEG is an extension of the neurologic examination and is a valuable tool for determining the presence of a cerebral disease, localizing it, determining its extent (focal or diffuse), differentiating between inflammatory and degenerative changes, and establishing a prognosis.

EEG has been used and reviewed extensively in human beings[52,53] and small animals,[8,49] but little work has been done in the horse.[54] This discussion presents a brief description of the use, interpretation, and limitations of EEG so that one may gain a better understanding regarding its use.

10.3.7.1

NORMAL PATTERNS

One should evaluate the EEG for symmetry, waveform, morphology, frequency, and amplitude. The bipolar montage, as discussed previously, allows comparison of cortex to cortex. One can compare the potentials generated by the left occipital region with those of the right occipital cortex, the potentials generated by the left frontal cortex region with those of the right frontal area, and the potentials generated by the left side (left frontal–left occipital) with those of the right side (right frontal–right occipital) (Figure 10.3-19). Normal EEG potentials in the horse consist of a dominant waveform in the awake alert horse of low voltage (8 to 15 μV) and fast activity (18 to 30 Hz) (Table 10.3-2). Usually this activity is superimposed over a low to medium voltage (10 to 40 μV) and slow activity (5 to 10 Hz). Muscle artifact occasionally interrupts the baseline, when the animal shakes its head, moves its eyes, or twitches the facial muscles.

Figure 10.3-19 Photograph of horse illustrating bipolar montage for electroencephalography. *RO,* Right occipital; *LO,* left occipital; *RF,* right frontal; *LF,* left frontal; *V,* vertex; *G,* ground; *ECG,* electrocardiogram position.

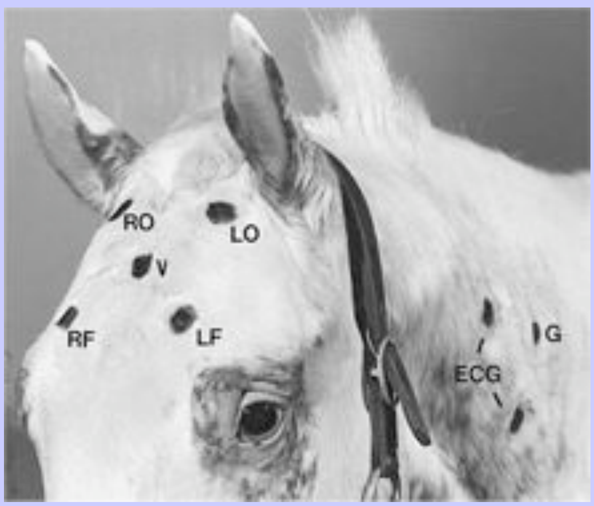


TABLE 10.3-2 Amplitudes and Frequencies Observed in Electroencephalographic Patterns in Normal Horses and Horses With Tranquilization

	PATTERNS	
	VOLTAGE (μV)	FREQUENCY (Hz)
Normal (awake)	8–15	18–30
	10–40	5–10
Xylazine HCl (Rompun)	10–80	10–15
	5–30	25–405
	10–90	0.5–4.05
Acetylpromazine	5–40	25–405
	5–10	1–4

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Figure 10.3-20 Electroencephalograph from a horse with cryptococcal meningitis showing generalized low-voltage, high-frequency activity and frequent spikes. *RO,* Right occipital; *LO,* left occipital; *RF,* right frontal; *LF,* left frontal; *V,* vertex; *G,* ground; *ECG,* electrocardiogram position.

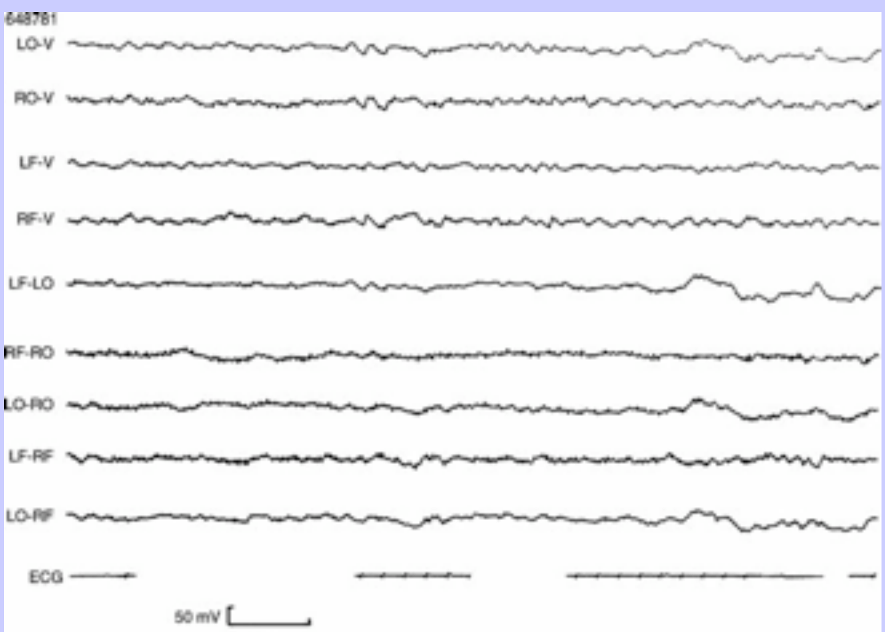
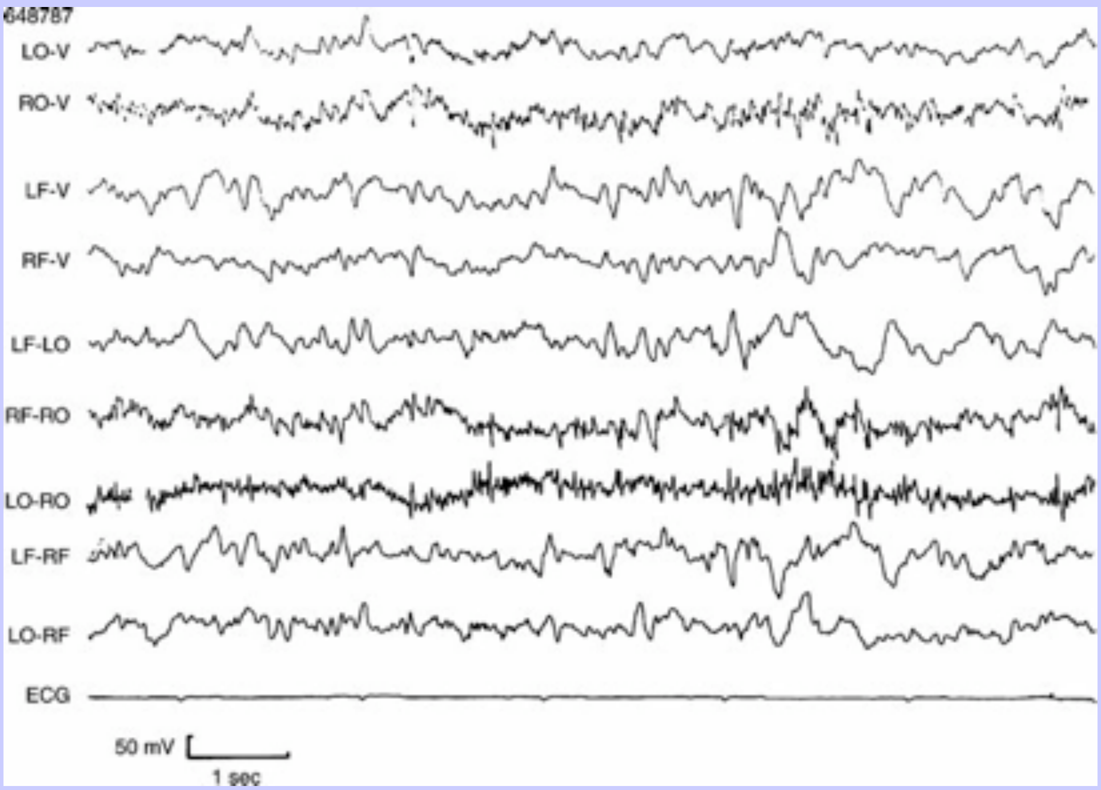


Figure 10.3-21 Electroencephalograph from a horse with a space-occupying mass in the left frontal area of the brain. The generalized high-voltage, low-frequency wave and asymmetry of left cortex are notable. *RO,* Right occipital; *LO,* left occipital; *RF,* right frontal; *LF,* left frontal; *V,* vertex; *G,* ground; *ECG,* electrocardiogram position.



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10.3.7.2 ABNORMAL PATTERNS

Diseases of the cerebral cortex can alter frequency, amplitude, and symmetry of EEG patterns. Low voltage–fast activity and spikes may occur with ongoing irritative processes such as seizures or inflammation (Figure 10.3-20). High voltage–slow activity indicates death of neurons in diseases such as brain abscess (Figure 10.3-21) and neoplasia. However, low voltage–fast activity and high voltage–slow activity are not pathognomonic for any disease process but suggest the various disease states previously discussed. Localized EEG changes indicate focal cortical disease such as infarcts, hemorrhage, early tumor, or abscessation (see Figure 10.3-21), whereas generalized EEG changes may indicate a diffuse cortical or subcortical disease such as infection (see Figure 10.3-20), trauma, space-occupying lesions (hydrocephalus, tumor), idiopathic epilepsy, or a systemic metabolic illness (hepatic encephalopathy). Serial recordings may be helpful in following therapy and the progress of disease. Generally, artifacts such as ocular movement and facial muscle twitches produce asymmetric and symmetric low voltage–slow activity, whereas hypothyroidism may produce

symmetric low voltage–medium-to-slow activity.[49] The difficulty in EEG in the horse is differentiating artifact (muscle movement, eye movement, head shaking, EEG artifact) from true EEG changes. For reemphasis, EEG alone is only one part of the diagnostic workup. One always should interpret the EEG examination along with the history, clinical signs, and neurologic examination.

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10.4
10.4—Seizures, Narcolepsy, and Cataplexy

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10.4.1
Seizures

Seizures are clinical manifestations of rapid excessive electric discharges from the cerebral cortex that result in involuntary alterations of motor activity, consciousness, autonomic functions, or sensation.[1–5] Seizures may be

referred to as *fits, attacks, strokes, convulsions,* or *epilepsy*. A true seizure, however, refers to a specific clinical event regardless of cause or morphology. Epilepsy refers to reoccurring seizures with nonprogressive intracranial alterations, which may be genetic or acquired. True inherited epilepsy probably does not occur in horses.[2] Convulsions, however, refer to seizures accompanied by tonic-clonic muscle activity and loss of consciousness. Convulsions may include a generalized seizure.

Seizures in horses can be classified into three broad forms based on clinical signs: partial seizure, generalized seizure, and status epilepticus. A partial seizure involves a discrete area of the cerebral cortex and results in localized clinical signs, such as facial or limb twitching, compulsive running in a circle, or self-mutilation. One may observe a partial seizure after cervical myelography, anesthesia, and cranial trauma that may spread throughout the cerebral cortex and produce a secondary generalized seizure. A generalized seizure involves the entire cerebral cortex and results in generalized tonic-clonic muscle activity over the whole body, with loss of consciousness. Generalized seizures are the most common form of seizures observed in adult horses and foals.[2,3] Status epilepticus is characterized by generalized seizures occurring in rapid succession and is uncommon in horses.[2]

10.4.1.1 PATHOGENESIS

Adult horses have a high seizure threshold. Severe damage must occur to the brain before adult horses have seizures. Foals, however, have a lower seizure threshold and are more susceptible to conditions causing seizures.[6] Intracranial or extracranial factors may cause seizures[2] (Tables 10.4-1 and 10.4-2). The most common causes of seizures in foals under 2 weeks of age are neonatal maladjustment syndrome, trauma, and bacterial meningitis.[6] The most common causes of seizures in foals less than 1 year of age are trauma and idiopathic epilepsy in Arabian foals.[2,3] The most common causes of seizures in adult horses older than 1 year 560
of age are brain trauma, hepatoencephalopathy, and toxicity.[2] Tumors, especially pituitary adenoma, may 562
cause seizures and blindness in horses older than 7 years.[2]

TABLE 10.4-1 Known and Suspected Causes of Seizures in Horses Less Than 1 Year of Age

	DIFFERENTIAL DIAGNOSIS		
CLASSIFICATION	EXTRACRANIAL	INTRACRANIAL	DIAGNOSTIC AIDS
Anomalies (congenital)		Hydrocephalus	
		Hydranencephaly	1–6, 8
		Benign epilepsy	11
Metabolic	Hypoxia, hyponatremia, hypoglycemia, hyperkalemia		2–4, 8, 11
Toxic	Organophosphates	Moldy corn	2–6
	Strychnine	Locoweed	9–11
	Metaldehyde		
Traumatic		Brain trauma	2–4
		Lightning	5, 6, 8, 10, 11
Vascular		Neonatal maladjustment syndrome (vascular accidents)	2–6
Infectious	Septicemia	Bacterial meningitis	2–4
	Endotoxemia	Cerebral abscesses	5–9
	Fever	Rabies	11
	Tetanus	Viral encephalitis	
	Botulism		

1, Breed; 2, onset; 3, clinical course; 4, physical examination; 5, neurologic examination; 6, clinical pathology: cerebrospinal fluid analysis; 7, serologic testing; 8, radiology (computed tomography scan, ultrasound, radiographs); 9, toxicologic testing; 10, electrodiagnostics (electroencephalography, electromyelography); 11, pathologic examination.

TABLE 10.4-2 Known and Suspected Causes of Seizures in Horses More Than 1 Year of Age

	DIFFERENTIAL DIAGNOSIS		
CLASSIFICATION	EXTRACRANIAL	INTRACRANIAL	DIAGNOSTIC AIDS
Metabolic	Hepatoencephalopathy, hypomagnesemia, hypocalcemia, uremia, hyperlipidemia		2–6, 11
Toxic	Organophosphates	Moldy corn	2–6
	Strychnine	Locoweed	9, 11
	Metaldehyde	Bracken fern	
		Lead, arsenic, mercury	
		Rye grass	
Traumatic		Brain trauma	8, 10, 11
Vascular		Strongylus vulgaris	2–4
		Cerebral thromboembolism	5, 6, 11
		Intracarotid injection	
Tumor		Neoplasia	2–5
		Hemarthroma	6, 8, 10, 11
		Cholesterol granuloma	
Infectious		Cerebral abscess	1, 3–6
		Rabies	7, 8, 12, 13
		Tetanus	
		Arbovirus encephalitides	
		Mycotic cryptococcosis	
		Protozoal myelitis	

1, Breed; 2, onset; 3, clinical course; 4, physical examination; 5, neurologic examination; 6, clinical pathology: cerebrospinal fluid analysis; 7, serologic testing; 8, radiology (computed tomography scan, ultrasound, radiographs); 9, toxicologic testing; 10, electrodiagnostics (electroencephalography, electromyelography); 11, pathologic examination.

The pathophysiologic mechanisms of seizures are not known thoroughly. Current research has focused on intracellular neuronal and synaptic events that initiate excessive and prolonged neuronal depolarization, known as "paroxysmal depolarization shift." Several mechanisms are thought to cause paroxysmal depolarization shifts and seizures and include increased excitatory neural transmitters, decreased inhibitory neural transmitters (γ-aminobutyric acid, GABA), alteration in neural transmitter receptor sites, or a derangement in the internal cellular metabolism of the neuron.[7,8] The most widely held hypothesis for seizure initiation is development of excitatory postsynaptic potentials. Seizures may develop because of a summation

of synchronous excitatory postsynaptic potentials in large groups of neurons that may be precipitated by an increase in excitatory neurons, a decrease in inhibitory neurons, a decrease in inhibitory neurotransmitters, or any combination of these.[7,9] Once a "critical mass" of neurons has fired, an uncontrolled spread of electric activity may occur over the cerebral cortex and precipitate a generalized seizure. Head trauma and decreased cerebral blood supply have been implicated in creating seizure foci. Head trauma may result in cerebral cortical hypoxia, which in turn may lead to necrosis of inhibitory neurons. Because cerebral inhibitory neurons are more sensitive to hypoxia than excitatory neurons, a loss of inhibitory neurons allows the spread of these excitatory postsynaptic potentials and seizure development.[10]

Inhibitory neurons surround excitatory neuron groups and check areas of intense stimulation. Decreased inhibitory neurotransmitters in these areas, such as GABA, have been implicated in seizure formation in animals. Application of penicillin directly to the brain of laboratory animals suppresses GABA and induces seizures.[11] These seizures can be blocked by use of other agents that potentiate inhibitory neurotransmitters. Phenobarbital and pentobarbital potentiate inhibitory neural transmitters, such as GABA, and block seizure foci caused by penicillin and hypoxia.

Alterations in the neuronal cell microenvironment may lead to seizure generation. Systemic and local neuronal electrolyte abnormalities may disturb excitatory neuron homeostasis and lead to spontaneous and excessive action potentials.[7] Intracellular potassium released during neuronal activity may reach sufficient concentration to move the resting membrane potential toward the threshold and generate a seizure focus.[12] Spontaneous action potentials generated by alteration in intracellular potassium concentration may spread to other parts of the cerebral cortex, causing a generalized seizure.

Furthermore, alterations in one intracellular neuronal electrolyte may alter the homeostasis of other intracellular electrolytes. This alteration is supported by the observation of increased intracellular potassium concentration, together with decreased intracellular calcium and chloride concentrations, in the long-duration changes in excitability known to occur during interictal epileptogenesis and the transition from interictal to ictal activities.[13,14]

Alterations in sodium conductance also have been implicated in causing seizures. Rapid influxes of sodium into the neuron may lead to hyperexcitability of the neuron and rapid firing. Phenytoin, a hydantoin derivative, blocks these rapid influxes of sodium into neurons and suppresses repetitive firing by hyperexcitable neurons.[15] Thus a complex interaction may occur between these electrolytes and the internal cell homeostasis that precipitates seizure formation.

10.4.1.2 CLINICAL SIGNS AND DIAGNOSIS

Seizure activity is manifested clinically in a variety of ways depending on the area and the extent of the cerebral cortex involved. In partial seizures, asymmetric twitching of a limb, facial twitching, excessive chewing, compulsive running, or self-mutilation may occur.[2] A localized seizure may develop into a generalized seizure.

In a generalized seizure, one may observe three distinct clinical periods. Just before the seizure (aura), horses may exhibit signs of anxiety and uneasiness. During the seizure (ictus), horses may become recumbent, unconscious, and have symmetric clonic muscle contractions (contractions and relaxations of muscles occurring in rapid succession), followed by symmetric tonic muscle contractions (continuous unremitting muscle contractions).[2,3] Horses also may show deviation of eyeballs, dilated pupils, ptyalism, trismus or jaw

clamping, opisthotonos, lordosis or kyphosis, violent paddling movements of the limbs, uncontrolled urination and defecation, and excessive sweating. A generalized seizure may last from 5 to 60 seconds.[2] After the seizure (postictus), horses may show depression and blindness for hours to days.[2,3]

Diagnosis of seizure is based on history, clinical signs, and ancillary diagnostic tests to determine an underlying cause, whenever possible (see Tables 10.4-1 and 10.4-2). In all paroxysmal, involuntary neurologic events, one should consider seizures first unless the cause is proved otherwise. Careful questioning of the owner can reveal information about the event, the time of day, relationship to feeding, date, unusual environmental circumstances (stimuli such as thunderstorms, fireworks, changes in housing), recent trauma, febrile episodes, exposure to drugs or toxins, recent behavioral changes, and the seizure history of the dam,
sire, and other siblings. One must rule out other conditions that mimic seizures, including painful conditions 562
(colic, limb fractures, exertional myopathy), hyperkalemic periodic paralysis, and syncope. In these conditions 563
horses do not lose consciousness but remain bright and alert. Horses with hyperkalemic periodic paralysis may show prolapse of the nictitating membranes of the eye and muscle fasciculations but remain anxious, alert, and have normal pain perception. This condition occurs in Quarter Horse and Quarter Horse crosses 2 to 3 years of age. Serum potassium concentration in these horses ranges from 5.5 to 9.0 mEq/L during or shortly before collapse.[16,17]

One can confuse narcolepsy and cataplexy with seizures. Most narcoleptic horses (except for some ponies) remain standing with the head hanging close to the ground. If recumbency occurs, loss of muscle tone and rapid eye movement (REM) sleep may follow.[18] Cardiac arrhythmias or severe murmurs may precipitate syncopal episodes. Auscultation of the heart may help determine the presence of severe murmurs, and electrocardiography may help determine the presence of arrhythmias. Icteric mucous membranes may be apparent in horses with hepatoencephalopathy, and diarrhea may be evident in horses after toxin ingestion.

One should perform a complete neurologic examination to determine the presence of other neurologic signs. One should peform the neurologic examination during the interictal period, for an immediate postictal examination may reveal depression, weakness, blindness, and crossed extensor reflex and may lead to false anatomic localization of lesions.[1–3]

Cerebrospinal fluid from the cisterna magna may be helpful in determining the cause of seizures. Increased cerebrospinal fluid protein, red blood cell count, white blood cell count, and abnormal differential white blood cell count may be helpful in determining the cause of the seizure.[1–3] Increased cerebrospinal fluid lactic acid concentration also may be evident in horses with cerebral abscess.[19]

TABLE 10.4-3 Anticonvulsant Drugs Used to Treat Seizure Disorders in Horses

REGIMEN	DRUG	50-kg FOAL	450-kg HORSE
Initial therapy (including status epilepticus)	Diazepam	5–20 mg IV[*]	25–100 mg IV
	Pentobarbital	150–1000 mg IV	To effect
	Phenobarbital	250–1000 mg IV	2–5 g IV
	Phenytoin	50–250 mg IV or PO q4h	—
	Primidone	1–2 g PO	—
	Chloral hydrate (±MgSO, barbiturate)	3–10 g IV	15–60 g IV
	Xylazine[†]	25–100 mg IV or IM	300–1000 mg IV or IM
	Guaifenesin (±barbiturate)	To effect	40–60 g
	Carbamazine	250–500 mg	
	Triazolam	1 g	
Maintenance therapy	Phenobarbital	100–500 mg PO b.i.d.	1–5 g PO b.i.d.
	Phenytoin	50–250 mg PO b.i.d.	500–1000 mg PO t.i.d. (low therapeutic index)
	Primidone	1 g PO s.i.d. or b.i.d.	

* IV, Intravenously; IM, intramuscularly; PO, by mouth.

† Should be used only in an emergency situation until an appropriate anticonvulsant agent can be started.

Skull radiographs may help determine the presence of traumatic skull fractures. Bone scan may reveal nondisplaced skull fractures. A fundic examination may reveal papillary edema, detached retina, or active inflammation that may suggest trauma or an infectious cause. Electroencephalography and a computed tomography scan may be helpful in localizing and determining the cause of a seizure.[20–22] If one cannot find an underlying cause, then one may make a diagnosis of idiopathic epilepsy.[2,3]

10.4.1.3

TREATMENT

One must base treatment of horses having seizures on medical considerations, client considerations, owner preference and compliance, and the long-term expense of medication. One should base starting anticonvulsant therapy on the frequency and severity of the seizures of the horse. One may extrapolate guidelines for anticonvulsant therapy from treatment guidelines used in human beings and small animals. Generally, anticonvulsant therapy is indicated in status epilepticus (which occurs rarely in adult horses and uncommonly in foals): one seizure occurring every 2 months; clusters of seizures more than 3 or 4 times per year; or several multiple seizures occurring over 1 to 3 days.[23] Foals with idiopathic epilepsy that have several seizures over 1 to 3 days also may require short-term (1 to 3 months) anticonvulsant therapy.[2,3] The chronic use of

anticonvulsants in horses is rare. Table 10.4-3 lists therapy guidelines.[2–4] One should decrease maintenance therapy slowly to determine if continued therapy is needed. The goals of anticonvulsant therapy are to reduce the frequency, duration, and severity of seizures without intolerable side effects. The complete elimination of a 563
seizure may not be possible. The best initial treatment for seizures in horses is diazepam and phenobarbital 564
sodium. One must treat each horse as an individual and tailor the treatment dosages to fit the individual.

Adult horses and foals in seizure also may benefit from 1 g/kg dimethyl sulfoxide given intravenously as a 10% solution with lactated Ringer's solution or 5% dextrose. Dimethyl sulfoxide scavenges free oxygen radicals released in damaged tissue and helps maintain cerebral blood flow by reducing thromboxane production, which may result in vasoconstriction and platelet aggregation.[24]

Corticosteroids may be effective initially as an anticonvulsant therapy. One should give a single dose of 0.1 to 0.2 mg/kg dexamethasone intravenously initially and should reevaluate the horse the next day.[25] Corticosteroids may stabilize neuronal membranes and decrease seizure foci. Corticosteroids and dimethyl sulfoxide may be synergistic.

Several drugs are contraindicated in seizures, and they include acetylpromazine, xylazine, and ketamine. Although acetylpromazine has been used to control seizures in foals, the drug is risky because of its ability to lower the seizure threshold.[2,3] Xylazine decreases cerebral blood flow and increases intracranial pressure, which may exacerbate cerebral hypoxia and worsen seizures. However, giving xylazine may be necessary in an emergency situation until a more appropriate drug becomes available. Ketamine increases cerebral blood flow, oxygen consumption, and intracranial pressure and may exacerbate seizures.

10.4.2 Narcolepsy

Narcolepsy is a rare, incurable sleep disorder of the central nervous system characterized by uncontrolled episodes of loss of muscle tone (cataplexy) and sleep.[1,3,4] The disease has been reported in Suffolk and Shetland foals (the fainting disease),[26] Welsh ponies, a Miniature horse, and in the Thoroughbred, Quarter Horse, Morgan, Appaloosa, and Standardbred.[1,3,4,27] A familial occurrence is thought to exist in affected Suffolk and Shetland pony foals.[3]

10.4.2.1 PATHOPHYSIOLOGY

Four components of narcolepsy are apparent in human beings and include excessive daytime sleepiness associated with short periods of REM sleep, cataplexy, hypnagogic hallucinations, and sleep paralysis.[4,28] Cataplexy or sudden collapse with complete inhibition of skeletal muscle tone occurs in horses with narcolepsy.[1,3,4] Respiratory and cardiac muscles are spared.[1]

The normal sleep cycle consists of two stages. Slow wave sleep (non-REM sleep), mediated by serotonin, occurs first and originates from the midline raphe of the pons. Slow wave sleep is followed by fast wave (REM) sleep, which is mediated by norepinephrine. These centers are located in the locus ceruleus of the pons.[1,29] Slow and fast wave sleep act through the ascending and descending activation system of the reticular formation.[1] In fast wave sleep, when REM occurs, the locus ceruleus activates the medial reticular formation nucleus, the axons of which descend the spinal cord to inhibit the lower motor neurons of the somatic efferent system. This inhibition produces atonia.[1,30,31] Electroencephalography recordings during fast wave sleep are

characterized by low-amplitude, fast activity. Electromyographic recordings during fast wave sleep show no recordable muscle tone.[1]

With narcolepsy and cataplexy a biochemical abnormality of the brainstem or sleep-wake center may be responsible for the disease. A recent study showed that decreased concentrations or turnover of serotonin, dopamine, or norepinephrine may play a role in narcolepsy and cataplexy.[1,32] A cholinergic mechanism mediated by acetylcholine also may play a role.[33] No morphologic lesion has been found to cause sleep dysrhythmia.[3]

Cataplectic episodes usually are not associated with exercise. Petting or stroking of the head and neck, hosing with cold water after exercise, leading out of a stall, the initiation of eating or drinking, and stall rest may precipitate a cataplectic episode.[4]

The onset of narcolepsy or cataplexy occurs at approximately 6 months of age.[1] However, adult onset has been described.[4] A predictable pattern of duration and frequency of attacks is set within the first 1 to 2 weeks following the onset of disease.[1]

10.4.2.2 CLINICAL SIGNS

Clinical signs of narcolepsy vary from mild muscle weakness to complete collapse. Adult horses may drop their heads, buckle at the knees, and stumble.[1,3,4] If forced to walk, the horse may be ataxic. Pony breeds are more likely to become recumbent.[4] Horses and ponies that collapse may show absent spinal reflexes and REM sleep. Occasional sudden contraction of a limb or trunk muscle can occur resulting in a spasmodic motion. Episodes may last from a few seconds to 10 minutes.[1,3,4] The horse maintains eye and facial responses, normal cardiovascular function, and normal respiratory function during the attack. Horses can be aroused from the attack with varying degrees of difficulty, and most recover and rise quietly without incident.[3] Affected horses are neurologically normal between attacks.[1,4]

10.4.2.3 DIAGNOSIS

Diagnosis of narcolepsy is based on history, clinical signs, pharmacologic testing, and the absence of other diseases. A complete blood count and serum biochemical profile may help determine underlying systemic and metabolic abnormalities. Cerebrospinal fluid is normal in affected horses.[4] Electroencephalography and needle 564

565 electromyography during an attack may reveal fast waves of REM sleep and the absence of postinsertional activity of resting muscle.[1]

A provocative test is useful in diagnosing narcolepsy or cataplexy in horses. Physostigmine salicylate, an anticholinesterase drug, given at 0.06 to 0.08 mg/kg slowly intravenously and 0.05 to 0.1 mg/kg slowly intravenously precipitates a cataplectic attack within 3 to 10 minutes after administration in affected horses.[3,4,27] This compound crosses the blood-brain barrier.[4] Careful monitoring of the horse after physostigmine administration is necessary because of untoward effects such as colic and cholinergic stimulation.

Atropine sulfate, a muscarinic blocker, given at 0.04 to 0.08 mg/kg intravenously or 20 to 60 mg intravenously reduces the severity of cataplectic attacks minutes after administration and can prevent their reoccurrence for

12 to 30 hours following administration.[3,4] Response to atropine sulfate supports a possible cholinergic mechanism for causing cataplexy. One must monitor horses given atropine sulfate for ileus and colic.

Neostigmine, a cholinesterase inhibitor, given at 0.005 mg/kg intravenously does not cross the blood-brain barrier and therefore has no effect on cataplectic attacks. However, neostigmine may help rule out conditions causing muscle weakness such as myasthenia-like syndromes and botulism. Horses with muscle weakness show increased muscle tone and a favorable response to neostigmine.

10.4.2.4 DIFFERENTIAL DIAGNOSIS

One should consider other causes of acute collapse in the horse. Acute collapse without warning is characteristic of cardiovascular collapse or syncope. Atrial fibrillation, ruptured chordae tendineae, myocardial infarction, myocardial fibrosis, aortic endocarditis, and pericarditis have been associated with syncope in horses. Cerebral hypoxia may occur in these conditions and may lead to coma, with or without signs of cardiac failure. One should consider seizures in ruling out acute collapse and also should consider botulism (shaker foal syndrome, forage poisoning), myasthenia-like syndrome, postanesthetic neuromyopathy, exertional rhabdomyolysis, and metabolic causes such as hyperthermia, shock, hypoglycemia, hypocalcemia, hypo- or hyperkalemia, endotoxemia, anaphylaxis, and snakebite. Horses with hyperkalemic periodic paralysis also may have attacks similar to those in horses with narcolepsy. These horses typically have muscle tremors and may become recumbent with hyporeflexia but are alert and anxious. Attacks in horses with hyperkalemic periodic paralysis may last up to 15 minutes, and the horse usually stands without incident. Diagnosis of this condition is based on clinical signs, serum potassium concentration, potassium chloride provocation, and electromyographic findings.[16,17]

Tight whole-body restraint may induce a cataplectic state in neonatal foals. This response is thought to be an inherent in utero mechanism that prevents violent movements, especially during parturition.

10.4.2.5 TREATMENT AND PROGNOSIS

Imipramine, a tricyclic antidepressant drug, is used to control narcolepsy and cataplexy.[1,3,4] The drug blocks the uptake of serotonin and norepinephrine and decreases REM sleep.[4] One can use imipramine at a dose of 0.55 mg/kg intravenously or 250 to 750 mg orally.[3,4] Oral administration produces inconsistent results.[4] As mentioned previously, atropine sulfate can provide relief from acute attacks for up to 30 hours, but one must weigh its use against the adverse gastrointestinal side effects it causes.

The prognosis for narcolepsy or cataplexy varies. Some newborn Thoroughbreds and Miniature horses may have severe attacks but recover fully.[3] In Shetland and Suffolk ponies the disease may persist throughout life, as is true with the adult-onset form.[1,3,4] In horses 1 to 3 years of age, several episodes may occur but are without permanent consequence.[3]

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10.5

10.5—Spinal Cord, Vertebral, and Intracranial Trauma

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10.5.1

Spinal Cord and Vertebral Trauma

Trauma to the central nervous system (CNS) is the most common cause of neurologic disease in horses, accounting for 22% of CNS disorders in one study. In the same study, 50% of horses with traumatic CNS disorders were diagnosed with cervical spinal cord disease. Trauma to the vertebral column frequently results in severe spinal cord damage and therefore should be considered in any acute case of neurologic disease. Commonly, trauma occurs because of falling or colliding with a large stationary object such as a jump, fence, or another horse and may be caused by a penetrating injury. Trauma to the cervical vertebral column often results from a fall on the flexed neck. Neurologic signs usually appear immediately after the accident but may occur weeks to months after the initial insult because of delayed damage to the spinal cord caused by instability, arthritis, or bony callus formation. Clinical signs in traumatic injuries reflect the neuroanatomic location and range from inapparent to severe incapacitating tetraparesis or tetraplegia. One study showed that lesions causing

recumbency mostly were found in the caudal cervical or thoracic spinal cord, whereas lesions of nonrecumbent horses mostly were found farther cranial in the cervical spinal cord.[1]

An age-related distribution in the location of trauma has been noted. Foals and young horses are more susceptible to vertebral trauma than adult horses, especially in the cranial cervical and caudal thoracic areas. An increased incidence of luxations, subluxations, and epiphyseal separations occurs in young horses and may be because cervical vertebral growth plate closure does not occur until 4 to 5 years of age. Adult horses are more prone to caudal cervical (C5 to C7) and caudal thoracic injuries, and considerable force is required for injury.[2] Table 10.5-1 lists the location, type of trauma, inciting incident, and clinical signs associated with the common types of vertebral trauma.

10.5.1.1 PATHOPHYSIOLOGY

Spinal cord damage following trauma is a dynamic process, and the severity is related to the velocity, degree, and duration of the compression or traction forces. The development of spinal cord damage does not coincide 566
with the clinical picture, because pathologic changes may progress in severity for approximately 1 week, even 567
in the face of clinical improvement.[3] Neurologic deficits following spinal cord injury occur through *primary* and *secondary* mechanisms of injury. The primary injury results from the immediate mechanical disruption of neural pathways, whereas secondary spinal cord injury occurs through alterations in the metabolic environment within injured spinal cord tissue in part from release of endogenous pathophysiologic factors. Mechanisms particularly involved in the secondary spinal cord injury are not understood fully; however, the disruption of cellular and subcellular membranes of glia, neurons, and vascular endothelial cells is believed to be the initiator of this autodestructive cascade of events.[4] Classically, initial spinal cord trauma results in changes in the center, or gray matter, of the cord. The reason for this is not entirely clear but is most likely related to the rich blood supply and increased metabolic requirements for oxygen and glucose of the nerve cell bodies in the gray matter. However, the edema, hemorrhage, and hypoperfusion of the gray matter progress outward and lead to central necrosis, white matter edema, and demyelination of the entire spinal cord.[3]

TABLE 10.5-1 Common Types of Vertebral Trauma

LEVEL OF INJURY	AGE	TYPE OF VERTEBRAL TRAUMA	COMMON TRAUMATIC INCIDENT	SYNDROME
Cervical	Foal to yearling	Fracture of dens, luxation C1-C2	Hyperflexion (e.g., somersault)	Tetraparesis, respiratory depression, death
Cervical	Young adult	Epiphyseal fracture	Hyperextension	Tetraparesis to tetraplegia
Cervical	Adult	Compression fracture	Head-on collision	Tetraparesis to tetraplegia
Cranial thoracic	Usually young	Fracture of dorsal spinous process	Flipping over backward	Often none
T2-S1	Any	Transverse fracture of vertebral arch, with dislocation	Somersaulting or falls	Paraparesis
Sacroiliac subluxation	Adult	Subluxation	Falls or slipping on ice	None
Sacral fracture	Any	Compression	Fall over backward or dog-sitting when backed	Urinary and fecal incontinence with or without posterior paresis; paralysis of the tail and anus

From Reed SM: Spinal cord trauma. In Robinson NE, editor: Current therapy in equine medicine, ed 2, Philadelphia, 1987, WB Saunders.

Early, often progressive hemorrhage is one of the hallmarks in acute spinal cord injury. Loss of microcirculation predominantly involving capillaries and venules spreads over considerable distance cranial and caudal to the site of injury.[5] Within minutes the spinal cord swells at the injury site, and ischemia occurs when cord swelling exceeds venous blood pressure. Multiple mechanisms likely are involved in secondary spinal cord injury, including vascular, inflammatory, biochemical, and molecular events.[4–6] Currently, one proposal is that the development of spinal cord ischemia is the most important cause of secondary spinal cord injury.[4,6]

Spinal cord ischemia develops over several hours. In other species a brief period of systemic hypertension followed by systemic hypotension has been demonstrated to occur immediately after initial spinal cord injury. The systemic hypotension can last for several hours and is referred to as spinal shock.[4,6] Furthermore, following injury, the spinal cord is incapable of maintaining constant blood flow independent of systemic blood pressure. This loss of autoregulation results in progressive spinal cord hypoperfusion through destruction of microvasculature, thrombus formation, vasospasm, and release of vasoactive chemicals.[6] Venous outflow obstruction in the spinal cord also occurs, leading to cavitation in the central gray matter.[3]

This ischemic hypoxic state is responsible for altered cell metabolism resulting in decreased aerobic metabolism and a shift to anaerobic metabolism, which is a less efficient method of energy production. Anaerobic metabolism results in lactic acid accumulation, causing acidosis in nervous tissue and thus decreasing glucose and oxygen consumption.[7,8] Lactic acid stimulates prostaglandin production, adenosine diphosphate release, platelet aggregation, thromboxane A_2 release, vasospasm, vasoconstriction, and the inhibition of neurotransmitter release.[7] Moreover, in hypoxic states, the Na^+,K^+-ATPase–dependent cell pump is inhibited or damaged, resulting in the inability of the cell to maintain its electric polarity.[7,8] Damage to the Na^+,K^+-ATPase (adenosine triphosphatase) pump allows for accumulation of potassium extracellularly and 567
sodium intracellularly. This mechanism is important in the development of edema.[7] 568

The sequence of biochemical events that occurs following primary spinal cord injury is complex, but a number of changes are well defined. First, extracellular concentrations of the excitatory amino acids glutamate and aspartate increase after acute spinal cord injury through the release of these amino acids from damaged neurons, the decreased uptake by ischemic astrocytes, and through depolarization-induced release.[6] The interaction of these excitotoxins with *N*-methyl-d-aspartate (NMDA) and non-NMDA receptors results in an increased intracellular sodium and calcium concentration. Intracellular calcium concentrations also increase through direct membrane damage and opening of calcium channels.[6] Particularly, the increase in intracellular calcium leads to impaired cell functioning and ultimately may result in cell death.[4,6] Impairment of cell metabolism occurs through binding of phosphates and subsequent depletion of energy, decreased mitochondrial function, vasospasm, and activation of phospholipase A_2 and other proteases. Excitotoxicity has been shown to affect neurons and oligodendrocytes. The oligodendrocyte injury occurs through effects on the glutamate α-amino-hydroxy-5-methyl-4-isoxazole propionic acid receptors and results in axon demyelination.[4] Free radical–induced lipid peroxidation of neuronal and glial cell membranes is a third important contributor to the secondary spinal cord injury.[4,6] Furthermore, lipid peroxidation of endothelial cell membranes results in platelet aggregation and propagation of spinal cord ischemia. The production of free radicals increases through the increased calcium concentrations, ischemia-reperfusion, and the presence of iron and copper complexes in areas of hemorrhage.[6]

The release of endogenous opioids resulting from trauma, especially dynorphin and other κ-receptor opioids, contributes to secondary injury by decreasing blood flow in the microcirculation.[9] However, κ-opioid agonists and antagonsists have been shown to improve outcome of acute spinal cord injury in rats.[6] The mechanism of these effects is not clear.

Currently much research is being performed on the role of apoptosis, or programmed cell death, in secondary spinal cord injury.[4,10] This form of active cell death has been shown to occur in neurons and oligodendrocytes, and protective treatment strategies are being investigated.

Controversy exists surrounding the role of inflammatory cells in acute spinal cord injury. First, the inflammatory response to CNS damage lags behind the development of secondary tissue damage, and the inflammatory response results in the development of cytotoxic and protective agents. The infiltration of inflammatory cells into damaged spinal cord tissue coincides with demyelination of surviving axons and chronic neuronal death. Inhibiting the infiltration of mononuclear cells has appeared beneficial, and a delayed pathologic effect of macrophages has been proposed to occur that is mediated through their production of quinolinic acid, which is a NMDA agonist.[6] Furthermore, the microglial cells can release cytotoxins such as

hydrogen peroxide, nitric oxide, proteinases, and cytokines such as interleukin-1 and tumor necrosis factor α within minutes of injury. However, their role in secondary spinal cord injury remains unclear.

10.5.1.2 NEUROLOGIC EVALUATION

One can localize observed clinical signs neuroanatomically to the affected area. Damage is worse in the large myelinated motor and proprioceptive fibers compared with the smaller or nonmyelinated nociceptive fibers. Therefore ataxia and loss of proprioception and motor function occurs before the loss of deep pain.[2]

One should direct initial evaluation of the patient toward stabilization and correction of any life-threatening problems such as airway obstruction, hemorrhage, cardiovascular collapse, pneumothorax, and open head injury. In addition, one must identify major long-bone fractures, because these may be the limiting factor for survival of the horse. One then should perform a systematic neurologic evaluation, as described in Chapter 10.1, to localize the site of injury.[11–13] In recumbent horses the use of a sling to assist standing may be a valuable diagnostic tool for localizing the site of injury and for assessing progression of disease and prognosis. Figure 10.5-1 shows the steps in evaluating and neuroanatomically localizing the lesion in the nervous system in a recumbent horse.[11]

Spinal shock (areflexia caudal to the lesion) and Schiff-Sherrington syndrome (extensor hypertonus in otherwise normal thoracic limbs with a cranial thoracic lesion) are infrequent and short-lived findings in the horse.[12,13] Both carry a grave prognosis.

Ancillary diagnostic aids that may be beneficial include radiography, myelography, scintigraphy, cerebrospinal fluid (CSF) analysis, nerve conduction velocities, electromyography, and computed tomography (CT).[14–16] Radiography may demonstrate fractures, luxations, subluxations, and vertebral compression. Myelography may be required to confirm cervical vertebral spinal cord compression.[16] Scintigraphy is helpful in diagnosing nondisplaced or occult fractures and soft tissue lesions.[17] Common CSF findings following spinal cord trauma include xanthochromia and mild to moderate increased total protein concentrations.[2,13] CSF analysis may be normal, especially in acute or chronic cases.[13] The use of nerve conduction velocity and electromyography testing thoroughly evaluates the lower motor neuron and aids in lesion localization. However, changes in the electromyography may not develop until 4 to 5 days following nerve damage.[18] 568

569 Currently, in human beings, CT or plain radiographs are the diagnostic aids of choice to evaluate skeletal injury, and magnetic resonance imaging is the diagnostic aid of choice to evaluate soft tissue structures such as the spinal cord and ligaments.[15] The use of CT in equine practice is increasing and appears helpful in identifying the nature and extent of spinal cord lesions.

Figure 10.5-1 Flow chart for localizing a lesion in the nervous system in a recumbent horse.

10.5.1.3

TREATMENT AND PROGNOSIS

Treatment to reverse primary spinal cord injury does not exist currently, but unless the spinal cord is severed, damage caused by stretch or laceration generally is minimal compared with the secondary damage that occurs thereafter. Treatment therefore aims at reducing the secondary tissue injury. The period of secondary injury 569
can be divided into three therapeutic windows.[6] The first 48 hours after acute spinal cord injury are dominated 570
by the vascular and biochemical changes that occur within the spinal cord. The second period results from the effects of inflammatory cells that occurs within hours of injury and peaks around 4 days after injury. The third period occurs 1 week after injury and is characterized by axonal regeneration and lesion repair. The goals of treatment are to stop the cascade of cellular events initiated by the traumatic insult: specifically, improvement in spinal cord blood flow; reduction in hemorrhage, edema, and membrane lipid peroxidation; and preservation of neural tissue.

Acute spinal cord injury often results in impaired cardiopulmonary function such as impaired ventilation, bradycardia, and hypotension.[19] Systemic hypotension may exacerbate spinal cord hypoperfusion and ischemia, and maintaining systemic blood pressure has been shown to improve spinal cord perfusion. However, hypertension has not been shown to increase spinal cord blood flow and may increase hemorrhage and edema formation.[6] Volume resuscitation clearly is indicated in shock and for restitution of tissue perfusion. The current recommendations are to maintain normotension. This point is important to consider during stabilization of the acutely injured horse but is particularly important when horses are placed under general anesthesia for the various diagnostic procedures.

Corticosteroids, alone or combined with other drugs, are the classic drugs of choice for acute spinal cord trauma. Reported dosages of dexamethasone for horses range from 0.1 to 0.25 mg/kg administered intravenously every 6 to 24 hours for 24 to 48 hours.[2,13] One should expect a favorable response within 4 to 8 hours after corticosteroid administration.[2] However, dexamethasone has not been proved to be efficacious and even may be detrimental in the treatment of spinal cord injuries.[6] One should monitor horses given corticosteroid therapy closely for the development of laminitis or *Aspergillus* pneumonia. If one observes improvement in clinical signs, one may place the horse on oral prednisolone therapy (0.5 to 1.0 mg/kg tapered over 3 to 5 days) to decrease the chance of laminitis.

The neuroprotective effect of corticosteroid is thought primarily to be mediated by free radical scavenging.[6] Furthermore, corticosteroid therapy–related effects have been attributed to a decrease in catecholamines or to a direct effect on the glutamic acid concentrations. Recently, dexamethasone, similar to methylprednisolone, has been shown to decrease apoptosis-related cell death in rats that were subjected to traumatic spinal cord injury.[20] Other potential beneficial effects of corticosteroid include reduction in the spread of morphologic damage, prevention of the loss of axonal conduction and reflex activity, preservation of vascular membrane integrity, and stabilization of white matter neuronal cell membranes in the presence of central hemorrhagic lesions. Furthermore, the antiinflammatory properties of corticosteroids may be useful in reducing edema and fibrin deposition and in reversing sodium and potassium imbalance caused by edema and necrosis. Another beneficial effect of corticosteroid is maintenance of normal blood glucose concentrations while maintaining electrolyte balance.[7]

In 1984 the first National Acute Spinal Cord Injury Study (NASCIS I) results demonstrated no significant difference between the effects of methylprednisolone sodium succinate (MPSS) and methylprednisolone on

outcomes of acute spinal cord injury patients.[21] However, results from the NASCIS II trial were more encouraging.[22,23] MPSS is a synthetic glucocorticoid with 4 times more antiinflammatory activity and 0.8 times less mineralocorticoid action compared with cortisol. MPSS is 40% to 60% bound to plasma proteins and has 2.4-hour and 12- to 36-hour plasma and biologic half-lives, respectively.[24] Beneficial effects of MPSS on neural tissue include inhibition of lipid peroxidation, eicosanoid formation, and lipid hydrolysis, including arachidonic acid release, maintenance of tissue blood flow and aerobic energy metabolism, improved elimination of intracellular calcium accumulation, reduced neurofilament degradation, and improved neuronal excitability and synaptic transmission. MPSS was selected for these trials rather than dexamethasone because the succinate radical has been shown to cross cell membranes more rapidly than other radicals.[23]

The results of this multicenter randomized, double-blind, placebo-controlled study in human beings compared the effects of MPSS, naloxone, and a placebo on the neurologic outcome of acute spinal cord injury patients.[22,23] MPSS was administered as a 30-mg/kg bolus followed by infusion at 5.4 mg/kg/hr for 23 hours. Naloxone was administered as a 5.4-mg/kg bolus followed by infusion at 4 mg/kg/hr for 23 hours. The patients' recovery was followed for 1 year. At 6 months, patients receiving MPSS within 8 hours of injury had significant improvement in motor function, sensation, and touch compared with the other groups.[22] This finding was true for patients with complete and incomplete lesions. At 1 year the MPSS group had significant improvement in motor function compared with the other groups.[23] Complications, such as wound infection and gastrointestinal bleeding, were not statistically different among the treatment groups. Mortality was approximately 6% among all groups.[22,23]

Apparently, the cell membrane antilipid peroxidation effect of MPSS is most beneficial. The dose of MPSS
used exceeds that necessary for activation of steroid receptors, suggesting that MPSS acts through 570
mechanisms unrelated to steroid receptors. Based on this study, the investigators concluded that high-dose 571
MPSS treatment within 8 hours of spinal cord injury improved neurologic recovery. The use of naloxone in spinal cord injury was not recommended.[22,23]

In a fifth trial of MPSS (NASCIS III), using essentially the same protocol for drug administration and neurologic assessment as NASCIS II, no significant benefit was found when the NASCIS II regimen of 24-hour therapy was extended to 48 hours.[25,26] Therefore the current recommendation for use of MPSS in acute spinal cord injury remains unchanged from the original recommendation of NASCIS II.[27] However, some reports dispute the usefulness of corticosteroids for treating acute spinal cord trauma and recommend further trials with MPSS.[28] The usefulness of high-dose MPSS treatment for spinal cord trauma in the horse remains to be investigated. Although more research should be performed, especially on the mechanism of action and optimal time of administration, MPSS seems to be a promising drug for treating spinal cord injury.

Because of the adverse effects of corticosteroid use and because the neuroprotective effects of methylprednisolone are independent of its glucocorticoid receptor actions, a group of steroid analogs was developed. These lazaroids, or 21-aminosteroids, lack glucocorticoid or mineralocorticoid activity and are potent antioxidants.[26,29] The 21-aminosteroid tirilazad mesylate is a potent inhibitor of iron-dependent lipid peroxidation and has been shown to be beneficial in cats and rats.[6] However, at the present time the clinical evidence is insufficient to recommend routine use of this drug, and further studies are required to show significant efficacy.[6,29] Currently the pyrrolopyrimidines are being investigated, which are antioxidants that have improved penetration of the blood-brain barrier.[29]

Dimethyl sulfoxide (DMSO) administered at 1 g/kg intravenously as a 10% to 20% solution for 3 consecutive days followed by three treatments every other day has been found to be of benefit in acute spinal cord trauma.[2,13] The pharmacokinetics of DMSO in horses indicates that twice-daily dosing is necessary to maintain adequate blood levels.[30] Reported benefits of DMSO include increased brain and spinal cord blood flow, decreased brain and spinal cord edema, increased vasodilating prostaglandin E_1, decreased platelet aggregation, decreased prostaglandins E_2 and F_2, protection of cell membranes, and trapping of hydroxyl radicals.[7,31] The exact mechanism of DMSO remains unknown. This treatment remains controversial because some researchers have found no positive effects on neurologic outcome from the use of DMSO.[32]

Although the free radical scavengers vitamin E and selenium have been shown to be beneficial in spinal cord injury, these antioxidants do not appear useful in acute spinal cord injury because of the length of time required to achieve therapeutic concentrations in the CNS.[6]

Osmotic diuretics such as 20% mannitol administered at 2 g/kg intravenously, dextran, and glycerol may be helpful in reducing brain edema. Currently, these diuretics have not been proved to be effective in spinal cord injury. One should not use these drugs continuously because their hypertonic nature may create dehydration, urine retention, and hypotension. One must maintain adequate patient hydration when using osmotic agents. Although some investigators and clinicians have found these agents to be helpful, others have found them to be of little benefit in spinal cord trauma.[7,13]

To counteract endorphin-mediated hypotension, the use of naloxone, a nonspecific opioid antagonist, in acute spinal cord injury was investigated. However, naloxone is more expensive and less effective than MPSS, and in light of the NASCIS II study, the use of naloxone is no longer advocated.[22,23] Currently, κ-opioid agonists and antagonists are being investigated because both may have beneficial effects in spinal cord injury.[6] Thyrotropin-releasing hormone (TRH) has been shown to improve functional outcome after experimental spinal cord injury, even when the administration was delayed. In one study, TRH at 2 mg/kg followed by 2 mg/kg/hr for 4 hours significantly improved the neurologic outcome in experimental spinal cord trauma in cats.[33] The beneficial results of TRH are thought to result from the neurotrophic effects of TRH and the effects of TRH on plasticity and facilitation of motor nerve firing. The effects of TRH have been reported to be better than those of naloxone or MPSS.[34]

Promising results have been obtained from research investigating the effects of calcium channel antagonists in spinal cord injury. Beneficial effects were seen with the use of nimodipine, nifedipine, diltiazem, and flunazirine but not with the use of verapamil.[6,33] One needs to administer calcium channel blockers with vasopressors to maintain systemic blood pressure. Although this class of drugs may be beneficial for treating spinal cord injury, clinical trials have not been performed and the clinical efficacy of calcium channel blockers still remains unclear. Other therapeutic strategies being investigated include CSF drainage, hypothermia,[6] the use of nerve growth stimulatory factors in the repair of the injured spinal cord in animal models.[35]

The use of nonsteroidal antiinflammatory drugs such as flunixin meglumine and phenylbutazone may decrease the inflammation associated with a traumatic episode. These compounds work by inhibiting cyclooxygenase, which converts arachidonic acid to inflammatory mediators (endoperoxides). They probably do not alter the course of the neurologic disease.

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Antibiotics are not always necessary for treating vertebral or spinal cord trauma.[2,13] However, they are indicated in treating open fractures and secondary complications associated with a recumbent horse, such as pneumonia and decubital sores. One should base antibiotic choice on culture and sensitivity testing. Good empirical choices for broad-spectrum coverage include trimethoprim-sulfamethoxazole at 30 mg/kg orally or intravenously every 12 hours or penicillin at 22,000 IU/kg intramuscularly every 12 hours or intravenously every 6 hours in combination with gentamicin at 6.6 mg/kg intramuscularly or intravenously every 24 hours. One should undertake appropriate monitoring for aminoglycoside toxicity with their use.

Nutritional support also plays a role in the outcome following neurologic injury. In human beings, neurologic recovery from head injury has been found to occur faster in patients receiving early adequate nutritional support.[36] If the horse is able to eat and the gastrointestinal tract is functioning normally, water and good-quality hay should be available at all times. One should feed small amounts of grain 3 to 4 times a day to boost caloric intake, and one should base the amount of grain fed on the condition of the horse and its ability to tolerate grain feeding. Horses with a poor appetite or those unable to swallow may have to be tube-fed using a gruel of alfalfa and complete feed pellets. One soaks the pellets in water, usually for 8 to 12 hours, until they have a soupy consistency and then pumps them into the stomach of the horse via a nasogastric tube. The horse requires approximately 1 gal of gruel fed 3 to 4 times per day. Horses that cannot tolerate this procedure, severely depressed or comatose horses, and horses not maintainable with other feeding methods are candidates for total parenteral nutrition.

Physical therapy is important in the rehabilitative process in spine-injured horses. Controlled exercise allows the unaffected parts of the nervous system to compensate for the affected parts by increasing strength and conscious proprioception. Exercise is especially helpful in improving weakness, ataxia, spasticity, and hypermetria. In recumbent horses, massage, therapeutic ultrasound, and hydrotherapy of affected muscle groups for 10 to 15 minutes at least twice a day is important. These measures help combat necrosis and muscle atrophy of the dependent muscle groups of the horse. Passive flexion and extension of all limbs is helpful in maintaining full range of motion in recumbent horses.[37]

Surgical intervention is warranted for stabilization, fracture repair, or evidence of a compressive lesion but is not routine practice. Chapter 10.8 describes ventral stabilization surgery for horses with cervical vertebral stenotic myelopathy. One always should institute medical treatment to stabilize the patient before performing surgery.

Prognosis is based on response to therapy and is related directly to the time from injury to the institution of treatment. Horses that show rapid neurologic improvement have a fair to good prognosis. Recumbent horses or horses suffering from fractures or luxations have a guarded to poor prognosis. Horses that have lost deep pain sensation have a functional or anatomic spinal cord transection and have a grave prognosis.[13] The longer the time from loss of deep pain to treatment, the poorer the prognosis. Partial or complete recovery of horses with spinal cord trauma may take weeks to months, so time and nursing care are required.

10.5.2 Intracranial Trauma

Head trauma in horses occurs less frequently than spinal cord trauma. In one study, 5 horses out of 22 (23%) that had traumatic neurologic disease were diagnosed with intracranial trauma.[1] Head trauma in horses, which may or may not be associated with a fracture, is caused by many of the same incidents that cause spinal cord trauma. In addition, penetrating wounds, kicks to the head, and rearing up and falling over backward, often resulting in

fractures of the basisphenoid and basioccipital bones,[38] may result in intracranial trauma. Other common fractures observed in association with head injuries include mandibular, maxillary, orbital, periorbital rim, and incisive bone fractures.[1,39,40] Clinical signs resulting from trauma reflect the extent and location of the injury.

10.5.2.1 PATHOPHYSIOLOGY

Following a traumatic insult to the brain, a cycle of events occurs that is similar to spinal cord trauma. The primary events occur at the time of injury and are primarily mechanical damage, including neuronal fiber and cell membrane disruption. Similar to what has been described for spinal cord injury, the secondary injury is characterized by free radical formation, excitatory neurotoxin release, endogenous opiate release, free fatty acid release, loss of high-energy phosphates, apoptosis, CNS acidosis, and CNS ion imbalance.[41–43] Other important secondary events in traumatic brain injury (TBI) include the development of an elevated intracranial pressure (ICP), brain edema, hypoxia, and seizures. The degree of these events depends on the type and extent of the initial injury.

The types of cranial trauma from least to most severe are concussion, contusion, laceration, and hemorrhage. Concussion is a short-term loss of consciousness and is caused primarily by a direct blow to the head. Concussion is reversible and occurs without an anatomic lesion.[39,40] Contusion is associated with the immediate primary mechanical injury and results from vascular and nervous tissue damage. Although 572
contusion occurs without major structural tissue disruption, it may or may not be reparable. Contusions may 573
be on the same or opposite side of the injury and result from blows or sudden accelerations or decelerations of the head. Contusion may result in intraparenchymal hemorrhage with later cavitation.[39] Cerebral hemorrhage and laceration result from penetrating wounds (gunshots) and fractures in addition to the previously mentioned injuries. Cerebral hemorrhage in horses may be epidural, subdural (rare), intracerebral, or subarachnoid (common). Hematoma formation is of special concern because of the potential for devastating expansion within the rigid calvaria, as can occur with edema.[13,39] These processes displace brain tissue, and herniation, pressure necrosis, and brainstem compression are possible sequelae.[13,39,40]

Blood flow to the brain is controlled by changes in diameter of resistance blood vessels. The cerebral blood flow (CBF) is controlled by autoregulation whereby the CBF is maintained within a pressure range of 60 to 160 mm Hg. Beyond these limits, CBF decreases at pressures below the lower limit and increases at pressures above the higher limit. Following TBI, an elevation of the ICP generally occurs as a consequence of hemorrhage and the development of edema. The increased ICP leads to a decreased CBF caused by collapse of dural veins and subsequently to a further rise in ICP.A reduced CBF results in areas of ischemia and subsequent restriction of the delivery of substrates to the brain such as oxygen and glucose. A reduced CBF has been associated with an unfavorable neurologic outcome in human beings and has been implicated in increasing the susceptibility of the brain to secondary injury.[41]

Loss of mitochondrial function and subsequent energy depletion leads to a loss in maintenance of membrane potentials resulting in depolarization of neurons and glia. As has been described for spinal cord injury, these events lead to an increase in intracellular calcium, the release of excitotoxins, and apoptosis.[43,44] Cytotoxic edema develops through the failure of the Na^+,K^+-ATPase pump in the presence of hypoxia and the subsequent influx of water that passively follows Na^+ and Cl^-.[41,45] Cytotoxic edema develops from swelling of the cellular elements of the brain (neurons, glia, and endothelial cells). This type of edema occurs in gray and white matter and decreases the extracellular fluid volume. If capillary endothelial cells are edematous, the capillary lumen size diminishes, creating an increased resistance to arterial flow. Capillary permeability

usually is not affected in cytotoxic edema. Major decreases in cerebral function occur with cytotoxic edema, with stupor and coma being common signs.[45]

Following TBI, disruption of the blood-brain barrier, including endothelial damage, degeneration of pericytes, and loss of astrocytes, is a common finding and results in extravasation of blood components and water, which is referred to as vasogenic edema.[41,45] This type of edema is the most common found after head trauma.[39] Cerebral white matter is especially vulnerable to vasogenic edema, possibly because of its low capillary density and blood flow. This type of edema also results in increased extracellular fluid accumulation. The edema fluid is a plasma filtrate high in plasma proteins. Vasogenic edema displaces cerebral tissue and increases intracranial pressure, which may result in brain herniation. The elevated intracranial pressure further decreases (CBF).[41,45] Many of the mechanisms decreasing blood flow in spinal cord trauma also apply to cerebral trauma. Blood flow interruption is responsible for disruption in ion homeostasis (especially calcium, sodium, and potassium), and a switch to anaerobic glycolysis resulting in lactic acid production and acidosis.[40,43,46] Cell membrane lipid peroxidation with subsequent prostaglandin and thromboxane synthesis, formation of reactive oxygen species, nitric oxide, and energy failure also ensue.[43,46] Because of the high metabolic rate and oxygen demand of the brain, disruption of blood flow and normal energy-supplying processes leads to impaired nerve cell function and even cell death.[40]

10.5.2.2 NEUROLOGIC EVALUATION

Clinical signs associated with head trauma range from inapparent to recumbency with profound depression, dementia, and tetraparesis. As with spinal cord trauma, one should attend to life-threatening injuries first and then perform a complete neurologic examination. Serial neurologic examinations are vital to ensure proper treatment and to predict prognosis. One should perform reflex and nociceptive testing to rule in or out a concurrent spinal cord injury. As with spinal cord trauma, one must identify concurrent long-bone fractures, which may limit the outcome. Table 10.5-2 provides an outline for localizing the level of brain injury based on the neurologic findings.[39]

Some horses may be intractable following a traumatic incident, and sedation may be necessary for examination. One should remember that xylazine and detomidine transiently cause hypertension, which may potentiate intracranial hemorrhage.[13,39] However, xylazine has been found to cause a minor decrease in cerebrospinal pressure in normal, conscious horses.[47] Although no studies were performed on horses with cerebral trauma, xylazine is believed to be a safe sedative to use in horses with head trauma.

Severe brainstem injuries can be associated with apneustic or erratic breathing and reflect a poor prognosis.[39,40] Bilaterally dilated and unresponsive pupils indicate an irreversible brainstem lesion. One should monitor horses with bilaterally miotic pupil size routinely, because a change to dilated and unresponsive indicates a progression of the lesion and the need for immediate medical treatment[39] and carries a grave prognosis. 573

TABLE 10.5-2 Signs Characteristic of Focal Brain Injury

LEVELS	CONSCIOUSNESS	MOTOR FUNCTION	PUPILS	OTHER SIGNS
Cerebrum	Behavior change, depression, coma	Circling	Normal	Blindness
Cerebellum		Ataxia and hypermetria, intention tremor		Menace response deficit without blindness
Diencephalon (thalamus)	Depression to stupor	Normal to mild tetraparesis, “aversive syndrome”[*]	Bilateral, nonreactive pupils with visual deficit	None
Midbrain	Stupor to coma	Hemiparesis; tetraparesis or tetraplegia	Nonreactive pupils, mydriasis, anisocoria	Ventrolateral strabismus
Pons	Depression	Ataxia and tetraparesis, tetraplegia	Normal	Head tilt, abnormal nystagmus, facial paralysis, medial strabismus
Rostral medulla oblongata (including inner ear)	Depression	Ataxia or hemiparesis to tetraplegia	Normal	Same
Caudal medulla oblongata	Depression	Ataxia, hemiparesis to tetraparesis, abnormal respiratory patterns	Normal	Dysphagia, flaccid tongue

From Reed SM: Cranial trauma. In Robinson NE, editor: Current therapy in equine medicine, ed 2, Philadelphia, 1987, WB Saunders.

* Deviation of the head and eyes with circling toward the side of a unilateral lesion.

General physical examination is important in cranial trauma because fractures and other concurrent injuries are common and require identification and treatment. Physical findings resulting from cranial trauma include blood coming from the nostrils, mouth, and ears or CSF draining from the ear (basilar fractures) (Figure 10.5-2).[38,39] With severe head trauma a brain-heart syndrome can develop consisting of cardiac arrhythmias, increased serum levels of heart-specific enzymes, myocardial necrosis, and sudden death from heart failure.[13]

Ancillary diagnostic aids helpful in further defining cranial trauma include radiography and CT to identify fractures and CSF analysis.[14] Cisternal CSF collection is contraindicated if one suspects increased intracranial pressure because of the possibility of brain herniation through the foramen magnum. Lumbosacral collection may be a safer alternative but can be normal despite a traumatic episode, especially in the acute phase, and because the sample is not obtained closest to the lesion, it may not reflect the changes that have occurred.[13] As with spinal cord trauma in human beings, CT and magnetic resonance imaging are also the diagnostic aids of choice in cases of head trauma.[1,48] If available, CT is an invaluable diagnostic aid.

10.5.2.3 TREATMENT AND PROGNOSIS

Based on the pathophysiology of events that occur during TBI, clearly a single-drug intervention for treating 574
TBI is not effective.[42] However, most research studies and pharmaceutical trials follow this single-drug 575
intervention approach. In horses, treatment of TBI aims at reducing brain edema, reducing intracranial pressure, and reducing free radical injury. The use of corticosteroid and DMSO for the reasons discussed under Spinal Cord and Vertebral Trauma are beneficial in treating cranial trauma. The dosages used in horses with intracranial trauma are the same as in spinal cord trauma. The neuroprotective effects of high-dose MPSS treatment also may be applicable to head injury.[24] Further research on the use of MPSS treatment in head trauma patients is needed to verify its usefulness.

Figure 10.5-2 Blood and cerebrospinal fluid draining from the ear following a basilar skull fracture.

Research in human and animal models has demonstrated that following TBI cerebral, mitochondrial function is impaired primarily through effects of ischemic hypoxia. However, the process of mitochondrial damage continues, and mitochondrial impairment may persist for 14 days after injury despite adequate delivery of oxygen and glucose. This indicates that restoring mitochondrial function might be as important as maintaining substrate delivery. Pharmacological interventions in animal models of experimental TBI that have been used to ameliorate mitochondrial function include the use of calcium channel blockers and antioxidants.[44] In horses, treatment aimed at reducing free radical damage is generally through administration of DMSO and vitamin E. However, the fact that lipid peroxidation occurs early on in TBI may be a limiting factor in the efficacy of free radical scavenger pharmacologic therapy.[49] Similar to spinal cord injury, treatment with 21-aminosteroids may be of benefit in TBI.[50] Hypothermia, and subsequent decreased cellular metabolism has been proven efficacious in treatment of TBI in experimental and clinical settings.[51,52] The neuroprotective effects include reduced release of excitotoxins, reduction of free radical and inflammatory mediator formation, and reduction of blood-brain barrier disruption. However, long-term benefits and improved functional outcome has not been demonstrated adequately.[53,54]

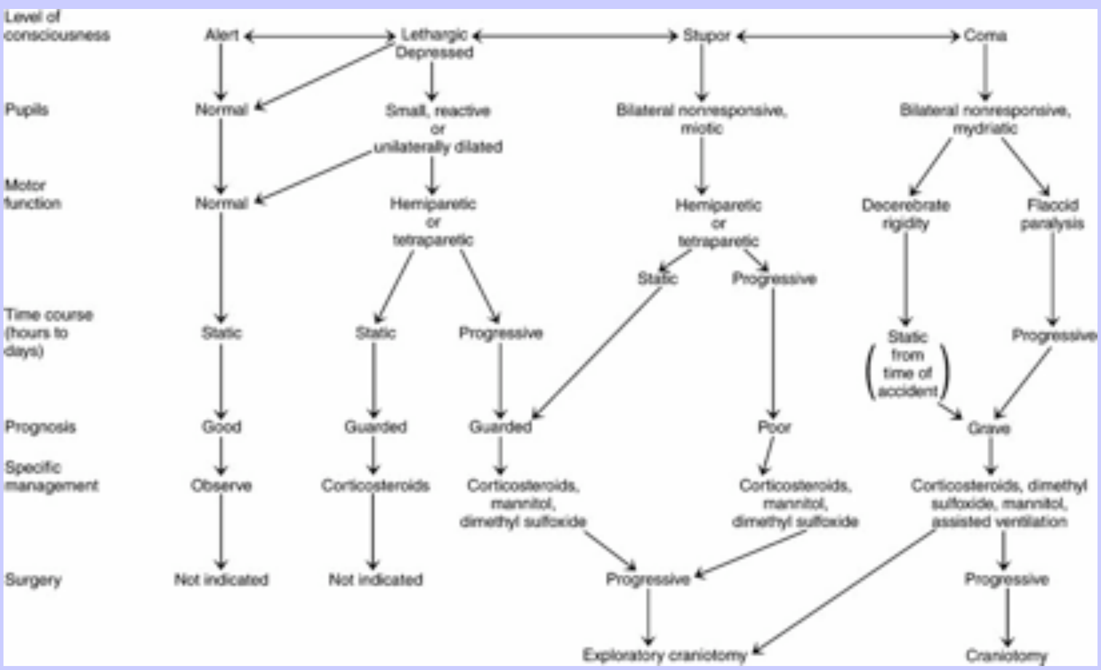
Osmotic diuretics such as 20% mannitol administered at 0.25 to 2.0 mg/kg intravenously over 20 minutes and glycerol administered at 0.5 to 2.0 mg/kg intravenously every 6 to 12 hours for 24 hours are effective in combating cerebral edema and increased intracranial pressure.[13,39] These diuretics have a rapid onset of action (10 to 20 minutes) and work because of their hyperosmolar nature. One should exercise caution in using these compounds because they can cause further hemorrhage by decreasing edema and allowing space for more bleeding. Leakage of these hypertonic solutions into the tissue also may increase cerebral edema.[45] Horses receiving osmotic diuretics should be hydrated adequately. The use of osmotic substances is warranted in any horse with worsening mental status, abnormal pupillary size, or inequality indicating transtentorial herniation, or development of paresis.[48]

Although mannitol administration is effective in reducing intracranial pressure, it has technical limitations for administration. Furthermore, the administration of multiple doses of mannitol may lead to intravascular dehydration, hypotension, and reduction of CBF.[55] Therefore current research focuses on the use of hypertonic solutions that reduce intracranial pressure and support intravascular volume.[56,57] Hypertonic saline administered early to treat shock associated with head trauma may enhance return of cerebral blood flow and cell membrane function.[58] Effects of hypertonic saline are due to its ability to move water out of cells and to decrease tissue pressure and cell size by osmotic plasma expansion.[58,59] These effects result in a lowering of intracranial pressure and cerebral water content. The beneficial effects of hypertonic saline prevail even with subsequent administration of isotonic fluids.[58,60] When 1.8% sodium chloride was compared with hypotonic Ringer's lactate solution as 24-hour maintenance fluids following experimental cerebral injury, the 1.8% saline provided the stated beneficial effects and adequate cardiovascular support, suggesting that hypertonic saline may be the maintenance fluid of choice in head injury.[61] Furthermore, multiple studies using 3% and 7.5% hypertonic saline have demonstrated similar effects on intracranial pressure.[62,63] Another study comparing the effects of a hypertonic saline hydroxyethyl starch solution and mannitol on increased intracranial pressure found that the hypertonic saline hydroxyethyl starch reduced the ICP more effectively than mannitol.[64] Recently, induction of prolonged hypernatremia using 3% hypertonic saline administered as a continuous infusion appeared to be a promising therapy for control of cerebral edema.[57] The authors have found 3% hypertonic saline useful in cases of suspected increased ICP, and furthermore, hypertonic saline is technically easier to administer than mannitol.

Experimentally, furosemide has been found effective in decreasing intracranial pressure. A 1-mg/kg bolus intravenous dose was given at 5-hour intervals, and a 0.5-mg/kg/hr dose was given intravenously for 4 hours, 1 hour after the initial bolus.[65] Normal hydration status is required before furosemide administration. One also may use furosemide concurrently with mannitol to increase the duration of intracranial pressure reduction provided by mannitol and to diminish the potential for rebound intracranial pressure elevation.

Other methods to lower intracranial pressure include elevation of the head by 30 degrees if no cervical fractures are present.[66] Hyperventilation to lower the partial pressure of carbon dioxide to 26 to 28 mm Hg in human beings decreases cerebral blood flow, thus lowering intracranial pressure.[48] One may consider 575
hyperventilation in cases of increased intracranial pressure in horses. Proper hyperventilation requires 577
monitoring of arterial blood gases and may require use of neuromuscular blockers if the horse is not comatose and is resisting the ventilator.

Figure 10.5-3 Flow chart for management of cranial trauma. (From Reed SM: Spinal cord trauma. In Robinson NE, editor: *Current therapy in equine medicine,* ed 2, Philadelphia, 1987, WB Saunders; modified from Kirk KW, editor: *Current veterinary therapy VII,* Philadelphia, 1980, WB Saunders.)



Barbiturate treatment or coma may decrease cerebral metabolism, thereby providing a protective effect against cerebral ischemia.[39,48] Barbiturates also may limit lipid peroxidation.[39] However, the actual benefits of barbiturate use on the neurologic outcome remain controversial. Concurrent hyperventilation enhances the effects of barbiturates on lowering intracranial pressure. An exact dosage regimen for barbiturate treatment in horses has not been investigated, but 5 to 10 mg/kg intravenously to effect is reported to be useful.[40] The

major side effect of barbiturates is hypotension, especially if mannitol and furosemide have been administered, so they must be used with caution and adequate blood pressure monitoring. One should reserve the use of barbiturates for those cases in which elevated intracranial pressure is refractory to other treatments.

Horses with intracranial trauma often need fluid therapy to maintain hydration and blood pressure. Isotonic crystalloid fluids administered in typical shock doses of 40 to 90 ml/kg/hr may produce worsening of cerebral edema and increased intracranial pressure. Comparison of isotonic crystalloid fluids with hypertonic saline solutions for fluid support of head trauma patients in shock has shown hypertonic saline to be the fluid of choice. Hypertonic saline is associated with significant decreases in intracranial pressure and cerebral water content compared with isotonic fluid treatment.[58] One may give hypertonic saline to horses with head trauma and in shock as a 5% or 7% sodium chloride solution in a 4- to 6-ml/kg intravenous bolus over 15 minutes. One then may use isotonic fluids for maintenance if needed. Monitoring central venous pressure to keep it in the normal range of 5 to 7 cm H_2O is important in comatose horses to avoid overhydration. Contraindications to the use of hypertonic saline include dehydration, ongoing hemorrhage, hypernatremia, renal failure, hyperkalemic periodic paralysis, and hypothermia.[59]

One should avoid using carbohydrate-containing intravenous solutions early in the treatment of head trauma patients.[39,67] Glucose suppresses ketogenesis and may increase lactic acid production by the traumatized brain, limiting the availability of nonglycolytic energy substrates.[67] Carbon dioxide liberated from glucose metabolism causes vasodilation and worsening of cerebral edema.[39]

Antibiotic treatment usually is given in cases of head trauma, especially when fractures are involved.[13,39] The presence of hemorrhage increases the possibility of septic meningitis. Broad-spectrum antimicrobials, such as those listed for spinal cord trauma, are effective. Because of the disruption of the blood-brain barrier, other antimicrobials probably penetrate into the central nervous system, and therefore their use also may be efficacious.

For horses sustaining cranial trauma to develop seizures is not unusual. The drug of choice in controlling seizures is diazepam. Doses range from 5 mg (foals) to 25 to 100 mg (horses) intravenously, repeated as necessary to control seizure activity.[13,39] Intractable seizures may necessitate general anesthesia. Agents useful for general anesthesia include guaifenesin, chloral hydrate, barbiturates, and gas anesthesia. One should not use ketamine as part of a balanced anesthesia regimen because it increases cerebral blood flow and intracranial pressure.[67,68]

Adequate nutritional support as discussed for spinal cord trauma also applies to head-injured horses. Additionally, the use of 1 g thiamine administered intramuscularly for 5 days may be of benefit in treating head injuries. Thiamine aids breakdown of lactic acid and is a necessary coenzyme in brain energy pathways.[37]

Emergency surgical treatment (although not commonly performed) is warranted in open cranial fractures and in the face of deterioration despite medical therapy. The surgical techniques for these procedures have been described elsewhere.[69] Once the patient is stabilized, one can consider repair of less life-threatening fractures. Figure 10.5-3 summarizes medical and surgical therapies for cranial trauma.[39]

As with spinal cord trauma, the prognosis for cranial trauma depends on early treatment and is gauged by response to treatment. Basilar fractures and severe brainstem injuries carry a grave prognosis.[38] Recumbency,

tetraparesis, and severe dementia carry a poor to grave prognosis.[39] Time, good nursing care, and adequate nutritional support, especially in the recumbent horse, are vital for a positive outcome.

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10.6

10.6—Vestibular Disease

Bonnie R. Rush

The vestibular system is a special proprioceptive system responsible for maintenance of balance and reflex orientation to gravitational forces. This system functions to maintain appropriate eye, trunk, and limb position in reference to movements and positioning of the head.[1,2]

10.6.1

Vestibular Apparatus

The afferent unit of the vestibular system comprises the receptor organ within the inner ear and the vestibulocochlear nerve (the eighth cranial nerve). The vestibulocochlear nerve processes auditory and vestibular input through a common physiologic mechanism. Acoustic stimuli and acceleration forces result in mechanical deformation of hair cells in the cochlea and vestibular receptor organs, which transform physical forces into electric impulses.[3] The inner ear is located in the petrous temporal bone and consists of the bony and membranous labyrinth. The membranous labyrinth is suspended within the bony labyrinth by perilymph, fluid similar to and likely derived from cerebrospinal fluid.[2] The membranous labyrinth comprises the cochlea, the saccule, the utricle, and the three semicircular canals.[4] The cochlea is responsible for auditory functions and has no input to the vestibular system.[5] Endolymph fills the membranous labyrinth and is derived from blood vessels along one side of the cochlea.[2]

The three semicircular canals are oriented at right angles to one another, which allows for detection of rotation in any plane at any angle. At one end of each semicircular canal is a dilation of the canal called the ampulla. A ridge of connective tissue called the crista is located on one side of the ampulla. The internal surface of the crista is lined with hair cells (neuroreceptor cells) and sustentacular cells (support cells) that are oriented perpendicular to the flow of endolymph.[2,6] These cells are covered with a gelatinous protein-polysaccharide material called the cupula, which extends across the lumen of the ampulla.[5] Rotation of the head in any plane causes endolymph to flow in one or more of the semicircular ducts. Each hair cell has 40 to 80 stereocilia and a single modified kinocilium. Movement of endolymph causes the cupula to move across the stereocilia. Deformation of the stereocilia by the flow of endolymph toward the kinocilia results in increased neuronal activity of the vestibular nerve. Inhibition of vestibular neurons occurs with deviation of stereocilia away from kinocilia.[5] The semicircular canals are responsible for dynamic equilibrium and respond only to changing forces such as

acceleration and deceleration but not to constant velocity or stationary positioning.[2] The semicircular canals can detect changing forces at a rate of 1 degree/sec^2.[5]

A similar receptor organ called the macula is located in the wall of the saccule and the utricle. The organ consists of a 2-mm oval plaque of dense connective tissue lined by neuroepithelial cells and covered with a gelatinous substance, termed the *otolithic membrane*. Calcium carbonate crystals called statoconia (otoliths) lie on the
otolithic membrane, and their movement initiates stimulation of the vestibular neurons.[7] The macula sacculi is 579
oriented vertically, and the macula utriculi is oriented horizontally. Gravitational forces continuously affect the 580
position of otoliths in at least one of the maculae, supplying constant information regarding the static position of the head.[8] These receptor organs can detect changes in head position of one-half degree from the stationary plane.[5] The maculae of the saccule and utricle also detect linear acceleration and deceleration and are responsible for maintenance of static equilibrium.[8]

The hair cells of the crista ampullaris and maculae synapse with sensory neurons of the vestibular nerve. The cell bodies of these bipolar neurons are located within the petrous temporal bone and constitute the vestibular ganglion.[5] Axons leave the petrous temporal bone through the internal acoustic meatus adjacent to the cochlear portion of the eighth cranial nerve. Fibers course to the lateral aspect of the rostral medulla and penetrate the brainstem between the caudal cerebellar peduncle and the spinal tract of the trigeminal nerve. The majority of fibers of the eighth cranial nerve terminate in the four vestibular nuclei (rostral, medial, lateral, and caudal) located in the lateral wall of the fourth ventricle of the rostral medulla oblongata.[1] Some of these fibers form the vestibulocerebellar tract and directly enter the caudal cerebellar peduncle to terminate in the fastigial nucleus and flocculonodular lobes of the cerebellum. The afferent supply to the vestibular nucleus is primarily from the maculae, the crista ampullaris, and the cerebellum. These sensory fibers synapse with second-order neurons in the vestibular nuclei that extend fibers to the motor neurons of the spinal cord, the nuclei of the cranial nerves that control eye position, the cerebellum, the autonomic nervous system, the reticular formation, and the cerebral cortex.[2,5]

The vestibulospinal tract courses in the ipsilateral ventral funiculus and terminates in interneurons of the ventral gray column. Stimulation of the vestibulospinal tract is facilitatory to α- and γ-motor neurons of the ipsilateral extensor muscles, inhibitory to the α-motor neurons of the ipsilateral flexor muscles, and inhibitory to contralateral extensor muscles.[2] The net result is ipsilateral extensor tonus and contralateral flexor tonus that act as an adaptive mechanism against gravity by catching the body and preventing a fall in the direction of vestibular stimulation.[6]

Axons from the medial vestibular nucleus project through the medial longitudinal fasciculus. The ascending portions of the medial longitudinal fasciculus course to the motor nuclei of the third, fourth, and sixth cranial nerves (oculomotor, trochlear, and abducens).[4,9] These fibers coordinate conjugate eye movement with changes in head position. This pathway, along with cerebellar input, controls physiologic (vestibular) nystagmus.[2] Physiologic nystagmus is a normal reflex that allows the eyes to remain fixed on a stationary object while the head moves.[5] Nystagmus is characterized by involuntary, conjugate, rhythmic eyeball oscillations with a fast and slow phase. The direction of nystagmus is defined by the direction of the fast phase and is induced by rapid movements of the head. Rapid dorsiflexion of the neck results in vertical nystagmus, whereas side-to-side movement of the head induces horizontal nystagmus. Turning the head to the left results in a horizontal nystagmus with the fast phase to the left. The accompanying slow phase is in a direction opposite to body motion and allows the eyes to fix on a stationary image. The fast phase is initiated when the eyeball reaches the lateral

limit of ocular movement and allows the eyeballs to jump forward and focus on a new image.[5,6] The slow phase is controlled by vestibular input, and the fast phase is a function of the brainstem.[2] Physiologic nystagmus induced by rapid manipulation of the head is called the oculocephalic reflex. This reflex occurs independent of vision.[10] Descending portions of the medial longitudinal fasciculus travel in the ventral funiculus of the cervical and cranial thoracic spinal cord segments and control the position and activity of the limbs and trunk in coordination with head position.[2,5]

Fibers from the vestibular nuclei that project to the reticular formation provide afferent nerves to the vomit center, which is the pathway for the development of motion sickness. Reticulospinal tracts also aid in the maintenance of extensor tone to support the body against gravity.[2,5]

Vestibular impulses enter the cerebellum via the caudal cerebellar peduncles. Fibers of the vestibulocerebellar tracts terminate primarily in the flocculonodular lobe and fastigial nucleus.[2] The flocculonodular lobe appears to be linked closely to the semicircular canals in the control of dynamic equilibrium.[5] The cerebellum functions to coordinate protagonistic, antagonistic, and synergistic muscle groups for controlled responses to gravity. The vestibular apparatus provides information to the cerebellum, dictating the relative degree of contraction necessary to maintain equilibrium.[5]

Vestibular signals travel through to the contralateral medial geniculate nuclei of the thalamus to the cerebral cortex. In addition to proprioceptive information from other parts of the body, the cerebral cortex facilitates conscious perception of orientation.[2]

The vestibular system is capable only of detecting movement and orientation of the head in relationship to the rest of the body. Afferent pathways from the neck allow the head to be cognizant of the orientation of the rest of the body.[5,8] Proprioceptive receptors in the joints of the neck that transmit signals to the reticular formation are essential to the righting reflex.[7,8] Exteroceptor receptors of the skin and proprioceptive receptors in other joints also are integrated in the cerebellum and reticular formation to aid in the maintenance of equilibrium. These 580
signals allow the vestibular system to know if the body remains in an appropriate position with respect to gravity 581
while the head is bent. Visual images can help to maintain balance by visual detection of the upright stance. In addition, slight linear or angular movement of the head shifts the image on the retina, which relays directional information to equilibrium centers. Visual compensation may be capable of maintaining balance in the face of complete vestibular destruction, if the eyes are open and motions are performed slowly.[5]

The seventh cranial nerve (the facial nerve) emerges from the lateral medulla ventral to the vestibulocochlear nerve at the level of the trapezoid body. The two nerves are associated closely with the petrous temporal bone and enter the internal auditory meatus together.[11] Within the internal auditory meatus, the facial nerve separates from the vestibular nerve and courses through the facial canal of the petrosal bone. The facial nerve exits the cranium from the stylomastoid foramen located immediately caudal to the external auditory meatus. Both nerves commonly are affected simultaneously by a single disease process because of the proximity of the facial and vestibular nerves.[12]

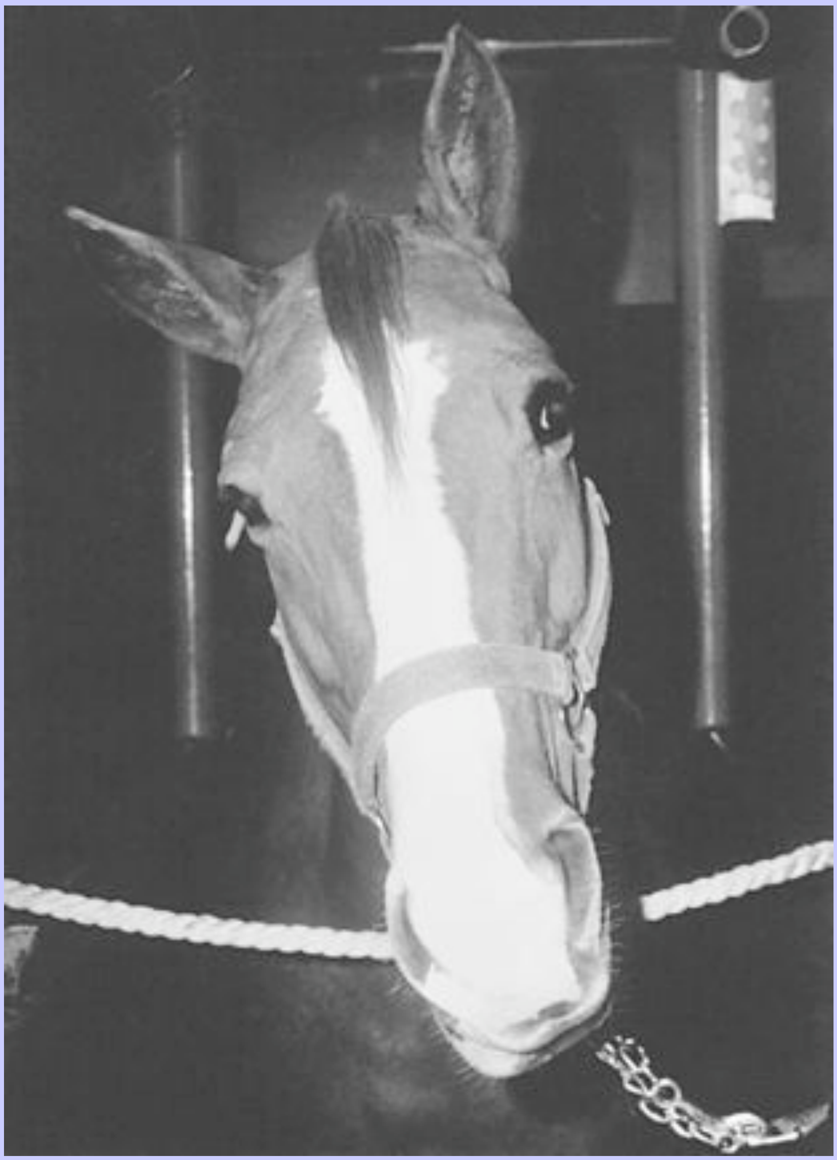
Sympathetic innervation to the eye also is associated anatomically with the petrous temporal bone. Damage to this nerve (Horner's syndrome), along with vestibular and facial nerve deficits, frequently occurs with petrous temporal bone trauma and otitis media in small animals.[10] The association of Horner's syndrome, facial nerve paralysis, and vestibular disease rarely is documented in the horse.[2]

10.6.2 Clinical Signs

Knowledge of the anatomy and function of structures related to the peripheral and central vestibular system aids in neuroanatomic localization of the lesion.[10] Differentiation of central versus peripheral vestibular disease is important for establishing a list of differential diagnoses, initiating therapy, and formulating a prognosis. A thorough physical and neurologic examination of a horse with vestibular disease may identify nonvestibular neurologic signs, lending significant insight into the location of the lesion. Historical information, including duration of condition, rate of onset, and disease progression, also may aid in differentiation of central from peripheral vestibular disease.

Signs of acute peripheral vestibular system dysfunction include head tilt, nystagmus, falling, circling, reluctance to move, and asymmetric ataxia with preservation of strength.[*] Horses affected with peracute vestibular disease are often violent because of disorientation.[1] A true head tilt is a consistent sign of vestibular disease and is characterized by ventral deviation of the poll of the head toward the affected side[4,14] (Figure 10.6-1). The horse prefers to lie on the side of the lesion and may lean on the wall toward the affected side when standing. When forced to move, the horse takes short, uncoordinated steps in a circle toward the direction of the lesion. The body may be flexed laterally with a concavity toward the lesion.[4,6] Extensor hypotonia ipsilateral to the lesion and mild hypertonia and hyperreflexia of the extensor muscles of the contralateral side result in asymmetric ataxia.[15] Extensor hypotonia occurs from loss of facilitatory neurons of the vestibulospinal tract to ipsilateral extensor muscles. Contralateral extensor hypertonia occurs from loss of inhibitory neurons and unopposed extensor tone of the contralateral vestibulospinal tract.[2,6,14] Central vestibular disease has similar clinical signs, but general proprioceptive deficits, weakness, and multiple cranial nerve deficits also may be present, resulting from damage to the surrounding neurologic structures. The onset of vestibular signs in a horse with an expanding space- 581
occupying central lesion is not as dramatic as peripheral nerve damage; adjustments by compensatory 582
mechanisms occur during slow development of the lesion. These lesions, however, are not likely to show significant clinical improvement after the onset of clinical signs, as may occur with peripheral vestibular lesions.

Figure 10.6-1 Thoroughbred with right-sided peripheral vestibular disease caused by a pathologic fracture of the petrous temporal bone following otitis media-interna. The head tilt, dropped pinna, and eyelid droop occur on the affected side, whereas the muzzle is pulled toward the contralateral side.



Pathologic nystagmus is involuntary, rhythmic oscillations of the eyes occurring while the head is in a normal position and indicates a lesion in the vestibular system or cerebellum.[12] As in physiologic nystagmus, one can identify a fast and a slow phase. The direction of nystagmus is defined by the direction of the fast phase.[2,5,12] Pathologic nystagmus may be spontaneous, occurring with the head in the resting position, or positional, which

is induced by elevation or lateral flexion of the head.[2] Nystagmus usually appears with the onset of other peripheral vestibular signs but may last only 2 to 3 days because of central compensation.[4,15] Concomitant blinking of the eyelid may hinder detection of nystagmus.[2]

Peripheral vestibular dysfunction may result in horizontal or rotary nystagmus. The fast phase of nystagmus is directed away from the lesion and does not change with changing head position.[2,3,6] The direction of rotary nystagmus is defined by the direction the limbus moves from the 12 o'clock position during the fast phase.[2] Horizontal, rotary, or vertical nystagmus can result from a central vestibular lesion. In addition, the type of nystagmus observed may change with changing head position in a patient with central vestibular disease.[1,12,14] Often the fast phase is directed away from the central lesion, but this is not a consistent finding.[2]

In the healthy animal a constant stream of electric stimulation arises in each vestibular end organ and transmits signals that control ocular position via the medial longitudinal fasciculus. These signals normally drive the eyes toward the opposite direction. The eyes are maintained centrally, however, because vestibuloocular pathways are opposed in an equal and opposite manner. Unilateral vestibular disease upsets this balance, resulting in slow deviation of both eyes toward the lesion. Rapid eye movements return the eyes to midposition. Individuals that are blind at birth or have been blind for an extended time may exhibit irregular eyeball oscillation with no slow or fast component.[16]

Ataxia and dysmetria are often severe with peripheral vestibular disease; however, strength is maintained. Postural reactions remain normal with the exception of the righting reflex. The motor system is unable accurately to control movement and identify the location of different parts of the body at a given time. Therefore the horse makes an exaggerated response toward the side of the lesion as it attempts to stand.[2]

Loss of hearing is a common finding with peripheral vestibular disease because of the proximity of the cochlea to the vestibular receptor organs. In the central nervous system, diffuse pathways control auditory signals and extensive central disease would be necessary to cause hearing loss.[14]

If vestibular signs are accompanied by depression, weakness, or conscious proprioceptive deficits, a central vestibular system lesion is likely. With a central lesion, abnormal conscious proprioception occurs because of damage within the brainstem of the descending upper motor neuron tracts to the limbs.[2] Damage to the spinocerebellar tracts or caudal cerebellar peduncles results in abnormal unconscious proprioception and hypermetria.[1] The nuclei of the trigeminal (fifth cranial nerve) and the abducens nerves (sixth cranial nerve) are in anatomic proximity to the vestibular nuclei and are damaged readily in a common disease process. Trigeminal nerve paralysis creates a loss of sensation to the head and atrophy of the muscles of mastication. Trochlear nerve damage results in medial strabismus.[10]

Destructive space-occupying lesions in the cerebellopontine angle or flocculonodular lobe may result in paradoxical central vestibular disease.[1,17] This syndrome is manifested by vestibular ataxia and a head tilt contralateral to the side demonstrating general proprioceptive ataxia and postural reaction deficits. When this unusual combination of neurologic signs is present, localization of the lesion is defined by the side of general proprioceptive deficits.[2,10]

Central or peripheral vestibular disease may produce strabismus. One observes ventrolateral strabismus ipsilateral to the vestibular lesion with elevation of the head and extension of the neck.[2,10] One observes mild ventral deviation of the eyes in normal horses when the head is elevated, but the finding is symmetric.

Ventrolateral strabismus of vestibular disease is not a sign of a cranial nerve deficit of the extraocular muscles[2] but is a reflection of abnormal upper motor neuron influences on the oculomotor nucleus from the ipsilateral vestibular nucleus via the medial longitudinal fasciculus. If the strabismus is purely vestibular in origin, normal ocular mobility is visible with manipulation of the head.[14]

Signs of vestibular disease may improve rapidly 2 to 3 weeks after onset because of visual and central accommodation.[2,13] Central vestibular lesions are slower to compensate than peripheral vestibular lesions; signs may even progress if the central lesion is an expanding space-occupying mass.[14] Human beings compensate satisfactorily for unilateral disease but are not required to be coordinated athletes performing at high speed.[4] Blindfolding a horse with compensated disease results in ataxia and a head tilt (Romberg's test). Blindfolding eliminates visual and limb proprioceptive orientation; the body is forced to rely on the impaired vestibular system for equilibrium.[2,10,11] This test is unreliable for localizing the side of the lesion.[3] Horses may 582
decompensate dramatically when the blindfold is placed over the eyes, resulting in anxiety, disorientation, and 583
falling.[2] One should perform the test with caution on a padded surface with good footing.

Horses affected with bilateral peripheral disease demonstrate no head tilt, circling, or pathologic nystagmus, and one cannot induce physiologic nystagmus by rapid manipulation of the head (the oculocephalic reflex) or by caloric testing. The head may sway with wide excursions from side to side.[4] As with all peripheral vestibular disease, strength is preserved.[2] Clinically, horses affected with bilateral vestibular disease exhibit more symmetric ataxia similar to generalized cerebellar disease.[1]

Facial nerve (seventh cranial nerve) paralysis frequently occurs concurrently with peripheral vestibular disease because of its proximity to the vestibular nerve within the petrous temporal bone. Facial nerve paralysis worsens the long-term prognosis and complicates the management of vestibular disease patients. The facial nerve innervates the muscles of facial expression, and damage to this nerve results in muzzle deviation away from the affected side, lack of menace and palpebral response, ear droop, decreased nostril flare impeding air flow, and buccal impaction of feed.[4,11,12] Keratitis and corneal ulceration are common because of the inability to blink and decreased tear production.[4,18] Decreased tear production results from damage to parasympathetic fibers to the lacrimal gland. Preganglionic fibers travel with the facial nerve through the internal auditory meatus and separate in the facial canal proximal to the geniculate nucleus.[12] The fibers split from the facial nerve to join the superior petrosal nerve, which carries fibers to the sphenopalatine ganglion. Postganglionic fibers join the sympathetic fibers to the eye and travel with the vasculature to the lacrimal gland.[2,12] Corneal ulcerations occur in the inferior portion of the cornea and are slow to heal because of ongoing exposure.[11] Lack of tear production aids in localization of the lesion, indicating the damage is within the petrous temporal bone, proximal to the geniculate nucleus.[2,12] Clinical signs of facial nerve paralysis may not appear for several days after the onset of vestibular disease, because damage to the nerve may result from hematoma, callus, or an extension of inflammation and secondary neuritis.[4] Because of the proximity of the nuclei of the facial and vestibular nerves, extensive lesions of the medulla may involve both nerves. If no improvement of facial nerve deficits occurs within 3 to 4 months after the onset of disease, the prognosis is poor for recovery. If one notes even mild improvement in the first 4 months, facial nerve function may return.[4] Horses may learn to retract the globe, allowing the eyelid and nictitating membrane to slide across the surface of the cornea, distributing lubrication and protecting the eye from trauma.[11] Careful observation is necessary to differentiate this adaptation from improvement of lid function.

* References 2, 4, 8, 10, 12, 13.

10.6.3

Peripheral Vestibular Disease

Acute onset of peripheral vestibular disease and facial nerve paralysis is not a rare occurrence in the horse.[15,19] Damage to the temporal bone is the most likely anatomic location when these nerves are affected concurrently. Otitis media-interna and traumatic skull fractures are the most common causes of these signs in large animals.[1]

10.6.3.1

OTITIS MEDIA

Otitis media-interna is less common in the horse compared with other species.[4] The disease occurs in adult horses with no breed or sex predilection. Rupture of the tympanic membrane and drainage of exudate from the external meatus can occur in the horse but is uncommon.[11,15] Instead, as in lambs the infectious process migrates ventrally, creating chronic inflammation of the tympanic bulla and proximal stylohyoid bone.[20] Inflammation induces bony proliferation of these bones with loss of the joint space and fusion of the temporohyoid joint. The hyoid apparatus is linked in series to the tongue and larynx; fusion of the temporohyoid joint results in impaired flexibility of the unit. A stress fracture of the petrous temporal bone, the stylohyoid bone, or both may result from eating or vocalization with normal tongue movement.[6,15] Petrous temporal bone fracture is most common. The fracture line extends into the cranial vault at the level of the internal auditory meatus, resulting in direct neural tissue trauma and hemorrhage into the middle and inner ear.[1,15] No neurologic signs are apparent during the formation of proliferative osteitis and temporohyoid joint fusion. The onset of neurologic signs corresponds with the occurrence of the stress fracture. Occasionally, the fracture extends to the foramen lacerum, caudal to the petrous temporal bone, where the glossopharyngeal and vagus nerves exit the skull. Trauma to these nerves by the fracture may result in dysphagia for several days, in addition to acute vestibular disease.[11]

As a result of extension of the inflammation through the internal acoustic meatus, focal suppurative meningitis may occur at the level of the pons, resulting in fever and depression.[21] Secondary meningitis complicates the treatment and management and worsens the prognosis for recovery.[15]

The origin of the infectious process of otitis media is presently unknown. Extension from otitis externa and tympanic membrane rupture is unlikely.[21] Hematogenous spread of bacteria to the inner ear and ascending infection from the eustachian tube are highly suspected.[4,11,15,21] Acute vestibular disease resulting from 583
extension of an aspergillosis guttural pouch infection to the stylohyoid and petrous temporal bone has been 584
documented.[22] However, otitis media rarely is associated with concurrent guttural pouch infection. In addition, microorganisms isolated from the guttural pouch did not correlate with microorganisms obtained from the middle ear of normal horses.[1,18] Pathogenic organisms isolated from the middle ear of horses include *Actinobacillus, Salmonella, Enterobacter, Pseudomonas, Streptococcus, Staphylococcus,* and *Aspergillus*.[1,18]

Radiographic examination of the skull is necessary for establishing the diagnosis of osteitis. Dorsoventral radiographs are most valuable in identifying the characteristic periosteal proliferation and sclerosis of the stylohyoid bone and petrous temporal bone[4,11,18] (Figure 10.6-2). The fracture line is difficult to identify because of minimal displacement of the fracture fragments, and lateral oblique radiographs at varying angles may aid in localization of a petrous temporal bone fracture. Several acute onset cases of otitis media initially did not demonstrate characteristic radiographic evidence of disease. Moderate bony proliferation may not

occur for several weeks and must be present to diagnose the condition radiographically.[18] Bone scintigraphy is a noninvasive technique that may allow for an immediate identification of early lesions of the petrous temporal bone. Radiography can identify only structural abnormalities of bone. Bone scintigraphy is capable of detecting dynamic characteristics of bone. Increased metabolic activity and blood supply to the bone, caused by infection or fracture, results in increased uptake of the radiolabeled compound (technetium-99m–labeled phosphate) before radiographic evidence of bony proliferation.[23–25]

Figure 10.6-2 Ventrodorsal skull radiograph demonstrating bony proliferation of the proximal left stylohyoid and petrous temporal bones *(arrows)* following chronic otitis interna-media.

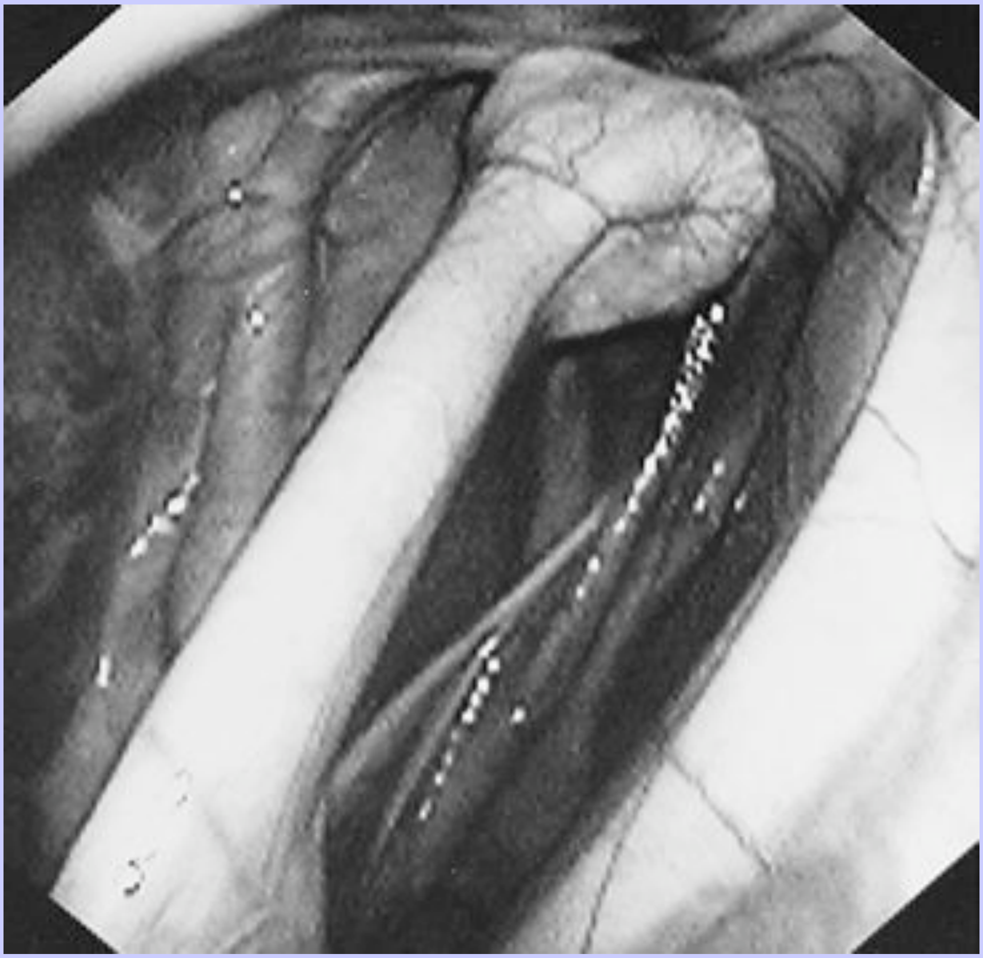
Otoscopy is difficult in the horse because of the long, bony, narrow, horizontal ear canal. The accumulation of ceruminous debris also impedes visualization.[12,18] Anesthesia allows for complete cleaning of the external auditory canal and is likely to be necessary for a thorough otoscopic examination given the uncooperative nature of the awake horse. Even with a thorough examination, detecting subtle changes of the tympanic membrane is difficult. Transtympanic lavage may aid in the diagnosis of otitis media. One infuses sterile saline (0.5 ml) via a myringotomy with immediate withdrawal of fluid. A cloudy or yellow tap is a positive finding. Fluid is submitted for cytologic examination, protein evaluation, and culture. Anaerobic and aerobic culture and sensitivity may allow for identification of the pathogenic organism and aid in appropriate antibiotic selection.[18]

One should perform cerebrospinal fluid analysis in all cases of acute vestibular disease.[1,2] In the case of otitis media, cerebrospinal fluid analysis, culture, and sensitivity help to reveal the presence of secondary bacterial meningitis, identify the causative organism, and direct the selection of an appropriate antibiotic.[15]

Endoscopy of the pharynx and guttural pouch is generally diagnostic in horses with proliferative osteitis of the stylohyoid bone. One should perform this noninvasive procedure on every horse suspected of having proliferative osteitis, because one often can identify bony proliferation of the proximal stylohyoid bone within the guttural pouch[1,22] (Figure 10.6-3).

Treatment should include long-term administration (30 days) of broad-spectrum antibiotics. Potentiated sulfa, 2 mg/kg ampicillin administered intramuscularly b.i.d. or t.i.d., and 15 to 25 mg/kg chloramphenicol administered orally q.i.d. have been recommended when one cannot obtain culture and sensitivity tests.[11,15] Two to 4 mg/kg phenylbutazone administered orally b.i.d. helps alleviate inflammation. If one identifies central nervous system involvement, 1 g/kg dimethyl sulfoxide administered intravenously in 10% solution is recommended to reduce brain inflammation and edema.[1] One should avoid corticosteroid therapy if one suspects an infectious process. Intensive supportive care and topical therapy for corneal ulceration also may be necessary. 584

Figure 10.6-3 Endoscopic view of the right guttural pouch of a horse with acute onset of right-sided vestibular disease and facial nerve paralysis. Bony proliferation of the proximal stylohyoid bone is consistent with chronic osteitis caused by otitis interna-media.



Diagnosis before acute neurologic signs is difficult because most horses may exhibit no clinical signs. Some horses may demonstrate early signs of otitis media, including ear rubbing, head tossing, chomping movements, and ear sensitivity before the onset of neurologic signs. Affected horses may exhibit signs of pain on palpation at the base of the ear. Head tossing is a common problem in the horse, with multiple causes; the primary problem rarely is identified.[18] One should perform ventrodorsal radiographs of the skull and endoscopy of the guttural pouches on the head-shaking horse to identify an early case of otitis media. Surgical removal of a 2-cm segment of the stylohyoid bone has been attempted on several head-shaking horses with bony proliferation of the petrous temporal bone in an attempt to prevent secondary stress fracture and neurologic signs. A fibrous nonunion of the stylohyoid bone should interrupt the transmission of hyoid forces to the temporohyoid joint. Long-term success of the surgery has not yet been established.[18]

10.6.3.2

HEAD TRAUMA

Traumatic fractures of the petrous temporal bone result in damage to the vestibular and facial nerves. Profuse aural hemorrhage or loss of cerebrospinal fluid from the external ear canal frequently is observed. Bleeding from the nose occurs if the fracture extends to the cribriform plate.[4] Clinical signs usually appear immediately following trauma and include vestibular disease, facial nerve paralysis, recumbency, or coma. Damage to nervous tissue may be caused by hematoma, callus formation, or displacement of fracture fragments resulting in delayed onset of clinical signs. Signs from brainstem contusion or concussion may be more severe than vestibular dysfunction. If blindness is present, the prognosis worsens because of the loss of visual compensation of vestibular disease in the future. If one cannot elicit the oculocephalic reflex (physiologic nystagmus), one should suspect damage to the medial longitudinal fasciculus, indicating extensive brainstem damage; a poor prognosis is indicated.[2] One may recall that bilateral peripheral vestibular disease also results in loss of the oculocephalic reflex; this, however, is an unlikely scenario for trauma.

Fractures of the basioccipital and basisphenoid bones occur most frequently in horses that rear over backward and strike the poll of the head. This fracture does not result from referred impact from the poll but is thought to be an avulsion fracture from the pull of the powerful ventral straight muscle of the neck (rectus capitis ventralis) on its insertion on the basioccipital bone.[26] Basioccipital fractures result in neurologic signs associated with damage to the brainstem; signs of vestibular disease are common.

Petrous temporal bone fractures are difficult to identify radiographically; tympanosclerosis appears as early as 20 days after trauma and obscures the fracture line.[4] One often can identify basioccipital fractures easily but can confuse them with the suture lines in the base of the skull.[26]

Treatment of vestibular signs following head trauma is similar to that of otitis media. Antiinflammatory drugs such as phenylbutazone and dimethyl sulfoxide are indicated to reduce swelling and edema. Broad-spectrum antibiotics are required to prevent secondary bacterial infection. One should administer dexamethasone intramuscularly at 0.1 to 0.2 mg/kg every 4 to 6 hours for 1 to 4 days if one suspects brain concussion or contusion.[1] Acepromazine maleate lowers the seizure threshold and is contraindicated in cases of head trauma.[27] Xylazine may potentiate an active bleeding process because of transient hypertension and should be avoided in the early period following head trauma.[28]

Drug toxicities can result in unilateral or bilateral peripheral vestibular disease and deafness. Degeneration of the hair cells within the peripheral receptor organs of the auditory and vestibular system occurs with prolonged administration of aminoglycoside antibiotics. Severely affected animals also develop neural degeneration.[3] A more common manifestation of aminoglycoside toxicity is renal failure. As renal clearance of the aminoglycoside decreases, the ototoxic effects of the antibiotics are potentiated.[1,10,21] Clinical signs of 585
vestibular disease appear before deafness. Early vestibular disease may be reversible or centrally compensated, 586
but loss of auditory function is permanent.[2,10,21] Streptomycin preferentially affects the vestibular system, whereas dihydrostreptomycin, kanamycin, gentamicin, neomycin, and vancomycin are more toxic to the auditory system.[2,15] Vincristine, a vinca alkaloid, can cause bilateral cochlear nerve damage in human beings. Auditory function improves several months after discontinuation of the drug.[3] This antimitotic drug is a common component of multiagent chemotherapy protocols in the treatment of lymphosarcoma in the horse.[29]

Vincristine also is used for immunosuppression and stimulation of platelet function in refractory cases of immune-mediated thrombocytopenia.[30] One should carefully monitor auditory function when using this drug.

Sudden loud noises can result in degeneration and necrosis of the sensory hair cells of the inner ear.[21] A lightning strike, although usually fatal, is reported to cause acute onset of unilateral vestibular disease in the horse. Facial nerve paralysis may or may not accompany the vestibular signs. Documentation of histopathologic findings in one case revealed hemorrhage and necrosis of the temporal bone, vestibular nerve, and adjacent tissue. Whether the mechanism of damage is electrocution or noise trauma is unknown.[1]

10.6.4 Central Vestibular Disease

Any inflammatory disease or space-occupying mass of the central nervous system may damage the vestibular nuclei and related tracts. Clinical signs vary with the type and extent of the disease process. One should suspect a central nervous system disease if abnormal mentation, seizures, blindness, or multiple cranial nerve abnormalities with general proprioceptive deficits are present. One should perform an electroencephalogram to detect the location and extent of the central nervous system lesion. The electroencephalogram can detect only cerebral damage and cannot identify lesions of the brainstem. Inflammatory, parasitic, and neoplastic diseases have been implicated in central vestibular disease of the horse.

Inflammatory disease affecting the central nervous system includes bacterial abscess, equine protozoal myelitis, polyneuritis equi, and viral encephalitis. One should perform cerebrospinal fluid analysis to identify the inflammatory process. In the case of brain abscessation a culture of the cerebrospinal fluid may identify the causative organism; *Streptococcus equi* is a common causative agent.[32,33] Equine protozoal myeloencephalitis is a common neurologic disease in the United States and Canada and should be suspected if multifocal disease is present.[1] For polyneuritis equi to occur with vestibular dysfunction is common, but the signs of cauda equina neuritis predominate.[2] Rabies may present as an encephalitis or spinal cord disease and should be considered in the differential diagnosis of any horse with neurologic disease. Spinal ataxia is the primary neurologic deficit observed in horses affected with equine herpesvirus myelitis, but the presence of concurrent vestibular disease is reported.[34] The major clinical signs observed with a togavirus (eastern, western, and Venezuelan equine encephalitis) infection are depression and seizure, although cranial nerve deficits are observed.[6,33]

Aberrant parasite migration of the central nervous system in horses results in acute onset of neurologic signs. Clinical signs vary, but progression of clinical signs occurs in most instances. Neurologic signs are generally asymmetric because of the random nature of migration. The parasites most frequently identified are *Hypoderma, Habronema, Strongylus,* and *Setaria* species. *Strongylus vulgaris* is most common and also may produce parasitic thromboembolism to the brain.[1,33] Migration of *Micronema deletrix* and *Setaria* may produce severe, diffuse brain and spinal cord disease.[35] Cerebrospinal fluid analysis may reveal eosinophilic or neutrophilic leukocytosis with evidence of hemorrhage. *Micronema* may be identified in the cerebrospinal fluid and urine.[1] Treatment includes antiinflammatory (flunixin meglumine, phenylbutazone, dexamethasone) and antiparasitic drugs (ivermectin, 200 mg/kg; fenbendazole, 50 mg/kg/day for 1 to 3 days; thiabendazole, 440 mg/kg s.i.d. for 2 days). Recovery may be dramatic.[1] Fungal granulomata caused by *Aspergillus* and *Cryptococcus neoforman* s have been reported as space-occupying masses within the cranium of a horse.[1,36] Cholesteatoma (cholesterol granuloma) could involve the vestibular system by extending from the choroid plexus of the fourth ventricle of the brain.[1] Neoplastic diseases of the central nervous system are rare in the horse. Any tumor affecting the

cerebellomedullary angle could result in vestibular signs.[2] Lymphosarcoma, ependymoma, meningeal melanoma, and melanotic hamartoma have been reported to affect the central nervous system of the horse.[2,37]

10.6.5 Ancillary Diagnostic Tests

10.6.5.1 CALORIC TESTING

The caloric test is a diagnostic aid that may be helpful in differentiating central from peripheral vestibular disease. The test is able to assess each peripheral vestibular sensory organ separately. In the normal animal, irrigation of ice-cold water (12° C) into the external auditory canal for 3 to 5 minutes induces a horizontal nystagmus with the fast phase away from the tested labyrinth.[2,3,5,6] The water cools endolymph closest to the tympanic membrane, increasing its density. A density gradient is created within the semicircular canal and the 586
cooled endolymph sinks, causing displacement of the hair cells. Warm water (45° C) irrigation of the external 587
auditory canal results in horizontal nystagmus with a fast phase toward the tested labyrinth. The warm water test is less reliable.[10,16] The test does not induce nystagmus in a nonfunctional labyrinth. Animals may resist the procedure, making the test difficult to interpret, and in some animals, one cannot induce nystagmus. If one obtains an asymmetric response, the depressed reaction indicates the abnormal labyrinth.[2] The test is difficult to perform and not entirely reliable, although it may be a helpful diagnostic aid in the anesthetized or comatose horse.[6]

10.6.5.2 BRAINSTEM AUDITORY EVOKED RESPONSE

The cochlea is damaged by trauma or inflammation of the peripheral vestibular receptor organs, and detection of hearing loss may help to differentiate central from peripheral vestibular disease. Unilateral hearing loss is difficult to assess subjectively in the horse. Brainstem auditory evoked response is a method of objective assessment of auditory function in the horse. This noninvasive, electrodiagnostic test stimulates the auditory system with a series of clicks. Far-field potentials of the brainstem auditory components are recorded via cutaneous electrodes and a signal averaging system.[6,38] The response is a series of evoked potentials occurring within 10 ms after the stimulus. In the horse the evoked potentials appear on the oscilloscope as a series of five waveforms.[39] In human beings, five to seven waveforms are present, and each corresponds to a specific neurologic structure.[38,39] Abnormalities of the specific waveforms can identify a lesion of the corresponding neurologic structure. In the horse, functional loss of the cochlea or eighth cranial nerve results in the loss of the entire waveform on the side of injury, and the presence or absence of the waveform can differentiate a central from a peripheral vestibular lesion. The test is reliable with sedation and general anesthesia. General anesthesia is not necessary to perform the test, but sedation is recommended.[38]

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10.7

10.7—Diseases of the Cerebellum

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587

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Cerebellar abnormalities have been reported in horses and are confined primarily to a small number of breeds. The cerebellum is essential for the coordination of movement. Afferent information arises from the general and special (vestibular) proprioceptive systems and the special somatic (auditory and visual) systems. The cerebellum is responsible for regulation of the rate, range, and strength of movement, as well as integration and coordination for balance and posture. Cerebellar abnormalities in horses are unusual; however, when present, they can have a profound affect on gait and posture.

10.7.1

Structure and Function

Knowledge of the structure and development of the cerebellum is important for understanding cerebellar function in health and disease. The cerebellum is located in the metencephalon dorsal to the pons and is attached

to the pons via three cerebellar peduncles (Figure 10.7-1). The caudal peduncle is composed primarily of afferent fibers arising from the medulla, the vestibular nuclei via the vestibulocerebellar tracts, and the spinal cord via the spinocerebellar tracts. The middle cerebellar peduncle contains only afferent fibers to the cerebellum that arise from the transverse fibers of the pons. The rostral cerebellar peduncle is the primary connection to the mesencephalon and carries the majority of cerebellar efferent fibers, although a few afferent fibers arise from the spinocerebellar tracts. The cerebellum consists of two hemispheres and a central region known as the vermis.[1] The extensive convolutions of the cerebellar cortex are termed *folia*. The cortex covers the surface of the cerebellum. On cut section, the cerebellar medulla is a central region of white matter with multiple projections called arbor vitae. These branches extend to the cerebellar cortex and form the white matter portion adjacent to the cerebellar cortex.

The cerebellar medulla has three nuclei: the fastigial, interpositional, and lateral nuclei from medial to lateral on each side of the cerebellum. The cerebellum also can be divided into three bilateral longitudinal regions in association with these nuclei.[1,2] The medial zone, containing the vermis and the fastigial nucleus, primarily regulates the tone, posture, and equilibrium of the body in general. The intermediate zones contain the interpositional nucleus and cortex adjacent to the vermis and adjust the orientation of limbs in space, maintaining balance, posture, and muscle tone during complex movements. The lateral zones, consisting of the lateral nuclei and lateral portions of the cerebral hemispheres, have a similar function but do not influence posture or muscle tone directly.[2]

The cerebellum arises from the alar plate region of the metencephalon and originates initially as a proliferation of cells in the rhombic lip that extend dorsally and medially to form the dorsal portion of the metencephalon. Germinal cells proliferating in the rhombic lip eventually migrate into the cerebellum and differentiate to form the specialized neurons of the cerebellar cortex. The cerebellar cortex has three layers: the outer molecular, the middle Purkinje, and the inner granular (Figure 10.7-2). The molecular layer is acellular and consists primarily of the dendritic zones of the Purkinje cells and axons of the granular cells.[1] The Purkinje layer is only one cell thick and consists of Purkinje neurons. The granular layer is densely cellular with granular neurons. All layers must be present and aligned in proper orientation for normal function.

Organization of the specialized structure of the cerebellar cortex allows integration and coordination of movement. The cerebellum primarily provides regulation of skeletal movement, allowing coordinated movement; it does not initiate muscular activity. Afferent information regarding movement and balance arising 588
from the mesencephalon, the brainstem, and the spinal cord enters the cerebellum via the cerebellar peduncles, 589
and regulation of movement is coordinated by the inhibitory influence of Purkinje neurons on the cerebellar nuclei. Information enters the cerebellum via the cerebellar peduncles and is carried on two major afferent nerves termed *mossy fibers* and *climbing fibers*.[1] Mossy fibers originate from the brainstem and spinal cord. Mossy fibers send collateral fibers to synapse with the cerebellar nuclei; they terminate by synapsing with granule neurons in the cerebellar cortex. These fibers are facilitory at these synapses. The axons that granule neurons send to the molecular layer course transversely through this layer to synapse with the dendritic zone of multiple Purkinje cells and also provide facilitory influence at these synapses. Climbing fibers originate in the olivary nucleus, which provides most of the extrapyramidal projections to the cerebellum. Similar to mossy fibers, climbing fibers send collaterals to synapse on neurons in the cerebellar nuclei; however, the axon continues through the cerebellar cortex to synapse with the dendritic zone of the Purkinje neurons in the molecular layer. As with mossy fibers, climbing fibers provide a facilitative influence at the synapses.

Figure 10.7-1 Schematic diagram of cerebellar efferent and afferent information pathways via the cerebellar peduncles. The arrow size reflects the relative contribution of each pathway. See text for details.

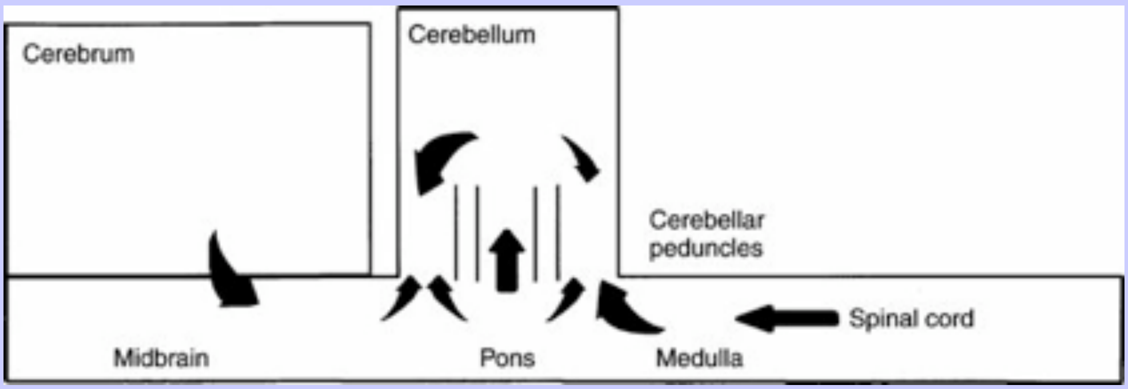
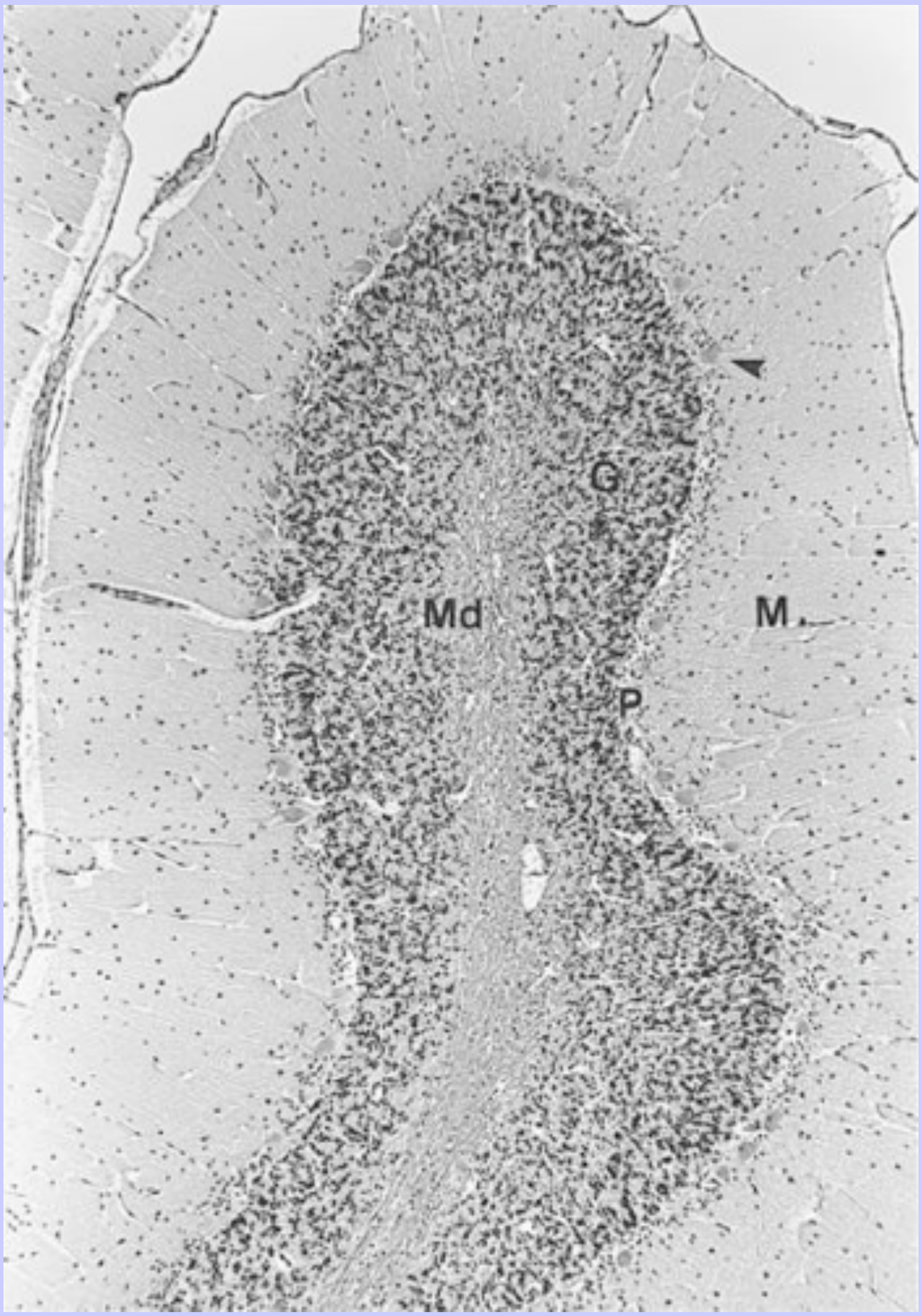


Figure 10.7-2 Photomicrograph of a normal cerebellum.*M,* Molecular layer; *P,* Purkinje layer; *G,* granular layer; *Md,* medulla; *arrowhead,* a Purkinje neuron. (Hematoxylin-eosin stain, ×55.) (Courtesy the Washington Animal Disease Diagnostic Laboratory, Pullman, Wash.)



Purkinje neurons provide the sole efferent fibers from the cerebellar cortex. The majority of Purkinje cell axons terminate on neurons in the cerebellar nuclei, although direct projections from these neurons to the vestibular nuclei occur via the caudal cerebellar peduncle. Purkinje neurons are inhibitory and use the neurotransmitter γ-aminobutyric acid.[1] Efferent nerves from the cerebellum are primarily from the cerebellar nuclei that facilitate activity of upper motor neurons originating in the brainstem.

Thus much of the influence of the cerebellum on skeletal muscle activity is to modulate the upper motor neuron. Information regarding movement and balance enters the cerebellar cortex via the cerebellar peduncles. This 589
afferent activity stimulates inhibitory Purkinje neurons by climbing or mossy fibers. Purkinje neurons in turn 590
modulate the activity of the cerebellar nuclei to regulate movement and muscular tone. Purkinje neurons also provide direct inhibitory input to the vestibular nuclei.

10.7.2 Clinical Signs

Clinical signs associated with cerebellar disease generally reflect the loss of coordination. Mentation is normal in horses with cerebellar disease, provided that other regions of the brain are unaffected and metabolic disturbances such as septicemia or endotoxemia are not present. Cerebellar disturbances result in ataxia and inability to regulate the rate, range, and force of movement.[1] Dysmetria refers to alterations in the range of gait. Hypermetria is an exaggerated range of movement. When moving, the limb has a higher or longer flight compared with normal. Hypometria is a diminished movement; for example, the arc of flight of a limb in motion may be lower. Horses showing hypometria tend to hit objects with the limb when stepping over them and may fail to extend the limb far enough to set the foot down on a step. Initiation of movement may be jerky and awkward, and the trunk may sway from side to side when the horse moves. Spasticity is caused by hypertonia and results in a jerky stiff gait. Paresis is not a feature of cerebellar disease because the cerebellum does not initiate voluntary motion, although a horse that is unable to regulate rate and range of motion sometimes may appear weak and drag its toes. Diffuse cerebellar disease results in bilateral signs. In general, unilateral lesions result in signs ipsilateral to the lesion.

Intention tremor is another prominent sign of diffuse severe cerebellar disease. Tremor is most obvious as vertical or horizontal head motions and can be observed readily as a horse approaches feed or attempts to nurse. The tremor is present only when a movement is initiated and tends to become more exaggerated as the horse approaches an object.

Cerebellar disease also may cause loss of the blink reflex and vestibular abnormalities. The horse may fail to blink in response to an ocular menace. The exact mechanism for this deficiency is unknown; however, a portion of the visual pathway from the visual cortex to the facial nucleus (which initiates the blink) likely travels through the cerebellum. Disruption of the flocculonodular lobe, located in the ventral cerebellum, or the fastigial nucleus may result in vestibular signs characterized by disequilibrium; a variable nystagmus, which may be positional; and positioning difficulties.[1] Unilateral lesions may result in a head and body tilt toward the side of the lesion and nystagmus with the fast phase away from the lesion. Paradoxical vestibular syndrome characterized by a head tilt away from the lesion and nystagmus with the fast phase toward the lesion is apparent with unilateral lesions involving the cerebellar peduncle.[1,2]

10.7.3 Diseases of the Cerebellum

10.7.3.1 CEREBELLAR ABIOTROPHY AND DEGENERATION

Cerebellar abiotrophy is the most commonly reported cerebellar disease in horses.[3–10] Abiotrophy in the nervous system refers to premature degeneration of neurons caused by some intrinsic abnormality in their structure or metabolism.[11] Cerebellar abiotrophy has been reported in Arabian, Gotland pony, and Oldenburg horse breeds. Degenerative cerebellar lesions have been observed in one Thoroughbred and two Paso Fino newborn foals.[12] Arabian and part-Arabian horses in North America are affected most frequently.[3–6,8–10] The incidence in some Arabian horse herds has been reported to be as high as 8%. In one report, colts were affected more frequently than fillies, although subsequent reports have not substantiated this finding.[5] The cerebellar abiotrophy that occurs in Oldenburg horses is progressive and fatal with atypical histologic lesions compared with the syndrome that occurs in Arabian foals.[13]

Cerebellar abiotrophy generally affects foals less than 1 year of age and occurs most frequently in 1- to 6-month-old foals. Adult-onset cerebellar abiotrophy has been reported in other species such as the dog and has been observed in two equine cases.[1] Many foals are born with no abnormalities and later develop disease; however, occasionally they are affected at or shortly following birth.[5,6,9]

Clinical signs associated with cerebellar abiotrophy include intentional head tremor, ataxia, wide-based stance and gait, dysmetria, and spasticity.[3–10] The most frequently reported initial signs noted by owners are an intentional head tremor, vertical or horizontal, or a hypermetric forelimb gait.[3,9] The neurologic examination reveals no change in mentation. One almost never observes nystagmus, which has been reported in only one case of abiotrophy in a Gotland pony.[7] A menace reflex frequently is absent or diminished.[6,9] One must interpret this finding with caution because normal foals may lack or have a depressed menace reflex until at least 2 weeks of age.[14]

Stance and gait abnormalities seen with cerebellar abiotrophy generally consist of a wide-based stance or gait and ataxia.[6,8,9] The foal may move stiffly and have a high goose-stepping gait. The horse may protract the limb when walking, resulting in slamming of the foot to the ground. Movement may be spastic with circumduction. Walking on an incline, asking the foal to step over obstacles, and blindfolding the foal exacerbate gait abnormalities. Generally, gait abnormalities are symmetric, although in a Welsh Cob–Arabian- 590
cross foal the initial signs were characterized by a stiff motion in the left front limb. Signs in this foal 591
progressed to severe ataxia.[4] Foals affected at birth may have difficulty rising.[5–7] Despite this finding, weakness is not a feature of the clinical signs associated with cerebellar abiotrophy. Spinal reflexes are usually normal. Some affected animals fall when startled or when raising the head. Signs are generally progressive for several months following diagnosis. Once the animal has reached maturity, the condition becomes static, although mild improvement has been observed.[10]

Ancillary testing is of limited value in diagnosing cerebellar abiotrophy but can be helpful to rule out other causes of ataxia. The complete blood count and serum biochemistry profile are normal in affected foals. Abnormalities in cerebrospinal fluid (CSF) are detectable. In one study, three of four foals had an elevated CSF creatine kinase activity. Values in affected foals ranged from 6.6 to 62 IU/μL (normal range, 0 to 8 IU/

µL).[9] CSF creatine kinase elevations generally are associated with neural necrosis or degeneration, although they are not associated specifically with a particular disease.[15–17]

In addition, CSF total protein may be elevated. In the study cited previously, three foals had elevated total protein with an average of 226 mg/dl (normal, 0 to 100 mg/dl) in all foals with cerebellar abiotrophy. As with creatine kinase, total protein elevations are not specific for abiotrophy and may occur with disruption of the blood-brain barrier or with central nervous system inflammation or degeneration. Many foals with cerebellar abiotrophy have normal CSF analysis. Electroencephalographic abnormalities, including increased synchrony and increased number of abrupt frequency changes, also may be detectable in affected foals.[9] In this study, these abnormalities were not observed in normal foals anesthetized under similar conditions. Skull and cervical radiographs are unremarkable. However, because mentation is normal in foals with cerebellar abiotrophy, electroencephalographic examination is not necessary to make a diagnosis and is primarily useful to exclude seizure disorders as a cause of the tremors observed.

Antemortem diagnosis of cerebellar abiotrophy is based on a typical history and the clinical signs of intention tremor, lack of menace, and failure to blink to bright light, and ataxia in Arabian or part-Arabian foals or Gotland pony foals. The differential diagnoses for cerebellar abiotrophy include cranial malformations; congenital spinal malformations, including atlantoaxial malformations and stenotic myelopathy; inflammation or infection of the cerebellum; and trauma. One can rule out these conditions based on the neurologic examination, CSF analysis, and radiography. The signs of characteristic ataxia and head tremor without weakness in the appropriate breed is nearly pathognomonic.

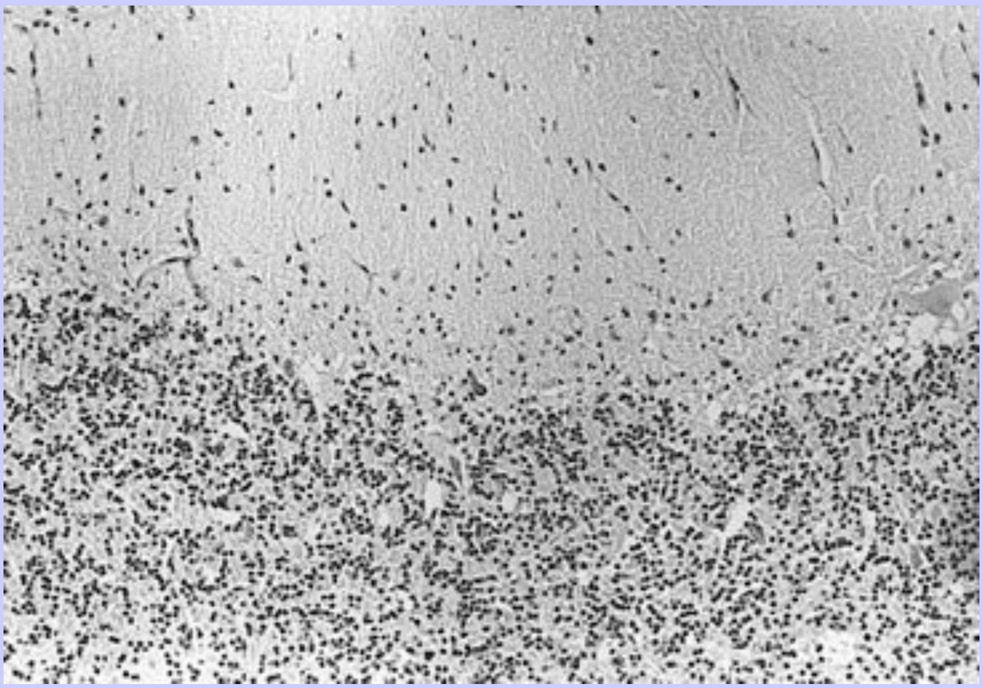
Postmortem examination provides a definitive diagnosis of this disorder. Generally, no gross abnormalities are notable; however, careful examination of the cerebellum may reveal an increased lobular pattern with prominent folia. In the Gotland pony, the weight ratio of the cerebellum to the cerebrum is reduced significantly in foals with cerebellar abiotrophy.[7] Normal foals had a 13% ratio, and affected foals had a 10% ratio. In the degenerative cerebellar condition in the Paso Fino and Thoroughbred foals, a decrease in the cerebellar-to-whole brain weight ratio was evident.[12] This ratio in normal foals was 8% and in affected foals was 6%.

Histologic abnormalities are consistent in cases of cerebellar abiotrophy. The most prominent finding is the widespread loss of Purkinje neurons[3–10] (Figure 10.7-3). Degenerative changes, such as shrunken and angular neurons with hyperchromasia and dispersion of Nissl's substance, are apparent. One may observe occasional “baskets” or clear spaces where the Purkinje neuron is lost. Thinning of the molecular layer occurs with gliosis. The granular layer is also thin with a loss of cellularity. Similar histologic findings were found in Thoroughbred and Paso Fino foals with vacuolation and proliferation of Bergmann's glia in the Purkinje cell layer.[12]

The pathogenesis of cerebellar abiotrophy is unknown. Viral, toxic, and genetic causes have been suggested.[3,5,6,9] To date no evidence has been found to support an infectious cause. No virus has been isolated from the CSF or brain of affected foals, and no viral inclusions have been observed on histologic examination. No toxin 591
has been associated consistently with cerebellar abiotrophy of Arabian foals. A genetic cause may be present. 592
Pedigree analysis of affected Arabian foals has shown a high degree of inbreeding of affected foals, although this breed is generally inbred.[5] Interestingly, in this study three of six foals from one mare suffered from cerebellar ataxia; all affected foals had different sires. A similar pedigree analysis was performed in the Gotland pony in which an autosomal recessive mode of inheritance was suggested.[7] Similar to Arabian foals, a high degree of inbreeding was noted, making a highly significant conclusion difficult. Attempts to breed

affected individuals in this study were unsuccessful. Although a definitive mode of inheritance has not been demonstrated in either breed, a familial tendency is suggested.

Figure 10.7-3 Photomicrograph of the cerebellum from a 9-month-old foal with cerebellar abiotrophy. The decreased number of Purkinje neurons is notable. (Hematoxylin-eosin stain, ×139.) (Courtesy the Washington Animal Disease Diagnostic Laboratory, Pullman, Wash.)



No treatment exists for cerebellar abiotrophy. As noted previously, signs may be progressive until the foal reaches maturity. Signs may stabilize or improve slightly with time.

10.7.3.2 GOMEN DISEASE

Gomen disease is a degenerative cerebellar condition recognized in the northwest part of New Caledonia.[18] Gomen disease is a progressive cerebellar disease that causes mild to severe ataxia. Horses that are indigenous or are introduced to the region may be affected. The disease occurs only in horses that are allowed to roam free, and signs may take 1 to 2 years to develop once a horse is introduced into an endemic area. Horses that are confined generally are unaffected. Clinical signs consist of ataxia, which is most prominent in the hindlimbs; toe dragging; and a wide-based stance. As the disease progresses, weakness becomes prominent and horses may have difficulty rising. The signs are primarily referable to involvement of the cerebellum; however, weakness likely is caused by brainstem or spinal cord involvement. Nystagmus is not observed. Ataxia is progressive over 3 to 4 years until the horse dies or is euthanized.

Mild cerebellar atrophy may be apparent on gross examination of the brain. Histologically, severe depletion of Purkinje neurons is evident throughout the cerebellum.[19] Purkinje neurons may contain lipofuscin pigment and vacuoles. One horse examined had moderate loss of granule cells. Moderate to severe lipofuscin pigmentation of neuron cell bodies occurs throughout the brain and spinal cord. Although lipofuscin accumulation may be considered a normal variation of aging, the degree of pigment accumulation is far more severe than that in horses of similar age.

The pathogenesis of this disease is unknown. Pedigree analysis has not revealed any genetic component for susceptibility to development of disease.[18] A condition of neuronal lipofuscinosis in dogs has some similarities to this disease.[19] The accumulation of lipofuscin pigment and association with free-ranging horses suggests a metabolic disorder, perhaps resulting from toxicity.[18,19]

10.7.3.3 DEVELOPMENTAL ABNORMALITIES

Dandy-Walker syndrome is characterized by a midline defect of the cerebellum and cystic dilation of the fourth ventricle, which separates the cerebral hemispheres.[20] Frequently, all or portions of the cerebellar vermis fail to form, and the corpus callosum may be absent. The condition is rare in horses and has been observed in Thoroughbred and Arabian foals.[21,22] Foals with this syndrome may be abnormal neurologically from birth, with difficulty rising, seizures, and absence of the suckle reflex.[21] The forehead may be domed excessively. Ataxia, nystagmus, aggression, and difficulty in training may persist as the foal ages.[21,22] Diagnosis generally is made at postmortem examination; however, one case was diagnosed antemortem using computed tomography.[21]

Several individual cases of equine developmental cerebellar abnormalities have been described. Cerebellar hypoplasia has been described in a Thoroughbred foal that had difficulty rising and developed seizures shortly after birth.[23] This report did not describe the histologic findings of cerebellar hypoplasia in detail; thus the relationship between this finding and cerebellar abiotrophy or degeneration in Arabian foals is unknown. Bilateral focal cerebellar cortical hypoplasia has been reported in a 6-year-old Thoroughbred gelding.[24] No gait abnormalities were detected in this horse, although it had fallen over repeatedly before euthanasia. The relationship between falling and the cerebellar abnormality is unclear. Possibly the abnormality in this adult horse resulted from a secondary problem, such as vascular injury, rather than a developmental defect.[13]

A single case of cerebellar dysplasia has been described in a 4-year-old Thoroughbred horse.[25] This horse had a 7-month history of circling and collapsing to the left side. In this case the horse had hyperplasia of the right side of the cerebellum with no associated central white matter. Histologically, the granule layer was thinning, with increased thickness of the molecular layer and cavitation of the white matter.

Additional reported developmental disorders include cerebellar hypoplasia with internal hydrocephalus and cerebellar aplasia with hydranencephaly in two fetuses from Haflinger mares with hydrops allantois.[26] Mild cerebellar degenerative changes consisting of Purkinje neuron granularity has been noted in a Standardbred filly with a chromosomal abnormality.[27] This abnormality was accompanied by mild spongiotic degeneration of the cerebrum. Abnormal neurologic signs in this filly included difficulty standing at birth, mental dullness, 592
and a head tilt. These signs were accompanied by growth retardation, small inactive ovaries, and a consistently 593
wrinkled muzzle.

10.7.3.4

INFECTIOUS CONDITIONS

Unlike in many other large animals, no infectious agents have the cerebellum as their primary target; however, a number of agents may affect the cerebellum. Any agent that targets the central nervous system, especially those that have a multifocal distribution, also may involve the cerebellum.

Equine protozoal myelitis is a protozoal disease caused by *Sarcocystis neurona*. This agent causes multifocal inflammation and necrosis of the central nervous system. The most common signs associated with *S. neurona* infection are spinal ataxia, weakness, and muscle atrophy, although this agent also can affect the cerebellum. Clinical signs associated with cerebellar involvement may reflect diffuse or focal involvement. A head tilt or asymmetric ataxia without weakness or lower motor neuron signs may be associated with focal disease. Diagnosis is based on multifocal neurologic disease and the presence of antibody to *S. neurona* in the CSF. Additional information regarding this disease may be found in Chapter 10.10.

Equine herpesvirus type 1 causes multifocal meningoencephalitis resulting from vasculitis, vascular thrombosis, and ischemia of nervous tissue. Although the primary signs associated with this agent include spinal ataxia, cranial nerve abnormalities, and signs associated with involvement of the cauda equina, this agent also may affect the cerebellum. As with equine protozoal myelitis, involvement may be focal, multifocal, or diffuse.

Occasionally, disseminated *Streptococcus equi* infection (bastard strangles) may result in a cerebellar abscess.[28] Neurologic abnormalities in one reported case included proprioceptive deficits in the right forelimb, nystagmus, and a head tilt. Meningitis contributed to other central nervous signs such as depression, blindness, and recumbency. Diagnosis of this condition can be based on a history of previous *S. equi* infection, evidence of severe suppurative inflammation in the CSF, and culture of *S. equi* from the CSF. Treatment consists of penicillin. The prognosis is guarded; however, successful surgical drainage of a cerebral *S. equi* abscess has been reported.[29]

Focal involvement of the cerebellum has been associated with aberrant parasite migration in the horse.[4,30] In a 6-year-old pony, infection with *Halicephalobus deletrix* resulted in severe ataxia.[30] Histologic study showed lesions scattered throughout the cerebellum, brainstem, thalamus, and pituitary gland, and nematodes were observed throughout the lesions. A second case involving a 1-year-old Thoroughbred colt had a sudden onset of severe ataxia.[4] Multifocal malacia with numerous eosinophils was observed throughout the cerebellar white matter. No nematode was detected, although parasitic involvement was suspected based on the eosinophilic inflammation.

10.7.3.5

MISCELLANEOUS CONDITIONS

A familial neurologic condition in newborn Thoroughbred foals has been reported.[31] This syndrome affected three of five foals of a Thoroughbred mare. The foals were normal at birth and developed signs of severe incoordination, a wide-based stance, and recumbency at 2 to 5 days of age. The condition appeared more severe when the foals became excited or struggled; consequently, they were treated symptomatically with diazepam. The signs would improve with this treatment and return as the sedation wore off. These foals improved with stall rest over 7 to 10 days. The cause of the clinical signs in these foals is unknown; however, the authors suggested possible viral or toxic causes.

Cerebellar ataxia in two Thoroughbred fillies has been associated with hematoma in the fourth ventricle.[32] These two horses demonstrated fever, dysmetria, spasticity, and weakness. Clinical signs most likely resulted from compression of the adjacent cerebellum. CSF analyses in these cases revealed xanthochromia, elevated red and white blood cell counts, and elevated total protein concentrations. The cause of the hematomas was not identified; damage to regional small vessels and a vascular anomaly were suspected.

Chronic methylmercurial poisoning in horses can cause a number of clinical abnormalities, including cerebellar ataxia.[33] Severe poisoning can result in incoordination, dysmetria, and gross head nodding in the experimental setting. Associated clinical signs include lethargy, anorexia, exudative dermatitis, and laminitis. Lesions in the cerebellum consisted of focal atrophy and cellular depletion in the granular layer with little to no involvement of Purkinje cells. Additional abnormalities included neuronal necrosis and gliosis in the cerebrum, lymphocytic perivascular cuffing, and swollen axons in the spinal cord. Preferential accumulation of inorganic mercury in the brain and resulting cell injury most likely led to the neurologic signs observed. Diagnosis of methylmercurial poisoning can be based on clinical signs and measurement of mercury in the liver and kidney (see Chapter 20).

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10.8

10.8—Cervical Vertebral Stenotic Myelopathy

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Cervical stenotic myelopathy (CSM) is a developmental disease of the cervical vertebrae characterized by stenosis of the cervical vertebral canal resulting in intermittent or continuous compression of the spinal cord.[1–3] Clinically the disease is characterized by weakness, ataxia, and spasticity of all limbs. Two general manifestations of this syndrome are cervical vertebral instability (CVI) and cervical static stenosis (CSS).[1,2] CVI is a dynamic condition in which spinal cord compression is intermittent and occurs when the cervical vertebrae are in the ventroflexed position.[4,5] The intervertebral sites most commonly involved in horses with CVI are C3 to C4 and C4 to C5.[6,7] In horses with CSS, spinal cord compression is continuous regardless of cervical position and occurs predominantly in the caudal cervical region: C5 to C6 and C6 to C7.[2,7] 594

CSM occurs most frequently in rapidly growing, young male horses.[4,8–10] Although CSM has been reported in most light and Draft breeds and in both sexes, it occurs more commonly in Thoroughbreds and Quarter Horses.[2,4,5,9] Early reports of CSM suggested that cranial and middle cervical lesions occurred in young horses (6 to 30 months) and caudal lesions in older horses (>5 years).[5] Subsequent studies, however, have not supported this observation. A retrospective study of 306 myelograms involving mostly Thoroughbreds reported 1- to 2-year-old horses to be affected most frequently and showed C3 to C4 to be the most commonly affected intervertebral site. In 594
most instances, no difference in site distribution of lesions exists between horses less than or equal to 2 years of age 595
and those greater than 2 years of age.[7]

10.8.1 Clinical Signs

Cervical vertebral stenotic myelopathy is characterized by symmetric ataxia, paresis, and spasticity, which are usually worse in the pelvic limbs than thoracic limbs.[4,9,10] At rest, affected horses often assume a wide-based stance and demonstrate delayed responses to proprioceptive positioning. Stumbling, toe dragging, circumduction of the hindlimbs, and truncal sway are often observable at a walk. These signs are accentuated by manipulation during neurologic examination using circles, hills, obstacles, and elevation of the head.[4,5,9] The laryngeal adductor response test may be abnormal. Clinical signs of ataxia often progress for a short period of time and then stabilize.[4,5] A history of a traumatic incident often is associated with the onset of CSM; however, ataxia was likely present before the traumatic incident. In many cases the fall results from mild neurologic deficits, and the traumatic incident exacerbates the stenosis leading to trauma of the spinal cord and resultant clinical signs of spinal cord compression.

Infrequently, evidence exists of gray matter and spinal nerve root damage such as cervical pain, atrophy of the cervical musculature, or cutaneous hypalgesia adjacent to the affected cervical vertebrae.[8,9,11] These signs are observed more commonly in horses over 4 years of age with significant arthropathy of the caudal cervical vertebrae (C5 to C7).

Clinical signs of concurrent developmental orthopedic disease of the appendicular skeleton, such as physeal enlargement of the long bones, joint effusion following osteochondrosis, and flexural limb deformities, are often present in young horses with CSM.[4,5,12] In addition, affected horses may have another concurrent neurologic disease such as equine protozoal encephalomyelitis.

10.8.2 Pathogenesis and Pathologic Findings

One of the most consistent pathologic changes observed in horses with CSM is narrowing of the vertebral canal as evidenced by decreased minimum sagittal diameter (MSD).[8,11,13,14] One can determine the MSD for each cervical vertebra by taking the smallest sagittal diameter of the vertebral canal via radiographic or postmortem examination.[8] Horses affected with CSM have a narrowed MSD at C3 to C6, regardless of the site of spinal cord compression.[8,11,13,14] In cases of CSS, narrowing of the canal diameter is exacerbated by thickening of the dorsal lamina, enlargement of the ligamentum flavum, and degenerative articular processes with thickened joint capsules.[2,12] Abnormalities of the dorsal lamina and ligamentum flavum are not observed in young Thoroughbreds affected by CSM[13] and appear to be more common in the older horses affected with CSM.[2] In some cases of CSS, flexion of the neck stretches the thickened ligamentum flavum and relieves spinal cord compression, whereas hyperextension exacerbates compression.[6,8] Pathologic changes of the cervical vertebrae most commonly observed in horses with CVI are instability and subluxation of adjacent vertebrae, malformation of the caudal vertebral physis or epiphysis (caudal epiphyseal flare), and malformation or malarticulation of the articular processes.[1,8] Histopathologic examination of the spinal cord reveals wallerian degeneration, malacia, and fibrosis at the sites of spinal cord compression and demyelination in the ascending and descending white matter tracts within adjacent segments of the spinal cord proximal and distal to the site of spinal cord compression.[8,9]

Many studies have identified an association between CSM and developmental orthopedic disease in horses, although no causal relationship has been established.[1,2,12,13,27] Osteochondrotic lesions are more severe in the articular processes of the cervical vertebrae and are more frequent and severe in the appendicular skeleton in horses affected with CSM than in clinically normal horses.[13] Instability between adjacent vertebrae, caused by osteochondrosis and subsequent degenerative joint disease of the articular processes, may lead to generation of excessive or inappropriate biomechanical forces on the ligamentum flavum, joint capsule, and dorsal lamina.[2] Thickening and fibrosis of these structures contribute to narrowing of the vertebral canal in horses with CSS. Osteochondrosis of the articular processes is not always present at the site of spinal cord compression in horses with CVI.[8,13] Physeal osteochondrosis may produce caudal epiphyseal malformation and vertebral instability in cases of spinal cord compression in which osteochondrosis of the articular processes is not present. Conversely, CSM and osteochondrosis may be two separate manifestations of developmental orthopedic disease characterized by an underlying inability to form normal cartilage and bone, and the occurrence of CSM may result from generalized failure of vertebral canal development rather than from osteochondrosis.[13] Nonetheless, the association between osteochondrosis and CSM indicates that the cause and pathophysiology of these two conditions are similar.

The cause of CSM appears to be multifactorial, involving genetic and environmental influences. No evidence indicates that CSM is directly heritable by simple mendelian dominant or recessive patterns.[15] Rather the mode of inheritance may involve multiple alleles and variable penetrance determining a genetic predisposition to CSM. Nutrition, rapid growth, trauma, and abnormal biomechanical forces likely contribute to the development of CSM in genetically predisposed individuals.[1,16–19] 595

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Dietary factors investigated in the pathogenesis of osteochondrosis and CSM include dietary copper, zinc, protein, and carbohydrates. Low dietary copper and high zinc concentrations produce severe osteochondrotic lesions in foals.[20,21] Additionally, diets containing 15 ppm copper produce 3 times as many osteochondrotic

lesions of the appendicular and axial skeleton compared with diets containing 55 ppm copper.[16–18,22–24] Correction of trace mineral balance including zinc, calcium, and phosphorus, along with copper supplementation, decreases the incidence of developmental orthopedic disease in foals.[16,17,22] Copper supplementation does not eliminate developmental orthopedic disease completely, suggesting other etiologic factors must exist. Excessive carbohydrates in the diet are hypothesized to contribute to the pathogenesis of osteochondrosis through endocrine imbalance.[19,25–28] A high-carbohydrate meal (130% of the National Research Council [NRC] recommendation) results in postprandial elevation of serum insulin concentration and depression of serum thyroxine concentration.[25] Insulin stimulates the zone of chondrocyte proliferation, and thyroxine stimulates the zone of chondrocyte maturation at the growth plate.[27,28] High insulin and low thyroxine concentrations are suspected to promote cartilage proliferation and retention without promoting maturation. Cartilage retention and failure of maturation are consistent with the histopathologic appearance of osteochondrosis. Endocrine alterations associated with a high-carbohydrate diet are the basis for the “paced diet” program for prevention and treatment of CSM.[29,30] A correlation between dietary protein concentrations and the incidence of osteochondrosis and CSM has not been identified.[16–18]

10.8.3 Diagnosis

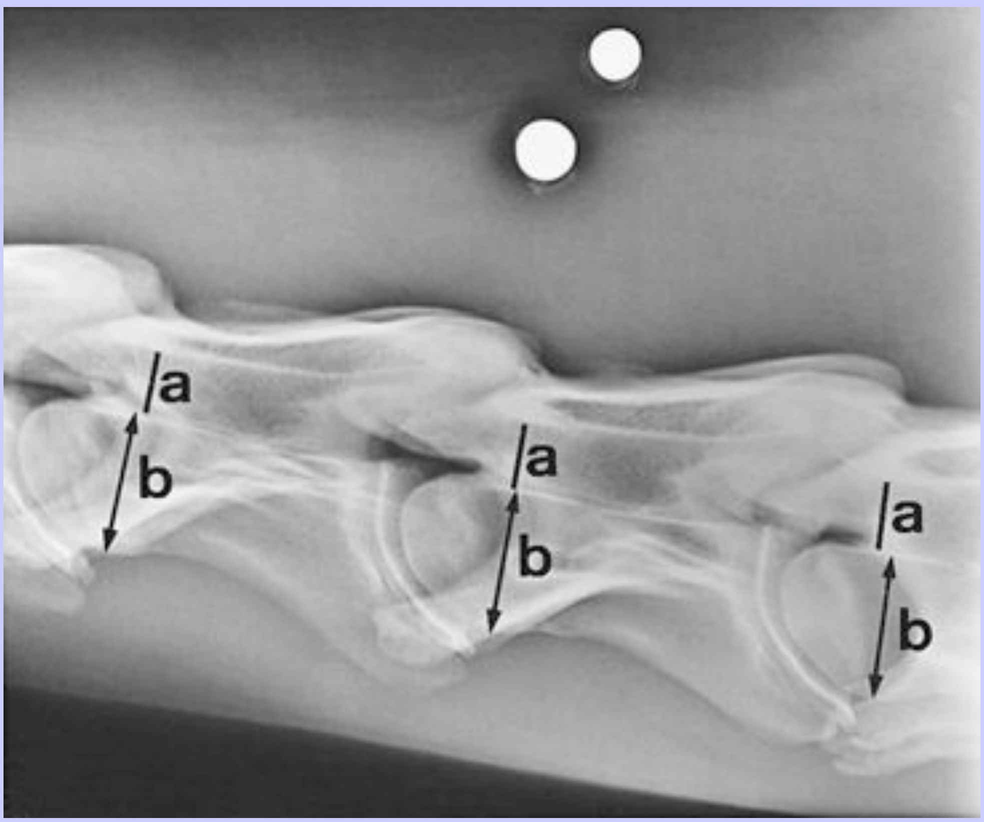
The differential diagnosis of CSM includes neurologic diseases that produce tetraparesis and incoordination, such as equine herpesvirus type 1 myeloencephalitis, equine protozoal myeloencephalitis, equine degenerative myeloencephalopathy, occipitoatlantoaxial malformation, spinal cord trauma, vertebral fracture or luxation, vertebral abscessation, and verminous myelitis.[4] Neurologic examination, cerebrospinal fluid analysis, and radiographic procedures are indicated in horses with symmetric tetraparesis and ataxia.[31]

One should examine lateral radiographic views of the cervical vertebrae to obtain objective determination of vertebral canal diameter and subjective assessment of vertebral malformation. Characteristic malformations of the cervical vertebrae in horses with CSM include flare of the caudal epiphysis of the vertebral body, abnormal ossification patterns of the articular processes, subluxation between adjacent vertebrae, extension of the dorsal laminae, and degenerative joint disease of the articular processes.[29,30] Degenerative joint disease of the articular processes is the most frequent and severe malformation observed in horses with CSM.[14] However, degenerative joint disease of the articular processes occurs in 10% to 50% of nonataxic horses,[8,14,32] and subjective evaluation of degenerative joint disease of the articular processes in the caudal cervical spine results in the false-positive diagnosis of CSM.[7] Although the presence of characteristic vertebral malformations supports the diagnosis of CSM, subjective evaluation of bony malformation from radiographs of the cervical vertebrae does not reliably discriminate between horses affected and unaffected by CSM.[7,14]

Objective assessment of the vertebral canal diameter is more accurate than subjective evaluation of bony malformation for identification of horses with CSM. The most reliable objective determination of vertebral canal diameter from standing radiographs of the cervical vertebrae is the sagittal ratio technique. One calculates the sagittal ratio by dividing the MSD by the width of the vertebral body.[14] One measures the vertebral body width perpendicular to the vertebral canal at the widest point of the cranial aspect of the vertebral body (Figure 10.8-1). This technique eliminates variability in absolute MSD values because of magnification. The vertebral body is located within the same anatomic plane as the vertebral canal; therefore the proportion of these two objects will be the same regardless of the degree of radiographic magnification.[33] The sagittal ratio technique has a sensitivity and specificity equal to or greater than 89% for vertebral sites C4 to C7 and should be used to 596 597

determine the likelihood of the presence of CSM in an individual with spinal ataxia.[14] If the sagittal ratio at a particular vertebral site is below the reference value for that site, one should perform myelographic examination to confirm the diagnosis of CSM. If the sagittal ratio is greater than the reference value, one should avoid or postpone myelographic examination until after performing diagnostic tests focused on alternative causes of spinal ataxia. The reference values for sagittal ratio measurements of the cervical vertebrae are 52% at C4 to C6 and 56% at C7. Accurate measurement of the sagittal ratio value requires a precise, lateral radiograph of the cervical vertebrae. Obliquity of the cervical vertebrae results in indistinct margins of the ventral aspect of the vertebral canal, producing erroneous values for MSD and vertebral body width.

Figure 10.8-1 Lateral radiographic view of the fourth, fifth, and sixth cervical vertebrae. One determines the sagittal ratio at each vertebral site by dividing the minimum sagittal diameter *(a)* by the width of the cranial aspect of the vertebral body *(b)* at its widest point.



A semiquantitative scoring system for assessing cervical radiographs has been developed to identify CSM in Thoroughbred foals up to 1 year of age.[29,30,34] The semiquantitative scoring system incorporates objective determination of vertebral canal stenosis and subjective evaluation of vertebral malformation. The maximum total score is 35 points, and a total score of 12 or higher constitutes a radiographic diagnosis of CSM. One assesses stenosis of the vertebral canal by determining the inter- and intravertebral MSD; the maximum score

designated for vertebral canal stenosis is 10 points. One corrects the inter- and intravertebral MSDs for radiographic magnification by dividing the MSD by the length of the vertebral body. One then determines malformation of the cervical vertebrae by subjective assessment of each of the following five categories: (1) enlargement of the caudal physis of the vertebral body, (2) caudal extension of the dorsal lamina, (3) angulation between adjacent vertebrae, (4) delayed ossification of bone, and (5) degenerative joint disease of the articular process. The maximum score allotted for each category of bony malformation is 5 points. This semiquantitative scoring system is a noninvasive method for predicting and diagnosing the presence of CSM in Thoroughbred foals up to 1 year of age.[34]

Myelographic examination is required for definitive diagnosis of CSM.[6,35] Use of the sagittal ratio method for determining specific sites of spinal cord compression overestimates the number of sites of spinal cord compression within the CSM-affected population.[8,11,13] In addition, subjective evaluation of bony malformation results in inaccurate diagnosis of the sites of spinal cord compression. The severity of vertebral abnormalities does not necessarily correspond to the site of spinal cord compression, and spinal cord compression can occur from stenosis of the vertebral canal at vertebral sites that lack bony malformation.[7,8,13] Therefore radiographs of the cervical vertebrae cannot replace myelographic examination for identification of the location, number of affected sites, and classification of spinal cord compressive lesions in horses with CSM.[31]

Definitive diagnosis of CSM is determined by 50% or greater decrease in the sagittal diameter of the dorsal and ventral dye columns at diametrically opposed sites.[6,35] This decrease is quantified by comparing it with the midvertebral site cranial or caudal to the compressed site. The ventral column often is obliterated totally at the intervertebral sites in normal studies, particularly in the flexed position; therefore a 50% or greater decrease in opposing dorsal and ventral columns must be present for diagnosis of CSM. In horses with obvious sites of spinal cord compression identified on myelographic views with the cervical vertebrae in the neutral position, one should avoid excessive manipulation of the neck so as to obtain flexed and extended myelographic views to prevent exacerbation of spinal cord injury.

Although myelographic examination has been shown to be a safe procedure,[6,7] it is not without risk. The contrast agent metrizamide may produce depression, increased ataxia, fever, seizures, meningitis, and muscle fasciculations.[36–38] Nonionic, water-soluble contrast agents such as iopamidol and iohexol provide superior radiographic contrast with fewer adverse effects than metrizamide.[35,39] Regardless of the contrast agent used, one should reserve myelographic examination for those cases in which the risk and the cost are justified. Indications for myelographic examination include confirmation of a definitive diagnosis of CSM at the owner's request, insurance purposes, before euthanasia, and identification of the exact site(s) of spinal cord compression before surgical intervention.

Analysis of cerebrospinal fluid shows the fluid often is within reference ranges in horses with CSM.[4,9,31] In instances in which cerebrospinal fluid is abnormal in horses with CSM, the alterations are consistent with acute spinal cord compression, such as mild xanthochromia or mild increases in protein concentrations.[9]

10.8.4 Treatment

Surgical intervention is the most widely reported treatment for CSM.[40–46] Stall rest, glucocorticoids, dimethyl sulfoxide, and other antiinflammatory drugs may provide transient improvement in clinical signs, but effective treatment must be directed at preventing further spinal cord injury.[5] The goals of surgical intervention in horses with CSM are stabilization of the cervical vertebrae and decompression of the spinal cord. Cervical vertebral

interbody fusion provides intervertebral stability for horses with dynamic spinal cord compression (CVI). Affected cervical vertebrae are fused in the extended position to provide immediate relief of spinal cord compression and prevent repetitive spinal cord trauma.[41,42,44,45]

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One achieves immediate decompression of the spinal cord in cases of CSS by subtotal dorsal laminectomy in which one removes portions of the dorsal lamina, ligamentum flavum, and joint capsule overlying the compressed site.[5,43,47,48] This procedure effectively decompresses the spinal cord but is associated with significant postoperative risk.[42] Interbody fusion of caudal cervical vertebrae in horses with CSS produces remodeling and atrophy of the articular processes, resulting in delayed decompression of the spinal cord over a period of weeks to months.[40,41] Decompression is immediate with dorsal laminectomy; however, because of its safety, interbody fusion is selected by some surgeons as the technique of choice for CSS and CVI.[42]

Cervical vertebral interbody fusion results in improvement in neurologic status in 44% to 90% of horses with CVI and CSS, and 12% to 62% of horses return to athletic function.[5,41,42,44,46] Subtotal dorsal laminectomy results in improvement in neurologic status in 40% to 75% of horses with CSS.[42,43] Subtotal dorsal laminectomy and cervical vertebral interbody fusion for CSS of the caudal cervical vertebrae are associated with fatal postoperative complications, including vertebral body fracture, spinal cord edema, and implant failure. The most important patient factor for determination of postoperative prognosis is duration of clinical signs before surgical intervention; horses with clinical signs for less than 1 month before surgery are more likely to return to athletic function than are horses with clinical signs of greater than 3 months' duration.[42] The number of spinal cord compressive sites and patient age do not affect the long-term surgical outcome of cervical vertebral interbody fusion.

The duration of convalescence and rehabilitation following cervical vertebral interbody fusion is approximately 6 to 12 months.[41,42] One should design an individualized exercise program—dependent on capability, projected use, and neurologic status of the horse—for promotion of muscular strength. Extended exercise at slow speed, including ponying and lunging on inclines, is recommended during the rehabilitation process. One should determine the point at which the horse is competent to return to athletic function following interbody fusion by neurologic examination. Significant improvement in neurologic status likely will not occur beyond the 1-year postoperative period.

Successful conservative management of CSM has been achieved using the paced diet program in foals less than 1 year of age. The goal of this dietary program is to retard bone growth, enhance bone metabolism, and allow the vertebral canal diameter to enlarge to relieve compression of the spinal cord.[29,30] This dietary program is restricted in protein and energy (65% to 75% of NRC recommendations) but maintains balanced vitamin and mineral intake (at least 100% of NRC recommendations). Vitamins A and E are provided at 3 times the NRC recommendations and selenium is supplemented to 0.3 ppm. Roughage is provided by low-quality (6% to 9% crude protein) timothy hay. Individual dietary plans are specially formulated according to the age and weight of the foal. Solitary stall confinement is recommended to reduce the risk of spinal cord compression caused by dynamic instability. This program of dietary management and restricted exercise has been successful in preventing the development of neurologic signs in foals with radiographic evidence of CSM and in treating foals demonstrating clinical signs of CSM.

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10.9

10.9—Equine Degenerative Myeloencephalopathy

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Equine degenerative myeloencephalopathy (EDM) is a noncompressive, diffuse, symmetric, degenerative neurologic disease characterized by ataxia, weakness, and spasticity (hypometria) in young horses of many breeds and both sexes.[1–5] Similar if not identical syndromes have been observed in Mongolian wild horses *(Equus przewalskii)*[6] and Grant's zebras.[2,7] Neuroaxonal dystrophy (NAD) can be considered a specific form of EDM in Morgan horses. NAD is clinically indistinguishable from EDM; however, histologic lesions are confined to the cuneate and gracilis nuclei of the caudal myeloencephalon, whereas in horses with EDM, lesions are found throughout the spinal cord and brainstem.[8,9]

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EDM has been diagnosed in horses in Europe and North America and is reported to occur more frequently in the northeastern United States.[4] The pathogenesis of the disease is unknown but likely is caused by many factors. Currently, a dietary vitamin E deficiency in genetically predisposed animals is thought to be the most important.[10,11] A familial tendency to develop EDM has been observed in the Arabian,[2] Thoroughbred,[10] Paso Fino,[10] Appaloosa,[12,13] and Standardbred[10] breeds and in Grant's zebras.[10] NAD in Morgan horses also appears to have a familial occurrence.[14] Although a familial occurrence suggests a genetic cause, this is so far unproven.

10.9.1 Clinical Signs

The age of onset of clinical signs varies from less than 1 month to several years. Most horses manifest signs at less than 6 months of age with the mean age of onset being 0.4 year.[1,3] However, in one study of 128 horses with EDM, age of onset ranged from 1 month to 20 years, with 16% of the horses showing signs at greater than 28 months of age.[14]

Onset of signs may be abrupt or insidious.[1,2,4] Signs are referable to upper motor neuron and general proprioceptive deficits and include symmetric ataxia, weakness, and spasticity of all limbs, often being worse in the pelvic limbs.[1–5] Signs may begin in the pelvic limbs and progress to the thoracic limbs. The gait is characterized by dysmetria and stabbing of the ground with the limbs. Hindlimb interference and dragging or scuffing of the toes often are present. When backed, the horse may resist or rock back on the pelvic limbs and sit like a dog.[2,4] Postural placing reactions may show conscious proprioceptive deficits. When circled, affected horses often pivot on the inside hindlimb and circumduct the outside limb.[4] Affected horses may have trouble stopping, and for them to fall while running in the pasture or being worked is not unusual.[3,4] Cranial nerve involvement, muscle atrophy, or changes in skin sensation or tail tone are absent in EDM.[1,2] One may find lower motor neuron signs such as hyporeflexia over the neck and trunk with diminished to absent cervical, cervicofacial, cutaneous trunci, and laryngeal adductor reflexes, especially in severe and long-standing cases.[15]

NAD in Morgan horses has similar clinical signs, but even in severely affected horses, only pelvic limb deficits may be apparent. Pelvic limb dysmetria, asynchrony, and ataxia are generally less severe than in EDM.[13]

10.9.2 Pathologic Findings

Gross necropsy findings in EDM are unremarkable. Classic histologic changes are evident in the caudal brainstem nuclei (medulla oblongata) and especially the spinal cord and include diffuse neuronal fiber degeneration (dystrophy) of the white matter. Generally, EDM affects the lateral and medial cuneate nuclei, gracile nucleus, lateral cervical and thoracic nuclei, and lumbosacral and cervical intermediate gray columns. Astrocytosis, astrogliosis, vacuolization, myelin loss, spheroid formation (axonal swelling), and lipofuscin-like pigment accumulation are present in these areas. With chronicity the dorsal and ventral spinocerebellar tracts and the medial part of the ventral funiculi of the thoracic segments are affected more severely.[1,2] Neurochemical studies of the spinal cord show a significant loss of myelin and component lipids. Demyelination occurs to a greater extent than axon loss.[1]

Histologic changes in NAD of Morgan horses lack the diffuse nature of the changes in EDM. In NAD, histologic changes may be confined to the accessory cuneate nuclei.[14]

10.9.3 Pathophysiology

The pathogenesis of EDM is unknown. However, EDM most likely is caused by a complex interaction of many factors. Degenerative myelopathy in other species, which bears clinical and histopathologic similarities to EDM, has been linked to vitamin E and copper deficiencies, hereditary factors, and toxic insults.[9] Three risk factors associated with the development of EDM were identified in a study involving 56 affected and 179 control horses: (1) use of insecticides, (2) exposure to wood preservatives, and (3) spending frequent time on a dirt lot.[16]

Spending time on green pastures was found to be a protective factor. Additionally, a foal was 25 times more likely to develop EDM if its dam had any other foals diagnosed with EDM.[15] These factors alone probably do not cause EDM but may interact with other factors to produce disease. One also should consider these factors to prevent or minimize the risk of horses developing EDM.

A hypothesis for the pathophysiology of EDM is exposure of genetically predisposed young foals to environmental oxidants and lack of antioxidants, including vitamin E.[11] Oxidative stress is caused by the imbalance between production of prooxidants and the antioxidant defenses. Reactive oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radical are formed during the reduction of oxygen to water in normal cellular metabolism (Figure 10.9-1, *A*). Aerobic cells have antioxidant defense mechanisms that protect them from oxidative stress (Figure 10.9-1, *B*). The high consumption of oxygen in the brain, high metabolic activity, and high concentration of polyunsaturated fatty acids, which can easily be oxidized to reactive oxygen species, makes the central nervous system vulnerable to oxidative attack by reactive oxygen 600
species.[9,17,18] Another source of reactive oxygen species is through the metabolism of excitatory amino acids 601
and neurotransmitters such as glutamate and aspartate. When present in excess, excitatory amino acids can trigger a series of events including an increase in intracellular calcium, which can lead to the production of free radicals and subsequent neuronal damage and death. Other sources of free radicals that arise from brain metabolism include cytochrome P-450 electron transport, monoamine oxidase activity, and endogenous guanidine compounds. Lipid peroxidation of cellular membranes and the direct oxidation of amino acids leading to inactivation of enzymes, receptors, and structural proteins are the main consequences of oxidative injury (Figure 10.9-2, *A*). More evidence indicates a role for redox signaling by oxygen radicals that targets mitochondrial cytochrome c release, DNA repair enzymes, and transcriptional factor NF-κβ. Neuronal damage occurs once these physiologic systems are disrupted.[19]

Figure 10.9-1 **A**, Physiologic metabolism of molecular oxygen to water. **B,** The mechanisms of action of four antioxidant systems. Superoxide dismutase may act as a prooxidant by increasing the formation of hydrogen peroxide and as an antioxidant by decreasing the superoxide radical concentration. Cofactors for superoxide dismutase are iron, zinc, and copper. The cofactor for glutathione peroxidase is selenium.

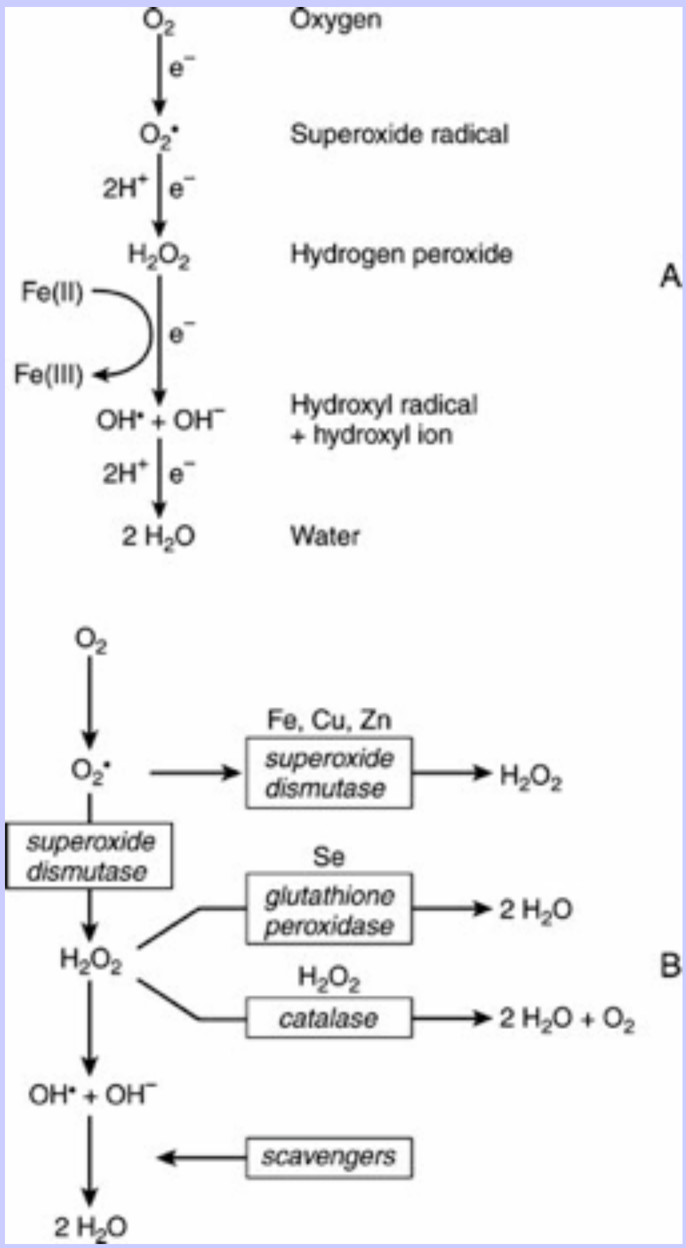
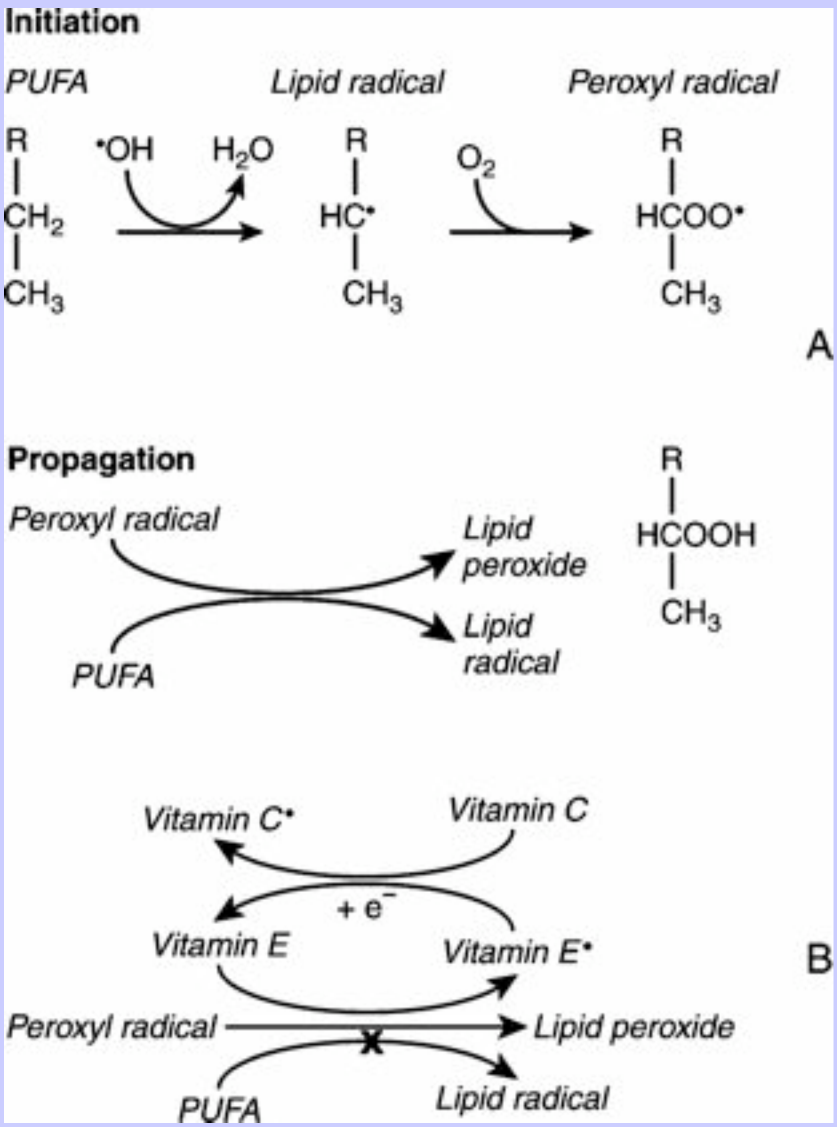


Figure 10.9-2 **A,** Mechanism of peroxidation of polyunsaturated fatty acids in cell membranes. **B,** Antioxidant mechanisms of α-tocopherol (vitamin E) and ascorbic acid (vitamin C). Polyunsaturated fatty acids are spared from oxidation because vitamin E is oxidized to a free radical instead. This prevents the propagation of lipid peroxidation in cell membranes and is referred to as the chain-breaking action of vitamin E. Vitamin C is a reducing agent that donates electrons to free radicals.



The main endogenous antioxidants are superoxide dismutase, catalase, glutathione peroxidase (that contains selenium), α-tocopherol (vitamin E), and ascorbic acid (vitamin C) (see Figures 10.9-1, *B*, and 10.9-2, *B*).[17,18] Another protective mechanism is the existence of iron-binding proteins that keep iron in a less reactive form and prevent iron from catalyzing free radical reactions. Vitamin E reacts directly with OH^- and prevents oxidant injury to polyunsaturated fatty acids and thiol-rich proteins in cellular membranes (see Figure 10.9-2, *B*).

Vitamin E deficiency has received much attention as a possible cause of EDM. This association is based on the presence of degenerative myelopathy in rats,[20,21] monkeys,[22] human beings,[23–25] and captive Przewalski's horses[6] with low serum vitamin E concentrations, the reduced incidence of EDM seen after prophylactic 601
treatment with vitamine E,[10] and the response to treatment with vitamin E in affected horses.[13] One study 602
reported a high incidence of EDM on two breeding farms in which low serum vitamin E concentrations were found. Vitamin E supplementation decreased the incidence from 40% to less than 10%. However, affected and unaffected horses on both farms were found to be vitamin E–deficient.[10] Another study found no significant differences in serum vitamin E and blood glutathione peroxidase concentrations between EDM affected horses and control horses.[26] A third study documented significantly lower vitamin E concentrations and clinical signs compatible with EDM in eight of nine foals sired by a stallion with EDM. Age-matched control foals raised in the same environment had normal serum vitamin E concentrations and no signs of EDM. Oral vitamin E absorption tests were performed on both groups, and no significant differences were found between the groups. Thus an inability to absorb vitamin E from the gastrointestinal tract does not appear to be a factor in the low serum vitamin E concentrations in horses with EDM.[27] Two other trials involving three[11] and nine[28] horses, respectively, support a role for vitamin E in the pathogenesis of EDM. Three foals developed neurologic disease and had low serum vitamin E concentrations after being housed on a dry lot. Two were examined post mortem at 6 months of age and had prominent NAD. The third foal was put onto grass pasture and recovered.[11] Nine horses with a presumptive diagnosis of EDM improved following treatment with vitamin E at 6000 IU/day.[28] The role of vitamin E or glutathione peroxidase in the development of EDM remains unclear.

Similar histologic changes and clinical signs have been observed in copper-deficient sheep,[29,30] goats,[31] guinea pigs,[32] young pigs,[33] calves,[34] and rats.[35] In one study plasma and liver copper concentrations were measured in 25 horses with histologically confirmed EDM and compared with values in 35 normal, age-matched control horses. This study found no significant differences in plasma and liver copper concentrations between the two groups.[36] Thus the role of copper in EDM remains unclear.

One also must consider hereditary factors in the pathogenesis of EDM and NAD in horses, because the diseases have been observed in familial clusters.[13] Heterofamilial NAD has been shown to occur in kittens[1,37] and Suffolk sheep[1,38] and is inherited as an autosomal recessive trait. However, the mode of inheritance has not been identified in horses affected by EDM or NAD.

Toxic compounds such as organophosphates,[1,39,40] diethyl-dithiocarbamate,[1,41] and cycad palm[42] have been reported to cause histologic changes and clinical signs similar to NAD in horses of several species. Their effect is caused by the neurotoxic properties of the compounds. No direct association of toxic compounds with EDM or NAD in horses has been shown.

10.9.4

Diagnosis

One can make a definitive diagnosis of EDM only with histopathologic examination of the spinal cord and brainstem. Antemortem diagnosis is based on clinical signs and ruling out other neurologic diseases (especially cervical vertebral malformation and equine protozoal myeloencephalitis). In EDM, cerebrospinal fluid analysis and cervical spinal radiographs are usually within normal limits, although increased cerebrospinal fluid creatine phosphokinase levels have been found in horses affected with EDM.[1] Serum vitamin E levels of less than 1 mg/ml in horses showing clinical signs of EDM may help support a diagnosis.[3]

The normal reference range for serum vitamin E concentration varies but is usually greater than 1.5 mg/ml.[3] Measuring of serum vitamin E concentrations may be unreliable when the animal is examined after the critical deficient period. Moreover, one should note a single serum vitamin E sample may not reflect the true vitamin E status of the horse adequately, for up to 12% variation in concentrations can occur normally.[43]

A diminished thoracolaryngeal reflex or slap test may raise the suspicion of cervical spinal cord disease by 50% to 70%; however, this test is not accurate, and negative results do not exclude the possibility of cervical spinal cord disease.[44]

10.9.5

Treatment and Prevention

No specific treatment for EDM in horses exists. Affected horses may benefit from oral vitamin E supplementation coupled with monitoring of serum vitamin E concentrations. The earlier vitamin E treatment is instituted, the better the chance for a response. Horses with clinical signs of EDM may benefit from large doses of vitamin E (6000 to 10,000 IU/day) for an extended time.[45] Horses with low serum vitamin E concentration also may benefit from 1000 (foals and yearlings) to 2000 (adults) IU of vitamin E in oil given intramuscularly every 10 days. One should maintain dietary vitamin E levels at 500 to 1000 IU/kg dry weight.[3] Heat-treated pellets, stored oats, and sun-baked forages have marginal vitamin E concentrations (0 to 5 IU/kg dry weight).[3,10] Horses fed a diet of these should have frequent access to fresh green forage or vitamin E supplementation at 600 to 1800 mg or tocopheryl acetate 1.5 to 4.4 mg/kg/day to meet their reported needs.[46] To date, vitamin E toxicity associated with supplementation has not been reported. Also, farms with a high incidence of EDM may benefit from prophylactic vitamin E supplementation.[10]

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10.9.6

Prognosis

EDM is generally progressive, necessitating euthanasia. Once clinical signs are evident, no improvement or remission occurs. However, signs may plateau.[3,10] Generally, horses with EDM do not progress to a state of recumbency.[1,3] Severely affected horses usually have an earlier age of onset and rapid disease progression. Mildly affected horses usually have a later age of onset, and the disease has a less rapid course.[4]

10.9.7

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10.10 10.10—Equine Protozoal Myeloencephalitis

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10.10.1 History

Equine protozoal myeloencephalitis (EPM) was identified initially as segmental myelitis by J.R. Rooney at the University of Kentucky in 1964.[1] The first cases were recognized among Standardbreds returning to Kentucky from racetracks in the northeastern United States. Subsequent cases have been reported among native horses in most of the United States and in Canada, Panama, and Brazil.[2–5] Several reports of the disease in countries in other than the Western Hemisphere were primarily in horses that originated from the Americas.[6–8] The disease was called *segmental myelitis* because discrete lesions were found distributed randomly throughout the spinal cord. As more cases were examined, the frequent involvement of the brain became apparent, resulting in the name focal myelitis-encephalitis. The lesions of EPM include multifocal areas of necrosis and hemorrhage along with nonsuppurative inflammation of the white and gray matter. Ten years later, a *Toxoplasma*-like protozoan was recognized in histopathologic sections, and the disease gradually became known as equine protozoal myeloencephalitis.[9–11]

Dubey, in a 1976 review of sarcocystosis in domestic animals, was the first to suggest that EPM was caused by a member of the genus *Sarcocystis*.[12] Another 15 years passed before he was able to culture the organism from the spinal cord of an affected horse.[13] He named the organism *Sarcocystis neurona* because it often develops within neurons. Until recently, little information was available regarding the life cycle of the organism, and only asexual stages of the parasite were known. Sporocysts presumed to be *S. falcatula* isolated from opossums were tested by polymerase chain reaction assay using primer sets derived from the 18S small subunit ribosomal gene sequence of *S. neurona*. Sequence analysis of the amplicon demonstrated 99.89% homology with the 18S small subunit ribosomal DNA sequences of *S. neurona*.[14] Recognition of the definitive host led to the erroneous conclusions that the parasite had a bird-opossum life cycle.[14,15] A few years later this error was rectified when the opossum turned out to be the host of at least three *Sarcocystis* spp.[16] Recently, another protozoan parasite *(Neospora caninum/N. hughesi)* was implicated as a cause of EPM in six cases; however, its role likely is limited.[17–22]

10.10.2 Epidemiology

Most of the information known about EPM was gathered from naturally occurring infections. Regional epidemiologic studies of EPM have yielded dissimilar results.[23,24] Although regional differences occur, the first

general information for North America was collected during an EPM workshop convened at the Veterinary Medical Forum of the American College of Veterinary Internal Medicine in 1988.[25] The information was based on 364 histologically confirmed cases from California, Florida, Illinois, Kentucky, New York, Ohio, Oklahoma, Pennsylvania, and Texas and Ontario, Canada. Data were compiled from histologically confirmed cases from animal diagnostic centers in each state or province. The average period reported was 6 years with a range of 4 to 12 years. In descending order of frequency, Thoroughbreds, Standardbreds, and Quarter Horses were affected most often, although many other breeds and ponies were represented. Usually only one animal in a herd or location was affected. The age of affected horses ranged from 2 months to 19 years, but more than 60% were 4 years old or less. No geographic or seasonal predilection could be established.

Preliminary seroprevalence data were collected using immunoblot testing to detect antibodies to *S. neurona*–specific proteins in equine serum samples (Granstrom, unpublished). Initial testing in Kentucky and Ohio 604
detected an average exposure rate greater than 20% among clinically normal horses. Subsequent investigations 605
conducted at the University of Kentucky laboratory on serum samples collected from horses in Ohio, Kentucky, and Pennsylvania have demonstrated exposure rates greater than 40% among clinically normal horses. Exposure rates on individual farms ranged from no exposure to 100%. The seroprevalence from a small study from one county in Pennsylvania was 45% of the horse population (95% confidence interval equals 36.3% to 54.3%) along with an increase in prevalence with age.[26] Another report from Oregon found an overall seroprevalence of 45% among horses with differences in seroprevalence among geographic regions.[27] The seroprevalence ranged from 22% in the eastern arid region of the state to 65% in the coastal region of Oregon. A third study from Ohio reported a prevalence of serum antibodies to *S. neurona* in horses at 53.6%. The study from Ohio demonstrated an increase in prevalence with age of the horse and greater prevalence in southwest Ohio versus northeast Ohio. These differences in prevalence may have been related to climatic differences based on freezing days in various regions of the state.[28] In a more recent study, antibodies to *S. neurona* were found in 33.6% of various equid serum samples submitted to a laboratory in Colorado. As in other seroprevalence studies, the prevalence increased with age; in 1- to 5-year-old horses, prevalence was 26.0% versus 37% in 10-year-old horses. Seroprevalence was lowest during the colder months, as has been determined in other states.[29] Results on clinical samples submitted to the University of Kentucky for EPM testing suggested that horses in the eastern half of the United States are exposed to *S. neurona* at a rate 10% to 15% higher than those in the western half of the country.[30] Recent reports suggest that the prevalence of *S. neurona* antibody in horses in Argentina and Brazil are 35.5% and 35.6%, respectively.[31,32] Little work has been performed regarding the prevalence of antibody to *N. caninum/N. hughesi* in horses. More recent work found a seroprevalence of 23.3% in sera examined from two horse slaughterhouses in the United States and no antibody detection in Argentina and Brazil. Because few horses were sampled, these studies may not reflect the true prevalence of *N. caninum/N. hughesi* antibody in horses.[31–33] These results suggest that exposure to *S. neurona* is common in some areas, but much more work needs to be done regarding *N. caninum/N. hughesi* exposure.

In 1978, the New York State Veterinary College at Cornell University, Ithaca, reported that 25% of all equine neurologic disease accessions were caused by EPM.[24] The number of cases diagnosed at the Livestock Disease Diagnostic Center at the University of Kentucky, Lexington, has been 8% to 9% of all neurologic accessions over the last few years. A recent report from the Ohio State University Veterinary Hospital, Columbus, revealed a 25% incidence of EPM in all horses with spinal ataxia from December 1991 to March 1994 based on Western blot analysis of serum and cerebrospinal fluid (CSF).[34] Determining whether the increase is real or is caused by heightened awareness resulting from the availability of immunoblot testing of serum and CSF is difficult. Horses that actually reach the postmortem room represent a small portion of the total number affected annually. Granstrom and Saville estimated the incidence of EPM based on accessions to the University of Kentucky

diagnostic laboratory was 1% of all horses or less each year.[35] Based on a recent national study conducted by the U.S. Department of Agriculture, the average incidence of EPM was 14 ± 6 cases per 10,000 horses per year. The incidence, based on primary use of the horse on the operation, found that the lowest incidence was in farm/ranch horses (1 ± 1 cases/10,000 horses/year). Incidence increased in pleasure horses to 6 ± 5 cases/10,000 horses/year, with a significant increase in breeding horses (17 ± 12 cases), racing horses (38 ± 16 cases), and competition/show horses (51 ± 39 cases), although the racing horses did not include horses at racetracks.[36] These estimates reflect a similar incidence of the disease as previously reported, if not lower. The incidence of neosporosis in horses is unknown at the present time because no controlled investigations have been performed. A total of six case reports of neosporosis in horses indicate the cause as *N. caninum (N. hughesi)*.[17–22] However, only four reports indicated neurologic signs were found; one was in an aborted fetus and another was related to an intestinal problem.[17–22]

EPM has been reported from Canada, Mexico, Panama, Argentina, and Brazil and from a number of states in the United States.[2,4–6,37,38] EPM also has been reported in England among horses imported from the eastern United States and in an 8-month-old Arabian horse in South Africa that had been imported from the United States approximately 5 months before the onset of signs.[7] The most recent report was a horse from California that developed clinical signs of EPM after 10 months in Hong Kong.[8] These reports suggest that EPM is primarily a disease of the Western Hemisphere.

Several authors have suggested Standardbred horses may have a higher prevalence of disease.[23,39–41] However, other reports suggest that this apparent prevalence may be caused by the environment in which horses were kept rather than breed characteristics.[23,40] In another case series, EPM was reported to be most common in Thoroughbreds; however, this was not a controlled investigation.[25] A more recent controlled investigation of risk factors for the development of EPM found that occupations such as racing and showing were associated with increased risk compared with breeding and pleasure horses.[42] This finding was corroborated by the incidence of 605
EPM reported in the National Animal Health Monitoring System (NAHMS) study.[36] 606

Early reports suggested that young horses had an increased disease risk; at least 60% of the affected horses were 4 years old or younger.[23,25,39] This increased risk in young horses also was found in the controlled epidemiologic study in Ohio.[42] However, the Ohio study also found an increased risk in horses older than 13 years of age.[43]

Historically, EPM has been reported as a sporadic disease with more than one case uncommonly reported on any one farm.[40,44] In contrast, reports of EPM cases from Panama stated that all affected horses were stabled at the same location.[4] A more recent report also described an outbreak on a farm in Kentucky.[45] In Ohio, previous diagnosis of the disease on the farm (>2.5 times higher) suggested an increased risk for EPM. This finding suggests clustering of cases may occur when all the risk factors for EPM are present.[42]

Several other risk factors for development of EPM were reported; for example, an increased risk if opossums were seen on the farm, if woods were present on the farm, or if seasonal effect or occurrence of a health event happened before development of clinical signs of EPM. Compared with the winter the seasonal effect increased the risk of EPM as the temperature increased, with the highest risk in the fall. The risk also decreased if a creek or river was present on the farm and if the feed was kept protected from wildlife access, according to the Ohio study.[42] In concert with the Ohio study, the NAHMS study also found, compared with never seeing an opossum on the premises, an increased risk if opossums were observed on the premises and even higher risk if the

opossums were seen frequently. Additional risk factors included an increased risk with increased numbers of horses, purchased versus home-grown grain, use of wood chips or shavings as bedding, presence of rats and mice on the premises, and increased human population density. A protective effect was apparent when woods were within 5 miles of the premises and where surface water was the primary drinking source. The NAHMS study also found the highest risk for disease was in the fall of the year.[36] The role that management may play in development of clinical EPM is patently obvious.

10.10.3 Cause and Life Cycle

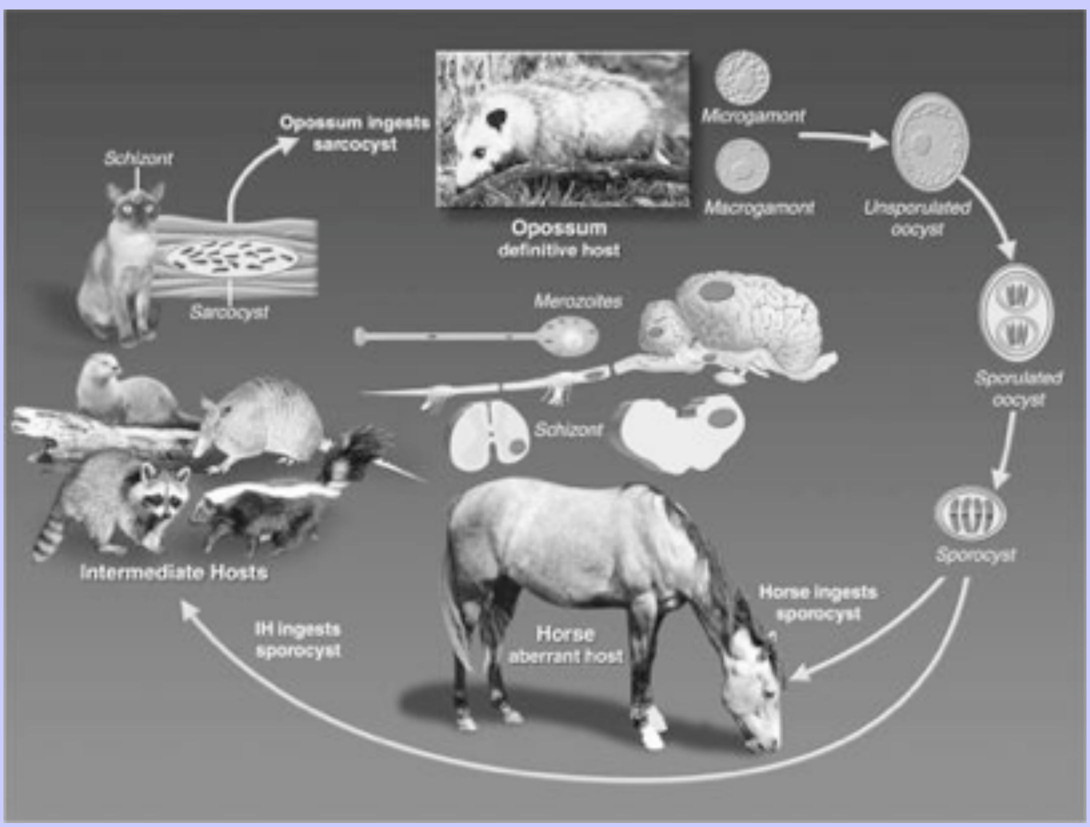
S. neurona has been cultured from CNS lesions of nine horses from several different locations: New York,[13] California (three cases),[46,47] Panama,[4] Kentucky (three cases),[48] and Missouri.[49] Preliminary morphologic, immunologic, and DNA comparisons have detected only minor differences among isolates.[4,48] Cultured organisms multiply asexually by a type of merogony (or schizogony) known as endopolyogeny, whereby many merozoites (or tachyzoites) are formed from a single nucleus.[13] Endopolyogeny contrasts with endodyogeny, the method of merogony that produces merozoites of *Toxoplasma gondii*. As the term implies, each individual *Toxoplasma* merozoite divides to form two new merozoites. The term *meront* (or *schizont*) refers to the small intracellular body of rapidly budding merozoites seen in histopathologic sections and in cell culture. Cultured merozoites contain a conoid, numerous micronemes, and a central nucleus, but no rhoptries.[4] This stage of *Sarcocystis* species is not known to be transmissible to other animals. Cultured merozoites have been injected epidurally, intramuscularly, intravenously, and subcutaneously without producing clinical signs in normal horses (Granstrom, unpublished). A recent study has corroborated that epidural injection of merozoites does not result in clinical signs of EPM.[50] Transplacental infection has not been reported but cannot be ruled out.

Sarcocystis belongs to the phylum Apicomplexa, which includes several genera of coccidia that use an obligatory predator-prey or scavenger-carrion life cycle.[3,51] The host range for an individual species of *Sarcocystis* is usually narrow. *Sarcocystis* species produce sporulated oocysts by sexual reproduction (gametogony) in the gut wall of the appropriate predator or definitive host. However, the oocyst wall is fragile and usually ruptures before being passed in the feces. Infective sporocysts are introduced into the food and water supply of the prey animal or intermediate host by fecal contamination from the predator. Birds and insects may serve as transport hosts to disseminate sporocysts further.[52,53] Once ingested by the intermediate host, sporocysts excyst, releasing four sporozoites that penetrate the gut and enter arterial endothelial cells in various organs. Meronts develop rapidly and eventually rupture the host cell, releasing merozoites into the bloodstream, which usually is followed by a second round of merogony in capillary endothelial cells throughout the body. Second-generation merozoites are released into the bloodstream and usually enter skeletal muscle cells where they develop into specialized meronts known as sarcocysts. Mature sarcocysts contain bradyzoites, which are able to complete the life cycle only when ingested by the appropriate predator or scavenger. *Sarcocystis fayeri* uses this general method to cycle between horses and canids and is not pathogenic in either host.[3] The prevalence of *S. fayeri* in North America has been estimated at approximately 30%, increasing with age.[54]

S. neurona may infect a large number of intermediate hosts aberrantly, unlike most *Sarcocystis* spp. Several species of animals and birds have been reported to exhibit symptoms similar to those in horses with EPM. 606
Reports indicate that an *S. neuron*–like organism infects and causes neurologic disease in dogs, sheep, cats, 607
mink, raccoons, a striped skunk, a golden hawk, pacific harbor seals, sea otters, chickens, and a Grant's zebra.[55–66] This wide host range is atypical for *Sarcocystis* spp, similar to that of *T. gondii,* which is phylogenetically close to *S. neurona*.[3,67]

Sarcocysts of *S. neurona* have not been found in affected horses, precluding transmission of the parasite to the definitive host.[3,54] The horse is an aberrant, dead-end host.[51,54] *S. neurona* cycles between the opossum and various intermediate host species (Figure 10.10-1). Sarcocysts are found in the muscles of infected domestic cats *(Felis domesticus)*, nine-banded armadillos *(Dasypus novemcinctus)*, striped skunks *(Mephitis mephitis)*, raccoons *(Procyon lotor)*, and sea otters *(Enhydra lutris nereis)*.[68–71] At first the domestic cat was thought to be only a laboratory intermediate host; however, a study in Missouri and another in Ohio suggest that the cat is a natural intermediate host as well.[72,73] The same holds true for the striped skunk. The life cycle of *S. neurona* has been completed in a laboratory setting.[70] However, previous reports of serum prevalence of antibodies to *S. neurona* in striped skunks (22 of 37) would suggest they are likely a natural intermediate host as well.[74] Muscle from wild-caught raccoons and road-killed armadillos fed to laboratory-raised opossums resulted in shedding of 607
sporocysts infective for ponies, horses, and IFN-γ/KO mice.[69,71] In addition, high seroprevalence of *S. neurona* 608
antibodies in armadillos (100%) tested from three states and raccoons (58.6%) tested from four states further suggests that these species are natural intermediate hosts.[69,75] Opossums shed sporocysts in their feces following ingestion of the infected muscle. Small numbers of sporocysts were found after feeding sea otter muscle infected with *S. neurona* sarcocysts to opossums; however, the sporocysts were infective for IFN-γ/KO mice. The role of the sea otter as a natural intermediate host likely is limited. This, however, demonstrates that many species may be potential intermediates hosts for this organism.

Figure 10.10-1 Life cycle of *Sarcocystis neurona*. (Photo courtesy Dr. William J.A. Saville, The Ohio State University, Columbus, Ohio.)



The life cycle of *N. caninum* or *N. hughesi* in horses is understood poorly. Recently, the definitive host of *N. caninum* has been demonstrated to be the dog,[76] but whether the dog is the definitive host of *N. hughesi* is not known. Similar to *T. gondii* in cats, the dog serves as the definitive host and one of several known intermediate hosts for *N. caninum*. Tachyzoites, as well as tissue cysts, have been found in other horse tissues in two of the horses reported to have EPM caused by *Neospora,* unlike EPM caused by *S. neurona*.[77] In one case of neosporosis, a foal was determined to have been infected congenitally, whereas congenital infections have not been demonstrated in horses infected with *S. neurona* up to the present time.[20]

The clinical severity of experimental sarcocystosis in appropriate intermediate hosts studied is related directly to the number of sporocysts fed.[78] The ability of any individual to resist infection appears to be related to the size of the infective dose, immunocompetence, environmental stress, and the species of *Sarcocystis*. Some individuals may be inherently more susceptible to infection, which may have variable heritability. Speculation about this possibility seems premature until more is learned about the pathogenesis of clinical infection.

Several studies have induced experimental infection in horses using *S. neurona* sporocysts. The first study was done at the University of Kentucky. Naïve foals were infected orally with 1×10^6 to 4×10^7 sporocysts, resulting in mild to moderate neurologic deficits. In this study, sporocysts were collected from wild-caught opossums, cleaned, and administered via nasogastric intubation. Parasites could not be cultured from the central nervous system tissues; therefore Koch's postulates were not fulfilled.[79] Three studies from the University of Florida were conducted using characterized sporocysts detected using molecular DNA probes.[80] One study administered 1×10^6 *S. neurona* sporocysts once, and another study was performed in which 5×10^5 sporocysts were administered orally once daily for 7 consecutive days; both resulted in mild to moderate neurologic deficits and no parasite was detected.[81,82] Sporocysts characterized as *S. falcatula* were administered to horses and resulted in no development of neurologic signs or seroconversion.[83] These findings corroborated that the opossum excreted more than one *Sarcocystis* spp. of sporocysts. Recently, another study attempted to infect horses using 8×10^4 *S. neurona* sporocysts with three different treatment groups. All nine horses in the study that were infected developed neurologic signs; however, the most severe signs (mild to moderate) were seen in horses in the transport stress group.[84] Unfortunately, parasites could not be cultured from the CNS tissues. All three studies attempted to mimic stress using dexamethasone; however, the clinical signs were less severe, and the clinical signs of the horses appeared to improve. Additionally, in all three studies, regardless of the dose of sporocysts administered, some horses demonstrated an improvement in their clinical signs in the face of no treatment.[79,82,84] The improvement suggests that horses are capable of clearing large numbers of these organisms, which may explain the high number of clinically normal horses with parasite-specific antibody in the CSF. More troublesome is the fact that after orally inoculating horses with 1×10^8 *S. neurona* sporocysts, clinical signs of neurologic deficits were readily detectable, but parasites were not found in the central nervous system at 7 or 14 days after infection (W.J.A. Saville and J.P. Dubey, unpublished observations). This result is unlike the *S. neurona* infection in the natural intermediate host, raccoon, in which the parasite was readily detectable in the CNS at 7 days after infection.[85] The life cycle of *S. neurona* in the horse remains enigmatic.

The organism may be transmitted via methods other than direct contact with opossum feces, based on the estimated numbers of opossums in North America, their poor survival, and limited individual range. Experiments performed by researchers in the 1980s suggest that birds may help disseminate sporocysts.[86] Secondary or vector transmission also was demonstrated by the recovery of sporocysts in the feces of budgerigars, canaries, mice, and chickens fed opossum feces. Recovered sporocysts then were fed to budgerigars to assess viability following

transit through the digestive tract of those species. Four of six budgerigars died, suggesting that sporocysts disseminated in this way may be transmitted to intermediate hosts.[52] Considering the apparent wide range of natural and aberrant intermediate hosts of *S. neurona* indicates that control of the disease may be difficult.

Insects such as flies and cockroaches also may be transport vectors for *S. neurona,* for early work demonstrated that flies may act as transport vectors for *T. gondii*.[87] The same group also found that cockroaches may act as transport vectors for *T. gondii*.[88] More recently, fatal pulmonary disease developed in psittacine birds that were fed cockroaches after the cockroaches had been fed opossum feces.[53] This suggests that insects may play a role in transmission of *S. neurona;* however, further investigation is necessary to determine which insects actually are involved in the life cycle of this organism, if any.

10.10.4 Clinical Signs

The clinical signs associated with *S. neurona* infection can vary. This variability simply reflects the multifocal or 608
diffuse distribution of the lesions that may occur randomly in the gray and white matter of the brain, brainstem, 609
or spinal cord. Usually the physical examination is within normal limits, and the horse appears bright and alert, although one may observe focal muscle atrophy. The onset of clinical signs may be gradual, but more typically mild signs develop acutely and may progress rapidly. The neurologic examination often reveals ataxia and incoordination in all limbs, which sometimes show lateralization, or gait abnormalities may be apparent with only one limb involved. Infection with *S. neurona* can result in brainstem and spinal cord signs and often causes damage to the lower motor neuron of spinal cord or cranial nerves, leading to muscle atrophy. Muscle atrophy is most common in the quadriceps and gluteal regions in the hindlimbs but also may mimic sweeney, radial paralysis, or polyneuritis equi. If the brainstem is involved, atrophy of the temporal-masseter muscles and occasionally the tongue may be evident along with head tilt, facial nerve paralysis, and difficulty swallowing.[89,90] Carefully examining for signs of muscle wasting and loss of sensation along the face, neck, or body is important.

A frequent complaint is obscure lameness that may progress to ataxia, spasticity, and incoordination of the limbs. Poor coordination and weakness worsen with walking with the head elevated or walking up or down a slope. The asymmetry of clinical signs may be an important early indication of EPM. Horses with brainstem involvement often have head tilt, facial paralysis, loss of sensation of the cornea and internal nares and also dysphagia, circling, and acute recumbency.[24,40] Some horses have a tendency to lean toward the side of the lesion and use the wall of the stall to balance themselves. At least three horses have been observed with seizures as the only clinical signs.[91] Visual deficits and behavioral abnormalities have been reported in some horses with EPM.[89] A recent case series reported head shaking in three horses diagnosed with EPM. After treatment for EPM, the head shaking resolved in these horses.[92]

10.10.5 Pathogenesis and Pathology

The pathogenesis of EPM is understood poorly, but one assumes that the horses ingest *S. neurona* and that the course of infection and disease is then similar to that observed in other host species infected with *Sarcocystis* spp. Sporocysts of *S. neurona* are passed in the feces of the opossum and introduced into the feed and water supply of intermediate hosts. On reaching the gastrointestinal tract, sporocysts excyst, releasing eight sporozoites that penetrate the gut and enter arterial endothelial cells in various organs. Meronts develop within host cells, resulting in cell rupture and release of merozoites into the bloodstream. A second round of merogony may follow in vascular endothelial cells throughout the body. In the appropriate intermediate host a final round of merogony

results in the formation of sarcocysts in various muscles. The predator or definitive host subsequently ingests the infected muscle tissue to complete the life cycle. At the present time, sarcocysts of *S. neurona* have not been found in affected horses, indicating that the horse is likely an aberrant, dead-end host.[35]

Many normal foals have been produced by mares suspected of having EPM. Recently, an infected pregnant mare was euthanized and the diagnosis was confirmed histologically. The foal, taken at euthanasia, died of pneumonia at 8 weeks of age without neurologic signs. No lesions were present in the CNS. Although the mare was immunoblot positive in serum and CSF, the foal was immunoblot negative. The earliest EPM case reported occurred in a 2-month-old foal.[25] If transplacental transmission does not occur, the minimum incubation period may be 8 weeks. However, a recent case suggests the incubation period may be much shorter. Serum and CSF collected 4 days after onset of clinical signs were negative for antibodies to *S. neurona*. Serum and CSF collected 3½ weeks later were positive. The results indicate that the parasite was ingested and caused clinical signs in the 10 to 12 days required to produce a detectable antibody response.

S. neurona has been recovered from CNS lesions in several horses and subsequently propagated in culture in the laboratory, which has enabled development of diagnostic tests. Cultured merozoites have not induced clinical disease in the horse when administered to horses parenterally or introduced via the epidural space.[93] The results have been corroborated by others.[50] The merozoite stage of *Sarcocystis* spp. is not known to be transmissible to other animals either[93]; however, nude mice have been inoculated intraperitoneally with cultured merozoites and subsequently have developed evidence of *S. neurona*–associated encephalitis.[94] These mice were immunosuppressed strains, and intraperitoneal injection likely would not be the normal route of infection with *S. neurona* in horses. Recently, another mouse model was developed by feeding sporocysts from feral opossums to IFN-γ/KO mice. Use of IFN-γ/KO mice also help to differentiate *Sarcocystis* spp. that are excreted in opossum feces, for at least three species appear to be present.[95] The mechanism is currently unknown by which the merozoites enter the CNS. The organism is believed to enter the CNS via infected leukocytes or directly through the cytoplasm of endothelial cells.[93]

Affected horses often have a history of recent stress; for example, shipment, heavy training or racing, and foaling. Many affected horses have been moved frequently, making determining where exposure occurred difficult. If the onset of clinical signs is stress-related, the incubation period and seasonal occurrence would vary 609
greatly. Clinical signs are confined to the central CNS and may include seizures and cranial nerve or spinal cord 610
signs. Affected horses may stumble and fall or show weakness, ataxia, and spasticity in one or more limbs.

One hypothesis suggests that stress may play a role in the development of EPM,[3,35] but limited evidence is available to support this hypothesis. The size of the infective dose, immune competency of the host, and the environmental stresses to which the horse is exposed may be related to the severity of EPM.[93] An association between immunosuppression and disease has been documented in other species with EPM-like symptoms; for example, recent mouse models for the disease have been developed using nude mice and IFN-γ/KO mice, both of which are immune-compromised strains.[94,95] Raccoons have been identified that were infected concurrently with a *Sarcocystis* spp. and canine distemper virus.[63,96] Canine distemper virus is known to be immunosuppressive and often has been associated with cerebral toxoplasmosis in dogs, foxes, and raccoons.[96] In a monkey that developed asymmetric neurologic signs similar to those in EPM, *Sarcocystis* spp. infection of the CNS also was identified with a concurrent simian immunodeficiency virus infection.[97] Immune-compromised human beings often are infected with *T. gondii*. Stress has been demonstrated to play a major role in the recrudescence of the clinical signs of *T. gondii*–associated encephalitis.[98] Infections with *N. caninum* or *T. gondii* can cause T cell

hyporesponsiveness to the parasite antigen in laboratory animals. In addition, an intact T cell response, specifically appropriate interleukin-12 and interferon-γ production, has been demonstrated to be necessary for resistance against *N. caninum* or *T. gondii*.[99] *S. neurona* behaves similarly to *N. caninum* and *T. gondii* in its wide range of intermediate hosts. Perhaps *S. neurona* facilitates further infection by compromising host immune responses. Recent evidence suggests that neuropeptides (neuroimmune proteins) are released from the CNS when an animal is stressed. Neuroimmune proteins may lead to suppression of lymphocyte production and function. Stress leads to high circulating glucocorticoid concentrations that are also immunosuppressive.[100] Perhaps the combination of high resting concentrations of glucocorticoids and release of neuroimmune proteins result in immunosuppression and facilitate development of clinical disease in horses infected with *S. neurona*. Recent evidence from a controlled investigation performed at Ohio State University demonstrated that health events before diagnosis of EPM were associated strongly with the disease.[42] Other evidence that stress may play a role in the pathogenesis of EPM results from transport stress and induction of the disease in an experimental equine model of EPM.[84] Transport has long been known as a stressor in horses and other species.[101–105] Horses are transported year-round to equestrians events in the United States. Further controlled investigations are needed to examine the role of stress in the development of clinical signs of EPM in horses.

S. neurona probably causes few pathologic changes in immunocompetent intermediate hosts. However, CNS lesions in the horse are often extensive.[9–11,24] Multifocal areas of hemorrhage to light discoloration of the brain or spinal cord may be visible on gross examination. Lesions may be microscopic to several centimeters wide. The brainstem and spinal cord are affected most often. Microscopically, lesions are characterized by focal to diffuse areas of nonsuppurative inflammation and necrosis with perivascular infiltration of mononuclear cells, including lymphocytes, macrophages, and plasma cells. Giant cells, eosinophils, and gitter cells also are present in inflammatory infiltrates. Gray or white matter or both are affected. Organisms have been found in neurons, leukocytes, and vascular endothelium, although they tend to develop most often in neurons[*]

* References 9, 10, 13, 24, 37, 38, 46, 106, 107.

10.10.6 Diagnosis

Immunoblot analysis of serum and CSF provides antemortem information regarding exposure to *S. neurona*.[108] The test uses cultured merozoites to detect antibodies directed against proteins considered unique to *S. neurona*. Antibodies produced to proteins shared with *S. fayeri* or other organisms found in North America can be differentiated. Other types of immunoassays are confounded by cross-reactivity with *S. fayeri* or other organisms that share antigens with *S. neurona*. Immunoblot testing of CSF samples has demonstrated greater than 90% specificity and sensitivity among approximately 300 neurologic cases that received postmortem examination. Approximately one half of the cases were confirmed histologically as EPM. Positive serum (specific antibody present) indicates exposure only. However, greater than 90% of histologically confirmed cases have tested seropositive.

Positive CSF indicates that parasites have penetrated the blood-brain barrier and stimulated a local immune response. If the integrity of the blood-brain barrier is compromised, circulating antibodies may leak across and produce a false-positive test result. Determination of total immunoglobulin G (IgG) and albumin in serum and CSF in such cases may be helpful. If the ratio of total IgG in CSF and serum is normal (IgG index) with an increase in blood-brain permeability (designated by the albumin quotient), leakage may have occurred. However, 610
if the albumin quotient is normal or increased, along with an increase in the IgG index, intrathecal antibody 611
production is likely, which may be caused by the presence of parasites in the CNS.[109] Unfortunately, immunoblot sensitivity exceeds that of the IgG index. CSF samples from horses with particularly high amounts

of circulating anti–*S. neurona* antibodies present the greatest potential for undetectable serum leakage using the IgG index. False-negative results have been rare but may occur. Some horses simply may fail to respond to the *S. neurona*–specific proteins identified.

The possible causes of false-negative responses are important to consider so that one does not misdiagnose affected horses. Horses that initially tested positive have become negative after several weeks of treatment and apparently have recovered. Chronically affected horses may test negative and still be infected, or the horse still may exhibit neurologic signs. The authors speculate that this may be caused by permanent CNS damage and that parasites are no longer present or antibody production is below test sensitivity. The use of polymerase chain reaction testing may aid in parasite detection when CSF antibody concentration is low. One should retest acute cases that test negative in 2 to 3 weeks. However, the incubation period appears to be sufficiently long to allow production of detectable amounts of IgG before the onset of clinical signs in most cases. As discussed previously, one exception with a short incubation period has been observed. Additional positive reactions were not observed when the immunoblot assay was modified for the detection of IgM.

10.10.7 Differential Diagnosis

Differential diagnoses for EPM include any disease affecting the CNS, although the results of neuroanatomic localization often make some more probable. For example, a horse with weakness, ataxia, and spasticity of all limbs and with no muscle atrophy or cranial nerve deficits suggests cervical vertebral stenotic myelopathy or equine degenerative myeloencephalopathy.[24,40,106,110] Both diseases affect young horses (1 to 3 years of age), but cervical vertebral stenotic myelopathy occurs more often in males. The clinical signs are often symmetric, with the hindlimbs usually a grade worse than the forelimbs.[24,106,110] Signs may be exaggerated by flexing or hyperextending of the neck.[24,110]

Equine herpesvirus (type 1) myeloencephalitis often has an acute onset following an episode of fever, cough, and nasal discharge or following one or more abortions on a farm. This condition often affects more than one horse on a farm. Herpesvirus has a rapid onset and often results in severe hindlimb weakness and ataxia along with bladder dysfunction. Urine dribbling sometimes may occur. Ataxia and weakness usually are symmetric and may result in recumbency. Occasionally, affected horses sit like dogs. Cranial nerve involvement is not common.[111–114]

Another disease one must consider in the differential diagnosis for EPM is polyneuritis equi. This disease can occur acutely and insidiously. Polyneuritis equi is more common in mature horses and usually starts with hyperesthesia progressing to anesthesia. Progressive paralysis of the tail, rectum, bladder, and urethra occurs, leading to urine dribbling. Rearlimb ataxia with gluteal atrophy may be present. Asymmetric cranial nerve deficits with involvement of cranial nerves V, VII, and VIII have been reported in 50% of the cases.[115–118]

One also should consider verminous myeloencephalitis, for the signs vary depending on the migratory pathway of the parasite. Diffuse or multifocal brain and spinal cord lesions have been reported. The onset is usually sudden, with rapid deterioration and death. The incidence of this disease is low, perhaps because of more intense parasite control.[119–121]

Over the last 3 years, another disease has become the number one differential diagnosis for EPM. West Nile virus was first reported in the United States in 1999, and the number of equine cases has gone from 25 in 1999 to 60 in 2000 to greater than 550 cases in 2001. Most of the equine cases in 1999 were diagnosed with EPM first before a definitive diagnosis of West Nile virus was determined, which demonstrates their similarity.[122]

Asymmetric neurologic deficits with profound weakness and ataxia makes differentiating West Nile virus from EPM difficult without the aid of ancillary diagnostic testing.

10.10.8 Treatment

Several regimens have been described previously for the treatment of EPM.[44,106,123,124] EPM has been described as successfully treated in 55% to 60% of the cases.[123] In the proceedings of the American Association of Equine Practitioners in 1994, 101 cases of EPM were diagnosed by clinical signs, Western blot analysis, and 22 postmortem examinations. One could speculate that the 79 (approximately 78%) that did not die responded to therapy. Treatment has included the combination of potentiated sulfonamides (trimethoprim-sulfas) and pyrimethamine. This combination causes a sequential blockade of folate metabolism in apicomplexan protozoa. The synergistic effect of this drug combination against *Toxoplasma* in other animal species suggested this therapy.[9,125] Many veterinarians at that time recommended a dosage of pyrimethamine of 0.25 to 0.5 mg/kg orally b.i.d. for 3 days, followed by the same dose once a day[123,124] or 0.25 mg/kg body mass orally s.i.d.[44] That dose was found not to be adequate. Based on a recent pharmacokinetic study of pyrimethamine in horses, the 611
dose required to reach the minimum inhibitory concentration for *T. gondii* in CSF is 1.0 mg/kg.[54,126] The 612
success rate of treated cases appears to be greater than 60% to 75%, with no complaints of anemia or thrombocytopenia.

Still others recommend pyrimethamine at a dose of 1 to 2 mg/kg s.i.d. for 90 to 120 days; however, the increased dosage for the full term may result in anemia. Previous work using the 1-mg/kg dose of pyrimethamine once a day did not result in anemia, but that dose was only administered for 10 days.[126] Because of the synergistic effect of the pyrimethamine–trimethoprim-sulfa combination on the parasites, perhaps the dose of pyrimethamine could be reduced and subsequently the incidence of anemia lowered. The trimethoprim-sulfa combinations have been recommended previously at a dose of 15 to 20 mg/kg orally b.i.d. or 15 mg/kg orally t.i.d.[44,123,124] The authors recommend sulfadiazine at a dose of 20 mg/kg orally s.i.d. for the full treatment period. Most of the treatments are administered for at least 5 months but must be extended sometimes. The activity of pyrimethamine, trimethoprim, sulfonamides, and combinations of the medications were tested against *S. neurona* merozoites in tissue culture. Pyrimethamine was demonstrated to be coccidiocidal at 1.0 μg/ml. Trimethoprim was coccidiocidal at 5.0 μg/ml; however, none of the sulfonamides alone had activity at concentrations up to 100 μg/ml. Sulfonamides at 5.0 or 10.0 μg/ml were used with pyrimethamine at 0.1 μg/ml. Activity against *S. neurona* improved.[127] These findings are based on in vitro studies, and further work is needed in controlled clinical trials in horses. Following completion of initial therapy, some clinicians recommend periodic treatments during periods of stress. Other therapies, such as once every 2 to 4 weeks or the first week of every month, also have been used.[44,124] However, careful controlled studies to determine the efficacy of these strategies have not been performed. Periodic treatment may lead to parasite resistance in that particular horse, resulting in the need for ever increasing doses.

Triazine derivative drugs used to prevent coccidiosis in other species in other countries have been used to treat EPM. These drugs (diclazuril and toltrazuril) originally were designed for use as herbicides. The response to therapy in horses with EPM was slightly better than the response documented for the standard therapy based on horses that previously had been treated with the standard therapy.[128] Recently, the pharmacokinetics of diclazuril and toltrazuril have been demonstrated in horses.[129] Currently, diclazuril is only available as a ration premix; therefore large volumes have to be given daily and its present form is not palatable. One advantage to using these compounds is an appreciably shorter duration of therapy, for most treatment regimens are approximately 30 days. One administers diclazuril at the rate of 5 mg/kg for a minimum of 28 days and may repeat it if necessary.

[130] Recent in vitro testing for activity of diclazuril against *S. neurona* has been demonstrated in the laboratory.[131] One may have to administer diclazuril by nasogastric tube daily because some horses refuse to eat the medication. Another anticoccidial drug, toltrazuril, has become popular because of its ease of use and good absorption orally in horses. Toxicity studies of toltrazuril in horses at 50 mg/kg for 10 days resulted in mild anorexia and depression; however, no serious side effects were observed. The current recommended dose is 5 to 10 mg/kg for a minimum of 28 days. More recently, a metabolite of toltrazuril called ponazuril (Marquis, Bayer Corporation, Kansas City, Missouri) has been approved by the Food and Drug Administration for use in treating EPM. One administers Marquis at the rate of 5 mg/kg for 28 days. No side effects have been reported. Nitazoxanide, another novel treatment, recently has been used to treat EPM. Nitazoxanide is a 5-nitrothiazole with a broad spectrum of activity against bacterial, protozoal, and helminth parasites and has been shown to kill *S. neurona* in cell culture. Toxicity studies were performed in horses. When horses were given 2 times the recommended dose, they became lethargic after 1 week of daily dosing; however, when horses were given nitazoxanide at 4 times the recommended dose, they became significantly ill with one death. The suggested dose schedule is 25 mg/kg once daily for the first week and 50 mg/kg once daily for the next 23 days.[130] Field trials currently are under way to gain Food and Drug Administration approval for diclazuril and nitazoxanide.

The prognosis for horses diagnosed with EPM appears to be similar regardless of the treatment used, because most reports suggest an approximate improvement rate of 60% to 75% with the standard therapy.[132–135] However, earlier work suggested the success rate of therapy was about 50%.[124] Some have suggested that less than 25% of affected horses may return to their original function; however, little objective information is available regarding this issue.[133] A recent study with diclazuril resulted in approximately 75% improvement among severely affected horses and approximately 30% (11 of 36) returned to their original level of performance or actually improved beyond the level of performance before illness.[128] An efficacy study of 70 horses given nitazoxanide found that 63% of the horses met the criteria for success after treatment by improving one grade in their neurologic signs.[130] A growing concern is the percentage of horses that have a relapse in clinical disease after cessation of therapy, for some horses relapse days, weeks, or even months after cessation of therapy. The mechanism of relapse is unknown but may be caused by recrudescence of a truly latent stage of the parasite, presence of a small persistent focus of infection, or perhaps re-exposure to the parasite.[54] Anecdotal estimates of 612
the relapse rates after standard therapy range from 10% to 28% of treated horses.[132–135] The relapse rate reported 613
using diclazuril to treat EPM was less than 5%.[128] Some apicomplexans have latent stages, most notably *Toxoplasma* pseudocysts and *Plasmodium* hypnozoites. *Sarcocystis* species are not known to form pseudocysts or hypnozoites. However, *S. faculata* encephalitis in birds may persist for several months without reinfection. This phenomenon simply may represent a low-level infection and not the development of a true latent parasitic stage. *Eimeria tenella* has been shown to develop latent stages in leukocytes, which reactivate after stress. A great deal concerning the life cycle of coccidia remains unknown. The ability to produce experimental infections helps determine whether *S. neurona* forms a latent stage or maintains a persistent, low-level focus of infection. Reinfection also may be responsible in some cases. The efficacy of preventive therapies is open to debate.

Antiinflammatory medications are recommended when acute onset results in dramatic and progressive clinical signs.[44,123,124] The use of flunixin meglumine or phenylbutazone may be helpful. The usual dose of flunixin meglumine is 1.1 mg/kg b.i.d. parenterally; intravenous administration of medical-grade dimethyl sulfoxide (Domoso, Syntex Animal Health, West Des Moines, Iowa) at a dose of 1.0 ml/kg (approximately 1 g/kg) in a 10% solution once daily for 3 days in a row; and although not uniformly recommended, some clinicians use dexamethasone parenterally in severely affected horses at a dose rate of 0.05 mg/kg b.i.d. or sometimes empirically at 50 mg b.i.d.[44,124] However, the authors believe one should use corticosteroids judiciously. The

exacerbation of signs in stressed patients and reports of horses with EPM showing a worsening of signs following the use of these medications suggest one should avoid immunosuppression.[40,106] Ancillary treatments may include padded helmets, slings, good supportive care, and a deeply bedded stall.

Because of the suspicion that protozoal infections occur more commonly in immunocompromised patients, immunomodulators or other therapies that may have a nonspecific enhancement of the immune system may be helpful. One clinician includes levamisole as part of the treatment regimen for her cases of EPM. Studies have shown that levamisole is a nonspecific immunomodulator that affects T cell–mediated immunity, including delayed-type hypersensitivity, and increases the phagocytic activity of macrophages.[136,137] Reaction to other *Sarcocystis* species in domestic animals includes delayed-type hypersensitivity with mononuclear cell infiltration.[3] The use of immunomodulators may have merit, but further investigation is necessary. Although unlikely, these drugs possibly also may enhance the immunopathologic effects associated with CNS infection.

One should monitor prolonged therapy with antifolate medications for signs of bone marrow suppression with resultant anemia, thrombocytopenia, or neutropenia.[44,124] The combination of trimethoprim-sulfamethoxazole and pyrimethamine also has an effect on reproductive function in pony stallions. The drug combination does not appear to affect semen quality, testicular volume, sperm production efficiency, erection, or libido of healthy stallions; however, it may induce changes in copulatory form and agility and alter the pattern and strength of ejaculation.[138] Therefore one should use caution when treating stallions with this combination for neurologic disease believed to be EPM.

Supplementation with folic acid, folinic acid, or brewer's yeast has been recommended for treatment of presumed folic acid deficiency in horses treated with the standard therapy, particularly pregnant mares.[6,124] However, some investigators have discouraged folic acid supplementation because of poor absorption and the potential for toxic effects on the bone marrow.[133] Toxicity has been reported in newborn foals born to mares that were treated for EPM. These mares had been treated with antifolate medications and concurrently supplemented with folic acid. The foals showed evidence of bone marrow aplasia and hypoplasia, renal nephrosis or hypoplasia, and skin lesions similar to the lesions seen in other species.[139] The cause-and-effect relationship between folic acid supplementation and these developmental abnormalities has not been demonstrated conclusively at the present time. However, particularly in pregnant mares, one should not use folic acid supplementation until controlled clinical trials can be performed to corroborate or refute these findings.

Some clinicians recommend the use of additional supplements such as vitamin E and thiamine that may facilitate healing of nervous tissue when treating horses with EPM.[133,134] However, clinical trials have not been performed to establish the efficacy of this supplementation.

10.10.9 Prevention

Because of the nature of the horse business, prevention of clinical cases of EPM will be difficult. Another complicating factor is the widespread distribution of the parasite throughout many parts of the United States. Although a vaccine is currently available, it remains unproven. In light of the difficulties experienced in the development of effective vaccines for other protozoan parasites, development of an efficacious vaccine for EPM most likely will be in the distant future.[140–143] Monitoring high-risk age groups such as young horses and old horses closely for evidence of neurologic disease may help detect EPM early. That EPM may be the cause of the clinical signs when horses are presented for neurologic disease in the warmer months should raise the index of suspicion. Because many major horse competitions take place in the fall of the year, monitoring of horses before 613 614

transport and competition may be helpful. Wildlife such as opossums and pests such as mice and rats should be denied access to feed by using rodent-proof containers to help prevent some cases of EPM. One also should protect forages from wildlife access by storage in enclosed facilities. Excluding birds from facilities may help prevent some cases of EPM, although the role birds may play in the pathogenesis of EPM is not certain. Sporocyst ingestion in birds may result in passage through the intestinal tract and infectivity for other species.[52,86] Case histories from affected horses indicate that the development of clinical signs often follows some other health event, and that has been borne out by a controlled investigation.[42] Close monitoring of broodmares close to foaling and horses that develop a major illness or injury is important, for it may help early diagnosis of EPM cases.

In addition to designing a prevention plan that minimizes risk factors associated with the definitive host, considering the role of the intermediate host in EPM is also important. As was mentioned previously, several species of mammals have been reported to act as natural or laboratory intermediate hosts in the life cycle of *S. neurona*.[68–71] These animals only represent a threat after death. Therefore veterinarians should encourage horse owners to pick up dead cats, armadillos, skunks, and raccoons on their property and dispose of the carcasses so the opossums cannot eat them and excrete more sporocysts. One should retrieve carcasses carefully with an inverted plastic garbage bag or something similar.

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10.11

10.11—Equine Herpesvirus 1 Myeloencephalopathy

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Equine herpesvirus 1 (EHV1) is an economically important pathogen of horses and exerts its major effect by inducing abortion storms or sporadic abortions in pregnant mares, early neonatal death in foals, and respiratory disease in young horses.[1–3] Myeloencephalopathy is an uncommon manifestation of EHV1 infection but can cause devastating losses during outbreaks on individual farms or boarding stables.[4,5] Although EHV4 rarely causes clinical manifestations of disease in organs other than the respiratory tract, isolated cases of myeloencephalopathy and sporadic abortions have been reported in EHV4 infections.[1–3,6,7] Clinical signs of neurologic disease reflect a diffuse multifocal myeloencephalopathy following vasculitis, hemorrhage, thrombosis, and ischemic neuronal injury. Sudden onset and early stabilization of signs including ataxia, paresis, and urinary incontinence; involvement of multiple horses on the premises; and a recent history of fever, abortion, or viral respiratory disease in the affected horse or herdmates are typical features, although considerable variation exists between outbreaks concerning epidemiologic and clinical findings.[8] Prevention is difficult because many asymptomatic horses are infected latently with EHV1, and vaccines do not confer protection against neurologic manifestations of infection.

10.11.1 Virologic Findings

Of the five distinct herpesviruses that are known to infect horses, three are typical α-herpesviruses with a double-stranded DNA genome and are designated equine herpesvirus 1 (EHV1; equine abortion virus, formerly known as EHV1, subtype 1), EHV4 (equine rhinopneumonitis virus, formerly known as EHV1, subtype 2), and EHV3 (equine coital exanthema virus), and two are γ-herpesviruses, designated EHV2 (formerly called equine cytomegalovirus) and EHV5.[2,3,9–11] In addition, three asinine α-herpesviruses (AHV1, AHV2, and AHV3) have been isolated from donkeys, and of these, AHV3 has been shown by many criteria to be related closely to EHV1. Indeed, EHV1 and AHV3 are related more closely to each other than either is to EHV4.[2,11–13] Phylogenetic analysis and epidemiologic evidence suggest that EHV1 recently has been derived from AHV3, and that donkeys may remain an alternate host for EHV1, serving as a reservoir to infect horses.[9,11] 617

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EHV1 and EHV4 are distinguishable from EHV2, EHV3, and EHV5 by biologic properties and virus neutralization tests and from each other by restriction endonuclease fingerprinting of DNA, DNA sequences, and several immunologic tests based on monoclonal antibodies to each virus.[1–3,9,10,14] EHV1 and EHV4 produce eosinophilic intranuclear inclusion bodies in infected cells in vivo and in vitro. Several strains have been identified within EHV4 and EHV1, although the epidemiologic, immunologic, and pathogenic significance of this finding is not known. The 1-p and 1-b subtypes of EHV1 likely are capable of inducing neurologic disease. Apart from differences in endotheliotropism, genetic and antigenic fingerprinting and experiments in baby mice have not yielded clear markers distinguishing EHV1 strains that induce neurologic disease or abortion or both.[9,15–19] Recently, a hamster model has been described showing some potential for discrimination between abortigenic and neuropathogenic EHV1 strains.[20]

10.11.2 Epidemiology and Immunity

EHV1 and EHV4 are enzootic in most horse populations, and the majority of horses show serologic evidence of exposure to these viruses. Most horses become infected via the respiratory tract with EHV1 or EHV4 (or both) during the first year of life. After an incubation period of 2 to 10 days, clinical signs of respiratory disease of variable severity develop and resolve within 1 to 2 weeks in uncomplicated cases.[1–3] Resolution of clinical signs is coincident with development of virus-specific neutralizing antibody directed primarily against surface viral glycoproteins. The development of cell-mediated responses is probably critical for recovery.[21] Resistance to reinfection with homologous virus is demonstrable after recovery but generally persists for only 3 to 4 months. Subsequent infections typically induce milder clinical signs or subclinical infection, although virus shedding from the nasopharynx occurs.[1,2] The immune response frequently is not successful in clearing herpesviral infection, and the majority of clinically recovered horses remain latently (asymptomatically) infected with EHV1 or EHV4 (or both) for life.[1,2,22,23] Recently, EHV1 has been shown to evade the host immune system by downregulating major histocompatibility complex class I expression at the cell surface. This process may be a prerequisite to the establishment of latency.[24]

Recrudescence of latent infection is important in the epidemiology of EHV1 and EHV4 and explains why these diseases can occur in closed populations without the introduction of new horses.[1,2,23,25] Signs of EHV1 infection may occur in the horse in which stress-associated recrudescence of infection has occurred, or the horse may remain asymptomatic but shed infectious virus in nasal secretions to infect other horses. Natural infection with EHV1 occurs by inhalation or ingestion of aerosolized infective virus or by direct contact with virus shed in the

products of abortion or in the nasal and ocular discharges and saliva of horses with overt clinical disease, subclinically infected horses, or shedding carrier horses.[1,2,14] Infectious EHV1 virus was detected in the feces of experimentally infected foals that developed diarrhea, suggesting that fecal spread is a possibility.[26] Virus may be shed by clinically affected and inapparently infected horses for 3 weeks or more, and EHV1 may remain infective in the environment for up to 14 days and on horse hair for 35 to 42 days.[2,27,28]

The first definitive association between EHV1 and myeloencephalopathy was made in 1966 in Norway with the isolation of the virus from the brain and spinal cord of a horse that showed signs of severe neurologic dysfunction.[29] The myeloencephalopathic form of EHV1 infection now is considered to have a worldwide distribution, having been recognized in Denmark, The Netherlands, Germany, Sweden, Austria, Britain, Ireland, Australia, India, the United States, and Canada.[4,5,8,18,30–52] In view of the ubiquitous occurrence of EHV1 infection in horse populations, outbreaks of EHV1 myeloencephalopathy are rare. In many instances, cases of neurologic EHV1 infection occur in association with outbreaks of abortion or respiratory disease, although some outbreaks occur in the absence of other manifestations of EHV1 infection and without the introduction of new horses into the group.[14,25,53,54]

The myeloencephalopathic form of EHV1 infection may occur as sporadic individual cases or, more often, as outbreaks involving multiple individuals over a period of several weeks on one or more premises within a limited geographic region[*] Secondary or tertiary waves of clinical disease may occur as previously unexposed horses become infected from a common source over short period.[5,14,35,51] Morbidity rates ranging from less than 1% to almost 90% of exposed individuals and mortality rates ranging from 0.5% to 40% of in-contact horses have been reported[†]Neurologic EHV1 infection can occur at any time of year but the highest incidence is in the late winter, spring, and early summer, perhaps reflecting the seasonal occurrence of abortigenic EHV1 infections during the same months.[43]

The neurologic form of EHV1 infection has been observed in pregnant mares, barren mares, geldings, stallions, and foals, although foals frequently do not show neurologic manifestations of infection during outbreaks that 618
involve severe neurologic disease in adult horses.[4,35] Pregnant mares and mares nursing foals appear be at 619
increased risk for developing neurologic manifestations of EHV1 infection, and the stage of gestation may be important in determining the outcome of infection in pregnant mares.[4,34,35,41,42] Mares infected during the first two trimesters of gestation appear to be more likely to develop neurologic signs without abortion, whereas mares infected during the last trimester are more likely to abort without showing neurologic signs.[14,32,34,42,49]

All breeds of horses are susceptible to the neurologic form of EHV1 infection, and other Equidae also may be affected. EHV1 was the suspected cause of myeloencephalopathy that developed in a zebra 1 week after an in-contact onager *(Equus hemionu onager)* aborted an EHV1 infected fetus.[57] The authors are unaware of reports of neurologic EHV1 affecting donkeys and mules, although donkeys and mules have shown seroconversion indicating infection with EHV1 while in contact with affected horses during outbreaks.[47,48,55] Indeed, donkeys and mules returning from a show were thought to be responsible for dissemination of EHV1 and propagation of multiple outbreaks of neurologic EHV1 infection in Southern California in 1984 and in several subsequent years, suggesting that a donkey-adapted variant of EHV1 with an increased neuropathogenicity for horses may have been involved.[55]

A modified live EHV1 vaccine of monkey cell line origin was shown to be associated with neurologic disease in 486 of 60,000 recipients, prompting its withdrawal from the U.S. market in 1977.[44] No reports of EHV1

myeloencephalopathy were associated with recent use of the modified live vaccine currently approved for use in horses in the United States.

* References 4, 5, 18, 29–36, 40, 43, 45–52, 55.

† References 4, 5, 18, 29–36, 40, 43, 45–52, 55, 56.

10.11.3 Pathogenicity and Pathogenesis

Natural infection with EHV1 occurs by inhalation or ingestion, after which the virus attaches to and rapidly replicates in cells of the nasopharyngeal epithelium and associated lymphoreticular tissues, causing necrosis, exudation, and infiltration of phagocytic cells. Bronchial and pulmonary tissues also become infected, particularly in foals, thus predisposing them to secondary bacterial pneumonia.[1,2,14,21,58] Migration of virus-infected phagocytes into the circulation results in viremia associated with mononuclear cells (primarily T lymphocytes) of the buffy coat.[1,2,14,59] The immunologically privileged intracellular location of the virus appears to protect it from inactivation by circulating antibody and permits dissemination to other tissues, including the central nervous system (CNS), even in the presence of high levels of antibody.[60] EHV1 is capable of spreading directly from one infected cell to contiguous cells without an extracellular phase.[14] Vascular endothelium is the initial site of infection in the central nervous system and appears to be the predilection site for replication of EHV1 after transfer of the virus from circulating leukocytes.[42,61,62] Viremia, which may be of prolonged duration, can occur during primary and all successive infections with EHV1, even when no clinical signs are apparent; thus all EHV1 infections pose a threat of inducing neurologic disease or abortion.[1,4]

The acute onset of clinical signs of EHV1 myeloencephalopathy appears to result from vasculitis and thrombosis of arterioles in the brain and especially the spinal cord. This causes functional impairment of blood flow and metabolic exchange and, in severe cases, hypoxic degeneration and necrosis (malacia) with hemorrhage into adjacent neural tissues of the white and, to a lesser extent, gray matter[*] This proposed pathogenesis, based primarily on interpretation of the prominent vasculitis seen histopathologically in infected horses and the lack of definitive evidence of viral multiplication in neural tissues, contrasts greatly with the well-established pathogenesis of encephalitis caused by herpesviruses in other species.[9] The propensity of certain EHV1 isolates to induce myeloencephalopathy does not appear to reflect specific neurotropism but rather a significant endotheliotropism[†] The finding of chorioretinopathy and neural lesions in experimentally infected specific pathogen-free ponies, however, suggests that at least some strains of EHV1 may exhibit neurotropism.[64] Furthermore, strong evidence exists that in addition to circulating T lymphocytes, epithelial cells of the respiratory tract, and lymphoid tissues draining the respiratory tract, trigeminal ganglia are important sites for establishing and maintaining the lifelong state of latency that occurs in most, if not all, horses during primary infection with EHV1 and EHV4.[22,23,65–68] The ubiquitous EHV2 has been proposed to play an important role in promoting reactivation of EHV1 and EHV4 from these sites in latently infected horses.[22]

No satisfactory explanation exists as to why some outbreaks of EHV1 infection are associated with a high incidence of neurologic disease, whereas others are not, or why different horses show different clinical manifestations of EHV1 infection during outbreaks.[4,5,36,51] The nature and extent of lesions resulting from EHV1 infection appear to be influenced by the age, sex, reproductive status (including stage of pregnancy), and immune status of the horse; the magnitude of challenge; strain variations; and perhaps the route of infection. [4,36,39] In one carefully monitored outbreak of EHV1 infection on a stud farm in England, less than 17% of 619
infected horses developed neurologic manifestations of infection even though almost 60% of the horses on the 620

farm were confirmed to have been infected.[4] Endothelial cell infection and perivascular cuffing within the CNS appeared to be at least as pronounced in foals that died during this outbreak without showing neurologic signs as in profoundly paretic mares with severe CNS lesions; however, parenchymal neural lesions were minimal in the foals.[4,36] A notable finding during this outbreak was that mares and stallions that developed neurologic signs had considerable antibody responses, whereas the majority of foals did not, despite experiencing a prolonged period of viremia.[4]

The majority of EHV1 infections that cause neurologic signs represent reinfection rather than a new infection.[30,33,69] Infection occurs in horses with significant preexisting serum EHV1 antibody titers, affected horses frequently have high titers at the onset of neurologic signs, and those horses that develop the most severe clinical signs are frequently the ones that show the most rapid increase in antibody titer after infection.[4,41,42] In addition, the characteristic vascular lesions in neural tissues of affected horses are typical of type III (Arthus) hypersensitivity reactions, and circulating immune complexes have been demonstrated at the onset of neurologic signs, suggesting that they may result from an immune-complex vasculitis[*] An immune-mediated mechanism is supported further by the difficulty experienced in isolating the virus from neural tissues of affected horses.[36,52,53] In addition, assessment of risk factors during outbreaks of neurologic EHV1 infection in Southern California in 1984 revealed that horses vaccinated with killed or modified live EHV1 vaccine within the previous year were significantly (9 to 14 times) more likely to develop neurologic manifestations of infection than were nonvaccinated horses.[55]

The finding of circulating antibodies to the neuritogenic myelin protein P2 in the serum of horses that died from EHV1 myeloencephalopathy but not in horses that recovered has led to the suggestion that an alternate immune-mediated mechanism may play a role in the pathogenesis of neurologic EHV1.[70] The presence of this antibody, however, may represent a response to leakage of the protein after damage induced by another mechanism.

Despite the foregoing observations, evidence for an immune-mediated pathogenesis for EHV1 myeloencephalopathy is by no means conclusive. In experimental EHV1 infections in which the onset of neurologic signs 8 to 9 days after infection correlated with a peak in the level of circulating immune complexes, vasculitis was not present in vessels in which endothelial cells did not support viral replication or in organs such as the kidney that one would expect to trap circulating immune complexes.[61] The finding of greatly depressed platelet counts several days before the onset of clinical signs, presumably the result of consumption in thrombi following endothelial damage, suggests that the neuropathologic changes are initiated before circulating immune complexes peak and that the action of immune complexes may be secondary and localized. Failure to isolate the virus from the CNS may be attributable to high levels of circulating antibody and to the endotheliotropism of the virus.[61]

* References 14, 41, 42, 45, 49, 53, 63.

† References 19, 26, 36, 41, 42, 61, 62.

* References 14, 33, 41, 42, 54, 62, 70.

10.11.4 Clinical Signs

In natural and experimental infections, neurologic signs appear 6 to 10 days after infection by the intranasal route[*] The onset of neurologic signs may be preceded or accompanied by signs of upper respiratory disease, fever, inappetence, or hindlimb edema within the previous 2 weeks although in many instances no antecedent signs are notable unless one routinely monitors rectal temperature. However, frequently one finds a herd or

stable history of current or recent cases of respiratory tract infection, fever, inappetence, distal limb edema, abortion, neonatal death, foal diarrhea, or neurologic disease, and for one to encounter different signs of EHV1 infection in different groups of horses on a particular farm is not unusual.[4]

Affected horses are occasionally febrile at the onset of neurologic disease, although most are normothermic and some are hypothermic. Neurologic signs are generally of acute or peracute onset, after which they tend to stabilize rapidly and generally do not progress after the first 1 or 2 days.[30,34,35,43,71] Clinical signs vary depending on the location and severity of lesions, but in most horses, signs reflect predominant involvement of the spinal white matter.[43] Ataxia and paresis of the limbs are the most common signs, with hypotonia of the tail and anus, tail elevation, and urinary incontinence being common but not invariable findings[†] Clinical signs are usually bilaterally symmetric or only mildly asymmetric, although hemiparesis or sudden onset of unilateral hind- or forelimb lameness progressing to unilateral or more generalized ataxia, paresis, and recumbency have been reported.[43,45,46,63,73] Lesions in peripheral nerves and spinal cord were observed in some of these cases.[45] The hindlimbs generally are affected more severely and earlier in the disease course than the forelimbs. In mildly affected horses, transient ataxia and stiffness of the pelvic limbs or dribbling of urine following overflow from a 620

621 distended atonic bladder may be the only signs noted.[34,43] One may note conscious proprioceptive deficits in these cases as reluctance to move, clumsiness, toe dragging, knuckling, stumbling, pivoting, and circumduction in one or more limbs on circling, with spasticity evident in some cases.[35,43,74] These signs are often subtle and may go unnoticed. More severely affected horses show profound limb weakness and swaying of the hindquarters, and a small proportion show complete paralysis of affected limbs, manifested as paraplegia and sitting like a dog, complete recumbency, or tetraplegia.[4,14,43,74]

Distention of the urinary bladder is common and may cause signs of colic or dribbling of urine, which frequently results in scalding of the perineum, legs, and other areas.[48] Cystitis is a frequent complication, particularly when repeated catheterization is necessary to relieve bladder distention.[71] Affected stallions and geldings may develop penile flaccidity and paraphimosis or repeated erections, whereas mare may develop vulvar flaccidity[*]In addition, stallions may experience reduced libido and swelling of the testes.[4,36] Scrotal edema may accompany hindlimb edema at the onset of neurologic signs in some cases.[4,34–36,49] Sensory deficits are uncommon, but perineal hypalgia or analgesia has been noted, and analgesia of the caudal half of the body was observed in one affected horse.[30,34,43,74] Consistent with predominant involvement of the white matter of the spinal cord, flexor reflexes are normal and perineal reflexes are preserved. In recumbent horses, spinal tendon reflexes can be tested and may be increased. Atrophy is rarely seen, even in the later stages of the disease.[43] Affected horses usually remain alert and have a good appetite, even when recumbent, although some show modest depression and inappetence.[35,72] Severe depression, when it occurs, is more often caused by secondary complications than by brain involvement.[72] Unequivocal signs of brain disease are rare, although infarction of the brainstem may cause depressed sensorium, altered behavior, and cranial nerve damage leading to vestibular signs and to lingual, mandibular, and pharyngeal paresis, which may manifest as dysphagia.[32,50,72,73,75] Strabismus, nystagmus, circling, and head tilt have been observed on occasion.[8,32,43,74]

Affected horses show variable progression of clinical signs. Those horses that are affected mildly frequently stabilize rapidly over a period of hours to a few days as edema and hemorrhage resolve and generally recover completely over a period of days to several weeks.[4,5,30,35,51] If recumbency occurs, it generally does so during the first 24 hours, with some horses showing such complete motor paralysis that they are unable to lift their

heads.[45] Severely affected horses may show progression of signs during the first few days and may die in coma or convulsion or be euthanized because of secondary complications.[4]

* References 5, 26, 41, 42, 46, 51, 62.

† References 5, 30, 34, 43, 51, 72.

* References 4, 14, 30, 35, 72, 74.

10.11.5 Laboratory Findings

Cerebrospinal fluid (CSF) analysis typically, although by no means always, reveals xanthochromia, an increased protein concentration (100 to 500 mg/dl), and increased albumin quotient (ratio of CSF to serum albumin concentration), reflecting vasculitis and protein leakage into CSF. The white blood cell count in CSF is usually normal (0 to 5 cells/μl) but occasionally is increased[*] Abnormalities in CSF are not present at the onset of clinical signs in some horses, and changes resolve rather quickly; thus the CSF may be normal within 2 weeks of onset of clinical signs.[42,43,71]

The presence of antibodies to EHV1 in the CSF of affected horses strongly suggests a diagnosis of EHV1 myeloencephalopathy, although such antibodies are absent in many cases.[8,32,42,43,78] One should take into account the albumin concentration, IgG concentration, and EHV1 antibody titer in serum and CSF when interpreting positive antibody titers in CSF.[76] Because the albumin quotient usually is elevated in affected horses and the IgG index is normal, the presence of EHV1 antibodies in CSF reflects leakage of protein across a damaged blood-brain or blood-CSF barrier following vasculitis rather than intrathecal antibody production.[70,76,78] Antibodies therefore are more likely to be present in the CSF of affected horses with concomitantly high serum titers.[42,43,70,76,78] Blood contamination during collection of CSF and other diseases that cause an increase in the permeability of the blood-brain barrier or bleeding into the subarachnoid space may elevate CSF antibody titers falsely if serum titers are also high. Isolation of EHV1 from the CSF of affected horses would confirm a diagnosis but is rarely successful.[43,48]

Virus isolation and identification of EHV1 from nasopharyngeal swabs or buffy coat samples strongly supports a diagnosis of EHV1 myeloencephalopathy in a horse with compatible clinical signs and should be attempted by submission of nasopharyngeal swabs in viral transport medium and an uncoagulated blood sample (citrated or heparinized)[†] Results of virus isolation may be negative, however, because the peak of virus shedding usually has passed by the time neurologic signs appear.[79] The likelihood of isolating EHV1 during outbreaks of 621
neurologic disease increases by monitoring in-contact horses and collecting nasal swab and buffy coat samples 622
during the prodromal febrile phase before neurologic signs develop.[35] Even so, interpretation of positive culture results can be confusing because EHV1 and EHV4 have been isolated from the respiratory tract of normal horses.[27] Application of new diagnostic methods such as polymerase chain reaction (PCR), in situ hybridization, antigen-capture enzyme-linked immunosorbent assay (ELISA), and dot immunobinding to nasal swabs or scrapings, buffy coat samples, or pathologic specimens have improved the speed and specificity greatly with which one can diagnose EHV1 infection.[80–88] Many conventional PCR protocols targeting specific genes of EHV1 have been published in recent years for molecular detection of EHV1 in nasopharyngeal swabs or buffy coat samples.[23,83–87] Although considerable progress has been made in developing PCR protocols for clinical use, quality control of nucleic acid amplification techniques remains an ongoing challenge because of lack of protocol standardization between laboratories.[89] Furthermore, the majority of PCR assays targeting genomic EHV1 DNA are unable to differentiate between a lytic and latent infection. Novel technologies such as the

TaqMan PCR that allow quantitation of viral DNA and detection at the level of gene expression likely will feature prominently as molecular diagnostic approaches to EHV1 infection are refined further in the future.

Serologic testing that demonstrates a fourfold or greater increase in serum antibody titer using serum-neutralizing or complement fixation tests on acute and convalescent samples collected 7 to 21 days apart provides presumptive evidence of infection.[*] Many horses with EHV1 myeloencephalopathy, however, do not show a fourfold rise in serum-neutralizing titer, and some actually show a decline.[48] This may be explained by the finding that when antibody titers rise, they do so rapidly within 6 to 10 days of infection and already may have peaked by the time neurologic signs appear.[†] Although serologic testing has limitations in confirming a diagnosis of EHV1 myeloencephalopathy in an individual horse, testing of paired serum samples from in-contact horses is recommended because a significant proportion of affected and unaffected in-contact horses seroconvert, providing indirect evidence that EHV1 is the causative agent.[‡] Interpretation of the results of serologic tests is complicated by the fact that the serum-neutralizing, complement-fixation, and ELISA tests in use at most diagnostic laboratories do not distinguish between antibodies to EHV1 and EHV4 because of cross-reaction between these viruses. A specific ELISA test based on the C-terminal portion of glycoprotein G of both viruses has been developed and should prove valuable in the investigation and management of disease outbreaks in the future.[91–93]

* References 41–43, 63, 71, 73, 76, 77.

† References 1, 2, 4, 8, 43, 79.

* References 4, 5, 8, 14, 30, 47, 50, 51, 72, 79.

† References 4, 5, 41, 42, 47, 51, 79, 90.

‡ References 4, 5, 8, 47, 48, 50, 51.

10.11.6 Diagnosis

The multifocal distribution of lesions results in variability of clinical presentation, which necessitates inclusion of a number of conditions in the differential diagnosis. These conditions include equine protozoal myeloencephalitis, cervical stenotic myelopathy, and cervical vertebral instability (wobbler syndrome), cervical vertebral fracture or other CNS trauma, neuritis of the cauda equina, fibrocartilaginous infarction, aberrant parasite migration, degenerative myelopathy, togaviral encephalitis (flaviviruses and alphaviruses), rabies, botulism, CNS abscess, and a variety of plant and chemical intoxications.[8,43] Sudden onset and early stabilization of neurologic signs including ataxia, paresis, and urinary incontinence; involvement of multiple horses on the premises; and a recent history of fever, abortion, or viral respiratory disease in the affected horse or herdmates is sufficient to make a tentative diagnosis of EHV1 myeloencephalopathy.[8] Antemortem diagnosis is supported further by ruling out other conditions; demonstrating xanthochromia and elevated protein concentration in CSF; identifying EHV1 in or isolating EHV1 from the respiratory tract, buffy coat, or CSF; and demonstrating a fourfold increase in antibodies using serum-neutralizing, complement fixation, or ELISA tests performed on acute and convalescent serum samples collected from affected or in-contact horses 7 to 21 days apart.[5,51,94] Antemortem confirmation of a diagnosis of EHV1 myeloencephalopathy is frequently not possible, however, particularly when an individual horse is affected, because the foregoing tests do not yield consistent results in all cases.

10.11.7 Treatment and Prognosis

Because EHV1 is a contagious and potentially devastating infection, horses suspected of being affected should be isolated promptly and strictly until EHV1 is ruled out by confirmation of an alternate diagnosis.[72] No specific treatment is available; thus management of horses with EHV1 myeloencephalopathy aims toward supportive nursing and nutritional care and reduction of CNS inflammation.[8] One should encourage horses that are not recumbent to remain standing and should protect them from self-inflicted trauma by the provision of good footing, such as a grass paddock; by placement of food and water in an accessible location at a convenient height 622 above ground level; and by other measures, including the use of padded hoods and elimination of obstacles. One 623 should maintain patients that become recumbent in a sternal position on a thick cushion of dry absorbent bedding and should roll them frequently (at least every 2 to 4 hours) to reduce the risk of myonecrosis and decubital ulcers. Whenever possible, one should lift and support the horse in the standing position using an appropriately fitting sling.[5] Slings are most beneficial for moderately affected horses that are too weak to rise but are able to maintain a standing position with minimal assistance.

Affected horses usually maintain a good appetite, even when recumbent, although hand feeding may be necessary to encourage some horses to eat. Maintenance of hydration is important, and provision of a laxative diet or the administration of laxatives such as bran mashes, mineral oil, or psyllium may be necessary to reduce intestinal impaction. One usually can meet the caloric and water needs of anorectic patients usually by feeding gruels of alfalfa-based or similar pelleted feeds in water or balanced electrolyte solution via nasogastric tube. If oral intake is insufficient to meet the daily water needs of 60 to 80 ml per kg of body mass per day, one can maintain hydration by intravenous administration of balanced electrolyte solutions.[43] One also can use partial or total parenteral nutrition to meet the caloric needs of anorectic, recumbent horses.

If affected horses are unable to stand and posture to urinate or if bladder function is impaired significantly, manual evacuation of the bladder by application of pressure per rectum may be necessary. If these measures are unsuccessful, judicious urinary catheterization is indicated and should be performed aseptically with the collection tubing attached to a sterile closed bag to minimize the risk of inducing urinary tract infection.[8,72,74] Cystitis is, however, a frequent complication, particularly in recumbent horses, and can lead to bladder wall necrosis, bladder rupture, and systemic sepsis. Urine scalding can become a major problem, particularly in mares that dribble urine. Prevention involves regular washing of the perineum, tail, and hindlegs with water, application of water-repellent ointments and braiding or wrapping the tail to simplify cleaning.[8] Administration of enemata or manual emptying of the rectum also may be necessary to promote defecation and improve patient comfort.[8]

Because vasculitis, hemorrhage, and edema are prominent early lesions and may have an immune basis, treatment with corticosteroids early in the disease course is recommended by most clinicians, although no objective data are available to document the efficacy of these or other antiinflammatory drugs.[35,43,72] A short course of treatment with prednisolone acetate (1 to 2 mg/kg/day) or dexamethasone (0.05 to 0.25 mg/kg parenterally twice daily) for 2 to 3 days with decreasing doses over another 1 to 3 days is recommended.[8,43,74] Flunixin meglumine (1.1 mg/kg body mass every 12 hours) is indicated to treat CNS vasculitis. Dimethyl sulfoxide at a dose of 0.5 to 1.0 g/kg administered intravenously as a 10% to 20% solution in normal saline or 5% dextrose once daily for up to 3 days commonly is used to treat horses with suspected CNS trauma or inflammatory disease, such as EHV1.[8] Although the efficacy of dimethyl sulfoxide for treating herpesvirus myeloencephalopathy has not been evaluated, its reported ability to inhibit platelet aggregation and scavenge free

radicals support its continued use. Because of the high risk of development of cystitis and other secondary bacterial infections, administering broad-spectrum antibiotics such as potentiated sulfonamides (trimethoprim-sulfamethoxazole 30 mg/kg body mass orally every 12 hours) or ceftiofur (2.2 mg/kg body mass intramuscularly or intravenously every 12 hours) is advisable, particularly when corticosteroid treatment is used.[5,8,43] One should base the choice of antibiotics for treating established secondary bacterial infections of the urinary tract, respiratory tract, or other areas on the results of culture and susceptibility testing.

Acyclovir, a synthetic purine nucleoside analog with inhibitory activity against several human herpesviruses, has been shown to exert an inhibitory effect on EHV1 in vitro.[95] Apparent efficacy of acyclovir was demonstrated by a successful treatment outcome in two of three foals with congenital EHV1 infection.[96] Similarly, four of six severely neurologically affected horses recovered completely, and a fifth horse showed significant improvement when treated with acyclovir during a recently reported outbreak of EHV1 myeloencephalopathy involving 19 horses in a riding stable.[51] Although the aforementioned results are promising, one should interpret them with caution because whether treatment with acyclovir actually influenced the outcome in these cases is not known. In addition, the author and his colleagues at the University of California have not experienced a similarly high rate of success. Data describing the pharmacokinetics, bioavailability, and safety of acyclovir in horses are lacking; therefore dosing protocols have been based on extrapolation from dosage regimens used in human beings to treat infections with herpes simplex virus or varicella zoster viruses. The low bioavailability of orally administered acyclovir in human beings necessitates frequent administration of high doses. Accordingly, acyclovir doses of 8 to 16 mg/kg administered 3 times daily for 7 to 12 days were used to treat congenital EHV1 infection in equine neonates,[96] and doses of 20 mg/kg administered 3 times daily for 5 days were used to treat horses with EHV1 myeloencephalopathy without apparent toxicity.[51] The primary route of elimination of acyclovir is renal, and some human beings with renal failure have developed adverse effects to acyclovir, primarily manifesting as 623

624 neurologic disturbances.[97] Therefore one should monitor and maintain renal function when administering acyclovir. Clearly, additional studies are needed to define the appropriate dose for acyclovir in horses and to document its efficacy for the treatment of EHV1 myeloencephalopathy. Another nucleoside analog, penciclovir, has been shown to have excellent activity against EHV1 in tissue culture and in a mouse model of EHV1 infection.[98]

Affected horses that remain standing have a good prognosis for recovery, and improvement generally is apparent within a few days, although a period of several weeks to more than a year may be required before horses with severe deficits show complete recovery.[*] In these instances, control of urination frequently returns before gait abnormalities resolve completely.[72] Some horses may be left with permanent residual neurologic deficits, including urinary incontinence and ataxia, that may necessitate euthanasia many months beyond onset of neurologic signs.[5,43,47,48] Horses that become recumbent have a greatly increased likelihood of developing complications such as myonecrosis, urinary tract infection, decubital ulcers, respiratory tract infection, gastrointestinal obstruction and ulceration, injuries, and complications of dehydration and malnutrition. Their prognosis for recovery is therefore poor, particularly if they remain recumbent for more than 24 hours and they are unable to stand after being lifted with a sling.[4,5] One should not elect euthanasia prematurely in valuable horses, however, because reports document horses standing again and recovering completely to race successfully after being recumbent for several days to 3 weeks.[35,45,49] Most mildly affected mares return to breeding soundness in the same season, whereas fertility is likely to be compromised in more severely affected mares that experience urinary retention.[4] Recurrence or exacerbation of neurologic signs in horses that have recovered completely has not been documented, even though the majority likely remain latently infected.[14,25,43,72]

* References 5, 30, 35, 47, 51, 71, 73, 74.

10.11.8 Pathologic Findings

When horses with suspected EHV1 myeloencephalopathy die or are euthanized, one should submit the whole carcass or at least the head, spine, spleen, thyroid, and lung for postmortem examination because lesions frequently are not confined to the CNS of horses with EHV1 myeloencephalopathy[†] Gross pathologic lesions in the CNS frequently are not found, but small (2 to 6 mm) focal areas of hemorrhage distributed randomly throughout the meninges and parenchyma of the brain and spinal cord may be observed[*] More diffuse dural hemorrhage is notable in some cases and may extend to spinal nerve roots and the cauda equina.[41,42,53] Small plum-colored areas of degeneration and hemorrhage are sometimes grossly visible in fresh tissue at various levels of the spinal cord (white matter), and malacic foci may be visible macroscopically in the gray and white matter in sliced fixed sections of brain.[41,42,49,50,53]

The gross and histologic lesions in the CNS reflect vasculitis, congestion, and secondary ischemic degeneration of nervous tissue[†] Although vasculitis is a consistent finding, degeneration of nervous tissue is evident chiefly in those horses with clinical signs of severe neurologic disease.[36,41,42] The vasculitis is often severe and has a widespread, random, multifocal distribution, with the most severe lesions usually in the brainstem and spinal cord. In the brain the meningeal and penetrating or radiating vessels in gray matter are the major sites of vascular involvement. Thus foci of axonal swelling and malacia develop in the grey and white matter, particularly adjacent to the meningeal surface and in the deep cortex adjacent to the white matter.[41,42,53] In the spinal cord a similar orientation to meningeal vessels results in degeneration of white matter within ovoid, linear, or wedge-shaped foci affecting predominantly the lateral and the ventral white columns.[42] In some instances, sheaths of nerve roots and nerves and capsules of ganglia also are involved.[36,41,42,53] Trigeminal ganglionitis may be present but usually is not manifest clinically.[45]

Ocular lesions, including uveal vasculitis with perivascular mononuclear cell cuffing in the ciliary body and optic nerve or extensive retinal degeneration, have been observed in foals showing signs of bilateral hypopyon and iritis or severe visual impairment and chorioretinopathy without anterior segment involvement during field and experimental EHV1 infections.[4,36,42,53,64] In some foals EHV1 appears to induce ocular and neural damage in the absence of gross signs of neurologic or visual impairment.[64] During paralytic infections, secondary viral replication occurs in blood vessels of the testis and epididymis in addition to CNS and may be responsible for reported signs of scrotal edema and loss of libido in affected stallions.[4,99]

EHV1 infrequently is isolated from the CNS of affected horses that show typical lesions[‡] Thus one should attempt to isolate the virus from other sites to support the diagnosis. Those sites most likely to yield virus or to contain viral antigen include the turbinates and nasal passages, lymph nodes draining the upper respiratory tract, 624
lung, thyroid, spleen, and endometrium, in addition to the brain.[36,43,90] Immunofluorescent antibody testing of 625
brain and spinal cord sections is considered to be more sensitive than virus isolation, but false-negative results have been observed.[36,43,52] An indirect immunoperoxidase method using orthodox light microscopy was described and was shown to be highly sensitive for identifying individual antigen-containing cells in the CNS and other areas of the body, even at sites containing few or no lesions or inclusion bodies and in which virus could not be detected by immunofluorescent antibody testing or virus isolation.[36] Similarly, the PCR technique has been shown to be more sensitive than virus isolation for detecting viral antigen in nasopharyngeal swabs collected from horses with respiratory tract disease caused by EHV1 or EHV4.[83] Both of these techniques—as

well as antigen-capture ELISA tests, dot immunobinding, and in situ DNA hybridization, which are sensitive and readily differentiate between EHV1 and EHV4—show great promise for routine application to samples collected ante mortem or at necropsy from affected horses.[36,62,81–84,88]

† References 4, 14, 27, 36, 41, 42, 53.

* References 14, 36, 42, 49, 50, 53.

† References 36, 41, 42, 49, 50, 53.

‡ References 32, 36, 39, 43, 46, 49, 50, 61, 90, 100.

10.11.9 Control and Prevention

Control measures during outbreaks of EHV1 infection aim at reducing spread by infectious aerosols, direct contact, and fomites and at reducing stress-induced recrudescence of latent EHV1 infection.[1,2,14] If neurologic signs or other clinical signs suggestive of EHV1 infection occur, one should isolate affected animals promptly and completely in a well-ventilated airspace separate from the remainder of the herd, and one should isolate in-contact horses in their current location as one or more small groups for at least 1 month and pregnant mares preferably until they foal.[1–4,14,35,75] On breeding farms, one should suspend covering.[4] Aborted fetuses and fetal membranes are rich sources of infectious virus and therefore should be collected at the site they are found and placed in leak-proof containers such as heavy gauge plastic bags for submission for diagnostic evaluation or for disposal by burning.[1,2] Similarly, bedding and dirt contaminated with fetal fluids should be disposed of or burned, and stalls or other areas occupied by infected horses should be cleaned thoroughly, disinfected with an iodophor or a phenolic product, and left empty for several weeks.[14] Equipment used to handle, groom, feed, water, muck out, or transport affected horses also should be cleaned and disinfected or disposed of. Thereafter separate equipment and personnel should be used for affected and unaffected horses, or at least caretakers should handle affected horses last and wear disposable gloves, surgical masks, and protective clothing that can be changed or disinfected between groups.[14] Although control measures are frequently successful in stopping further spread of infection during outbreaks, one should note that transmission of infection before control measures are implemented may result in a secondary waves of disease 1 to 2 weeks later.[4,14,35]

Traffic of horses and human beings on the premises should be minimized, and movement of horses onto and off the infected premises should be suspended until at least 3 weeks after resolution of acute signs in the last clinical case or until tests show that virus transmission is no longer occurring.[1–4,8,14] Collection of nasal swabs and uncoagulated blood (buffy coat) samples from clinically affected and exposed horses within each group and demonstration of stable or declining antibody titers in serum samples proved to be helpful in determining patterns of exposure and spread and in establishing when virus transmission had ceased in one reported outbreak. Protracted viremia lasting several weeks or months occurs in some horses and extends the period during which movement of horses should be restricted.[4]

If horses must enter the farm, they should be current on EHV1 vaccination and should be isolated away from the resident population. Although giving booster vaccinations to exposed pregnant mares during outbreaks of abortigenic EHV1 infection is common practice,[1,2,14] vaccination of exposed horses during outbreaks of EHV1 myeloencephalopathy has not been investigated and cannot be recommended at this time because of the possibility of an immune-mediated pathogenesis. Administering booster doses of inactivated EHV1 vaccine to all unexposed horses that have not been vaccinated within the previous month is common practice, however.

Preventive measures should include routine management practices aimed at reducing the chances of introducing and disseminating infection.[3,14,63,101] New arrivals should be isolated for at least 3 weeks before joining the herd, distinct herd groups should be maintained based on the age and use of horses, and care should be taken to minimize or eliminate commingling of resident horses with visiting or transient horses. In particular, pregnant broodmares should be maintained in groups separate from the remaining farm population. In addition, minimizing stress associated with overcrowding and handling procedures is prudent in an attempt to reduce recrudescence of latent EHV1 infection.[14,25,63,101]

No method is known that reliably prevents the neurologic form of EHV1 infection. None of the EHV1 or EHV4 vaccines currently available carry a claim that they prevent EHV1 myeloencephalopathy, and the disease has been observed in horses vaccinated regularly at 3- to 5-month intervals with inactivated or modified live vaccines.[8,43,48,50,51] Repetitive administration of currently available EHV1 and EHV4 vaccines appears to induce some immunity to respiratory disease and reduce the incidence of abortion but does not block infection 625
and induction of viremia or eliminate the possibility of clinical disease and establishment of the carrier state. 626
[1,2,14,50,101–106] Although the protection induced by inactivated EHV1 vaccines is incomplete and of short duration, the vaccine reduces virus excretion in horses that do become infected.[103,106] To maintain appropriate vaccination procedures in an attempt to reduce the incidence of other manifestations of EHV1 infection and reduce the magnitude of challenge experienced by in-contact horses is logical. This indirectly may help prevent EHV1 myeloencephalopathy.[43] An attenuated live virus vaccine based on the temperature-sensitive and host range mutant clone 147 of EHV1 has been evaluated recently. The novelty of this vaccine lies in the fact that a low dose administered intranasally replicated in conventional target species and conferred exceptional efficacy against respiratory disease, virus shedding, viremia, and abortion caused by a severe EHV1 challenge.[107,108] The intranasal vaccine also claims to protect against less common manifestations of EHV1 infection such as paresis and jaundice (Patel, et al., unpublished).

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10.12 10.12—Polyneuritis Equi

William J. Saville

Polyneuritis equi is an uncommon neurologic disease of all equine species that is characterized by tail and anal sphincter paralysis, often accompanied by cranial and peripheral nerve damage.[1–13] Previous reports referred to the disease as neuritis of the cauda equina because of the susceptibility of this region, but frequent involvement of the 628
629 cranial and peripheral nerves led to the term *polyneuritis equi*.[2] Although the disease has been recognized more readily in Europe, where it was first reported by Dexler in 1897, cases have now been reported in Great Britain, Canada, and the United States.[3,4,6,14] The disease does not appear to have a breed, sex, or age predilection, but the youngest horse affected was 17 months of age.[2,5,6,13–15]

The cause of this disease is unknown. Primary immune reaction and viral inflammatory disease have been suggested, although possibly one may be a consequence of the other.[2] Several infectious agents have been suggested, such as equine herpesvirus type 1, equine adenovirus, and streptococcal bacteria.[10,12,16] The pathologic lesions resemble those of Guillain-Barré syndrome in human beings, and the disease is also similar to experimental allergic neuritis in rats.[10,13] Evidence suggests that the immune system is involved because horses with polyneuritis equi have circulating antibodies to P2 myelin protein, which is present in rats with experimental allergic neuritis.[10,17] The significance of this is that polyneuritis equi may be an inflammatory and an immune-mediated disease.

10.12.1 Clinical Signs

The disease often manifests itself in two forms: (1) the acute signs include hyperesthesia of the perineal or head region or both, and (2) in the chronic form, horses show paralysis of the tail, anus, rectum, and bladder. Paralysis often is accompanied by fecal and urinary retention, urinary scalding of the hindlimbs, and in male horses, penile paralysis[*]

The hindlimb signs in affected horses are often symmetric, whereas the head signs are often asymmetric.[6,13,15] Muscle atrophy in the gluteal region is sometimes present along with mild degrees of ataxia.[2,3,5,6,9] Muscle atrophy associated with cranial nerve involvement may occur in the head region. Damage to peripheral motor nerves may result in gait deficits and abnormal use of forelimbs or hindlimbs.[1,12,13,15]

Although cranial nerve involvement is reported primarily to affect cranial nerves V, VII, and VIII, any of cranial nerves II, III, IV, VI, IX, X, and XII also may be involved.[2,4,5,8,13] The horses may have trouble with mastication and swallowing.[12] A head tilt, ear droop, lip droop, and ptosis are common signs.[8,12,13] One report describes a horse with brachial neuritis along with involvement of cranial nerves V, VII, and XII. The horse also exhibited mild ataxia and weakness in all limbs. The horse performed the hopping test poorly on the right thoracic limb, and the horse resented palpation in the right caudal cervical and prescapular region.[2]

Colic caused by fecal retention may be the primary sign when one initially examines horses with polyneuritis equi. Fecal retention leads to an impaction caused by the flaccid anal sphincter, often accompanied by an atonic, distended bladder.[1] If the clinician sees these signs in the acute or hyperesthetic form, they usually progress to the chronic form of hypalgesia or anesthesia. An area of hyperesthesia may surround the area of anesthesia.[1,2,8]

* References 1–3, 6, 8, 9, 12, 13, 15.

10.12.2 Diagnosis

The definitive diagnostic test is a postmortem examination. The peripheral white blood cell count usually reveals a mature neutrophilia with hyperfibrinogenemia, mild to moderate anemia, and an increased total protein—all indications of a chronic inflammatory process.[3,5,13,15] Examination of the cerebrospinal fluid may reveal an elevated protein (70 to 300 mg/dl) along with an elevated white blood cell count, which indicates a mononuclear inflammatory reaction, although cytologic examination of cerebrospinal fluid may be normal, particularly in the acute stage of the disease[*]

Radiography may be required to rule out trauma to the tail head or cranial nerve involvement such as a fractured petrous temporal bone.[2,8]

Some horses with clinical signs exhibit circulating P2 myelin antibody in the serum.[10,17] However, the presence of this antibody only supports the diagnosis; the same antibody has been detected in horses with equine herpesvirus 1 and equine adenovirus infections[†]

Classically, the primary pathologic lesions involve the extradural nerve roots but also may involve the intradural nerve roots.[2,3,5,12,13] The lesions are granulomatous with various degrees of inflammation and infiltration of lymphocytes, eosinophils, macrophages, giant cells, and plasma cells. This inflammation leads to myelin degeneration, subsequent axonal degeneration, and thickening of the epineurium, endoneurium, and perineurium with proliferation, which causes obliteration of the neural architecture by the fibrous tissue.[1,2,9,13] The most severe lesions are in the cauda equina, but swelling, edema, and hemorrhage of cranial nerves may occur. The fibrous tissue formation may lead to adhesions between the meninges and the periosteum of the vertebral bodies.[13] Reports describe involvement of the autonomic nervous system, but no changes in clinical signs have been reported (post mortem only).[2,4]

The polyneuritis lesions are typical of the Guillain-Barré syndrome in human beings, experimental allergic neuritis in rats, and coonhound paralysis in dogs.[2,5,11–13] This similarity may indicate a combination of 629
inflammatory and immune-mediated mechanisms in the pathophysiology of polyneuritis equi. 630

* References 2, 3, 5, 6, 8, 11, 13.

† References 1, 2, 10, 13, 16, 18.

10.12.3 Differential Diagnosis

The most important differential disease is trauma to the sacrococcygeal area of the spinal canal, which can be differentiated by radiography of the area looking for fractures or displacements.[2,6,8]

Equine protozoal myeloencephalitis is the second most common disease in the differential diagnosis of polyneuritis equi. The usual signs of equine protozoal myeloencephalitis include asymmetric damage in the limbs and brain and brainstem lesions causing cranial nerve deficits with alterations in attitude, whereas the cranial nerve deficits of polyneuritis equi are peripheral, with no change in attitude.[6] This disease may be differentiated from polyneuritis equi by Western blot analysis of the cerebrospinal fluid.[19]

Equine herpesvirus (type 1) myeloencephalitis often has an acute onset following an episode of fever, cough, and nasal discharge or following one or more abortions on a farm. This condition often affects more than one horse on a farm. Herpesvirus has a rapid onset and often results in severe hindlimb weakness and ataxia along with bladder dysfunction. Urinary dribbling sometimes may occur. The ataxia and weakness is usually symmetric and may result in recumbency. Occasionally, the horses sit like a dog. Cranial nerve involvement is not common.[20–23]

One should consider verminous myeloencephalitis; the signs vary and depend on the migratory pathway of the parasite. Diffuse or multifocal brain and spinal cord lesions have been reported. The onset is usually sudden with rapid deterioration and death. The incidence of this disease is low, perhaps because of more intense parasite control.[24–26]

One should consider equine motor neuron disease in the differential diagnosis. Horses with motor neuron disease have symmetric muscle wasting or atrophy and weight loss with significant weakness, sweating, and muscle fasciculations. However, these horses are not ataxic and their unique clinical feature is that they walk better than they stand. This disease is a denervation atrophy of type 1 muscle fibers only and may be diagnosed by a spinal accessory nerve biopsy or sacrodorsalis caudalis muscle biopsy.[27]

10.12.4 Treatment

The primary therapy is palliative. No treatment for the disease is known. Removing feces from the rectum and evacuating the bladder are usually necessary. If cystitis caused by bladder distention occurs, systemic antibiotics may be indicated. Some attempts have been made at treating the inflammation with corticosteroids, but the effects have been short lived. The prognosis is usually poor, but the progression of the disease is slow. Some animals may be maintained for many months[*]

* References 2, 4–6, 8, 12, 13, 15.

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10.13

10.13—Togaviral Encephalitis

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Togaviridae are small, lipid- and protein-enveloped RNA viruses. Several insect-borne viruses (arboviruses) of the genera *Alphavirus* and *Flavivirus* have been associated with encephalitis in horses. The structure of the viruses and associated clinical presentations are similar among the viruses, but the epizootiology and antigenicity are distinct. *Alphavirus* species (formerly arbovirus group A) tend to be more infectious and more often are associated with epidemics compared with *Flavivirus* species (formerly arbovirus group B). *Flavivirus* species tend to be associated with sporadic outbreaks. In the Togaviridae, equine arteritis virus, a *Rubivirus* species, has been associated with some neurologic deficits and central nervous system edema,[1] which is discussed elsewhere.

In general, birds, rodents, and reptiles act as reservoirs. Mosquitoes often play a role in transmitting the disease among these species. Mosquitoes and, less commonly, other insects feed on sylvatic hosts and subsequently transmit the disease to horses and human beings.

10.13.1

Alphavirus Encephalitides: Eastern, Western, and Venezuelan Equine Encephalomyelitis

The predominant togaviral encephalitides in the Western Hemisphere are associated with eastern (EEE), western (WEE), and Venezuelan (VEE) equine encephalitis (Table 10.13-1).

10.13.1.1

CAUSE

EEE and WEE are specific and discrete togaviral particles. North and South American antigenic variants of EEE exist. WEE variants include eastern, western, and Highlands J strains. The various strains have equivocal differences in antigenic properties and biologic behavior, and extensive geographic overlap occurs.[2] The molecular basis for the antigenic variation between EEE and WEE has been described.[3] VEE virus has four subtypes. Type I variants A, B, and C are associated with disease and epidemics. Type I variants D, E, and F and types II, III, and IV are endemic and usually not associated with clinical disease.[4–7]

10.13.1.2

EPIZOOTIOLOGY

Encephalitis similar to the viral encephalitides has been reported in the United States for many years with high morbidity and mortality rates.[8,9] Evidence that an eastern and western virus exist and are antigenically distinct was first reported in 1933.[10] Horses immunized with strains of virus isolated from infected horses from the East or West were protected differentially when vaccinated with attenuated virus from one location and exposed to virus from the other.[10–12]

10.13.1.2.1 Distribution

In general, disease associated with EEE, WEE, and VEE is restricted to the Western Hemisphere and ranges from temperate to desert climates. Each virus and incidence of associated equine disease has a characteristic distribution. The range of positive serologic tests for the viruses is often far greater than the range of clinical disease[13–39] (Figure 10.13-1). For example, clinical disease associated with VEE was identified in southern Texas in 1971. However, asymptomatic horses with significant titers for VEE (type II) often are identified in Florida where the disease syndrome has not been evident.[16] The WEE virus is recognized in reservoir avian hosts in the eastern United States; however, clinical disease rarely is identified there. Geographic variation in viral virulence may be an explanation. Some importance of the vector in eliciting a more virulent virus lies in the fact that the vector for WEE in the East is *Culiseta melanura,* which is the common vector for EEE. This mosquito may not be able to generate the virulent state of WEE, for clinical disease in that region is rare. Equine disease associated with WEE is rare on the eastern seaboard of the United States but is recognized.[40] Outside the Western Hemisphere, EEE has been identified in the Philippines,[41] and some indications point to its presence in Europe.[42]

10.13.1.2.2 Epidemic

Several requirements must be met for epidemics associated with EEE, WEE, or VEE to develop. Long 631
lapses can occur between outbreaks if all of these conditions are not met. These prerequisites include 632
adequate and adjacent numbers of reservoir animals, sufficient quantities of virulent viruses, infected intermediate hosts, insect vectors, and susceptible horse and human populations.[43,44] Prediction of outbreaks has been attempted but without success,[36,45,46] which indicates that other, unknown factors may exist.

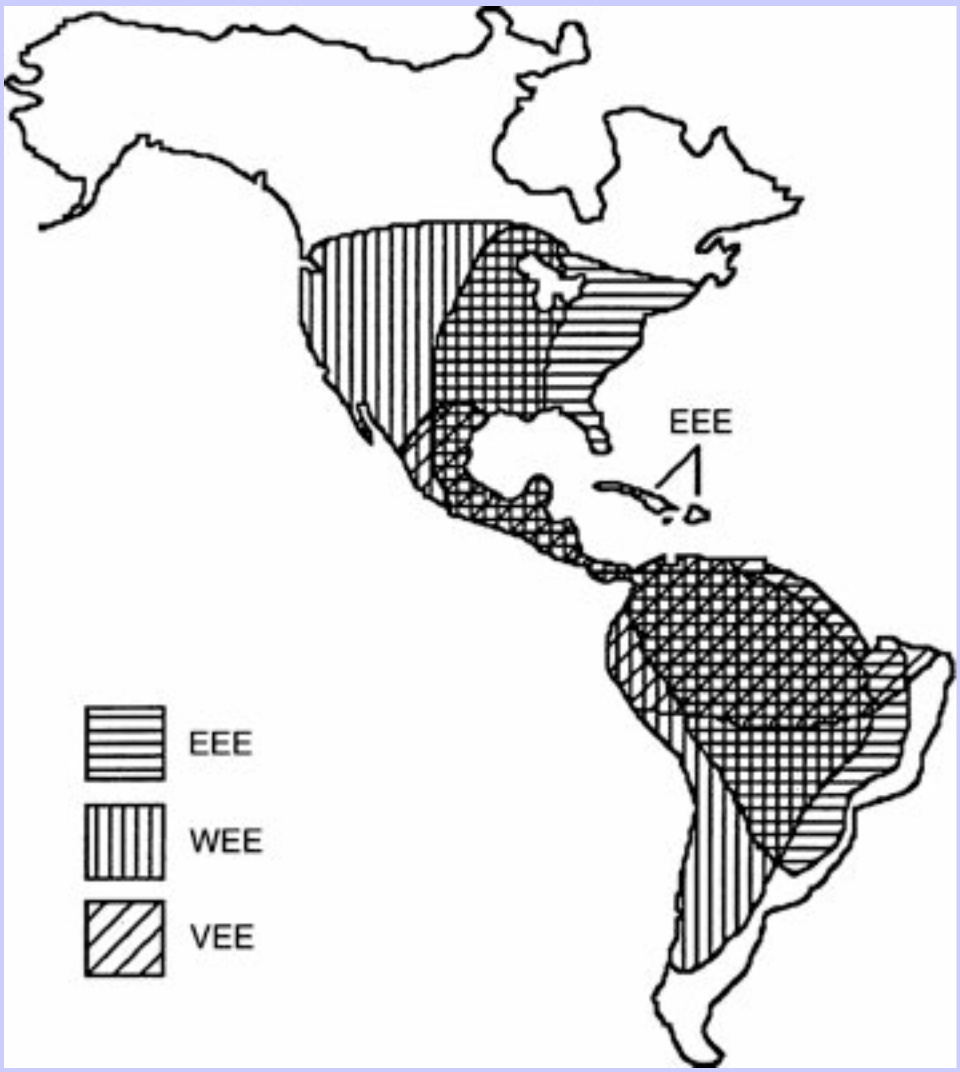
TABLE 10.13-1 A Summary of the Major Togaviral Equine Encephalitides[*]

VIRUS[†]	MAJOR DISEASE VECTOR	ZOONOTIC POTENTIAL	AMPLIFICATION FROM HORSES	DISEASE SPREAD	VIREMIA	EQUINE MORTALITY	HUMAN MORTALITY
EEE	Aedes spp.	Unlikely	Unlikely	Vector	Low	75%-100%	50%-75%
WEE	Culex tarsalis	Unlikely	Unlikely	Vector ±secretions	Low	20%-50%	5%-15%
VEE	Culex melanoconium, Aedes spp. Phosphora spp.	Occurs	Occurs	Vector ±secretions	High	40%-80%	1%

* The statements made are generalizations, and some degree of variation occurs.

† EEE, Eastern equine encephalitis virus; WEE, western equine encephalitis virus; VEE, Venezuelan equine encephalitis virus.

Figure 10.13-1 Predominant distribution of *Alphavirus* spp.–associated equine encephalitis in the Western Hemisphere. This figure represents the disease distribution. Positive serologic results for the diseases are more widespread. *EEE,* Eastern equine encephalitis virus; *WEE,* western equine encephalitis virus; *VEE,* Venezuelan equine encephalitis virus.



10.13.1.2.3

Reservoirs

With minor exceptions, Togaviridae persist by asymptomatically infecting wild animals (sylvatic hosts) such as birds, small mammals, and reptiles by unknown mechanisms.[47] The viruses survive during the winter or nonvector season in sylvatic populations.[46,48]

10.13.1.2.4

Vectors

Specificity of the viruses for particular vectors occurs. Vector distribution explains viral distribution to a large degree. The vectors for EEE include *C. melanura* and *Aedes* species.[13,29,49,50] *C. melanura* for the most part is confined to freshwater swamps, feeds primarily on swamp birds, and rarely is found in areas of increased horse populations.[13] In general, *C. melanura* serves as the vector for the enzootic cycle, which involves swamp birds. *Aedes* species appear to be more important in epizootics and epidemics.

In Florida, WEE persists continuously in *C. melanura* (see the previous discussion).[13] Important WEE vectors include *Culex tarsalis, Dermacentor andersoni,*[51,52] and *Triatoma sanguisuga* (assassin bug).[53,54] The cliff swallow bug *(Oeciacus vicarius)* may be an overwintering reservoir[51,55] (see the following discussion).

The vectors for VEE include *Culex melanoconium,* and *Mansonia, Aedes,* and *Phosphora* species.[16,17,23,24]

10.13.1.2.5

Virulence Induction

Some puzzles exist in light of the ability of some mosquitoes and other insects to carry two or three viruses and the inability of these viruses to induce disease. Some form of virulence induction specific to the viruses may occur. The existence or mechanism of virulent mutation is controversial. The intermediate host may play a key role in this process, but that is speculative.

10.13.1.2.6

Vector Ecology

Vector transmission is the most important way infection spreads. WEE and VEE may spread by nasal secretions, but this is less likely.[16,17,54,56] 632

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Vectors transmit viral particles between sylvatic hosts when taking a blood meal. If the virus is able to penetrate the gut of the vector, it may pass through the hemolymph to oral glands, multiply, and subsequently be shed in saliva and other oral secretions. If the blood meal contains adequate numbers of viral particles, multiplication may not be required for transmission. In most instances and judiciously assumed, the mosquito remains infected for life.[50,57]

10.13.1.2.7

Seasonal Incidence

In most instances the diseases occur during the height of the vector season. In temperate climates the highest number of cases occurs between June and November. In warm climates, where the vector season is longer, the disease problem lasts longer[*]

* References 23, 27, 30, 31, 50, 54, 57.

10.13.1.2.8
Zoonology

Clinical infection in human beings usually involves old or young persons. Signs, symptoms, and morbidity and mortality rates are virus-specific.[58]

Circulating virus concentrations are often too low for transmission of EEE from infected horses to human beings, mosquitoes, and other animals. Human disease most likely is associated with insect vector contact and often coincides with or is preceded by equine epizootics.[45] In the acute stages of equine disease, a transient, substantial viremia occurs. Therefore if vector density increases, an acutely infected horse could be a transient amplifier of EEE. Spread from horse to horse is possible.[49] Clinical signs in human beings include acute fulminant encephalitis, headache, altered consciousness, and seizures. The mortality rate is 50% to 75%.[58]

Human beings and horses are terminal hosts for WEE. Human cases of WEE occur yearly, but fatality and incomplete recovery are rare. These cases are associated with vector contact. Environmental conditions that decrease exposure to insects decrease the incidence of disease.[59] Increased numbers of animals with clinical equine disease are an indication of heavy sylvatic concentrations of virus and are not a potential source of infection for human beings. Generally, increased numbers of horse cases precede cases in human beings by 2 to 5 weeks.[60] Thus horses are sentinels for human beings in a given area. Clinical signs in human beings include fever, headache, confusion, stupor, and seizures, with a 5% to 15% mortality rate.[58]

Horses with VEE have sufficient circulating viral concentrations that they act as amplifiers of disease.[23,61] Ocular and nasal secretions from infected horses contain high concentrations of VEE.[16,17] Infection via entry through the respiratory tract may occur by direct contact with infected animals. Equine and human survivors of VEE infection and clinical disease may develop chronic relapsing viremias and serve as chronic disease amplifiers.[61] Clinical signs in human beings include fever, headache, myalgia, and pharyngitis. The mortality rate is 1%.[58]

With any of the alphaviral equine encephalitides, sufficient viral particles for infection may be present in central nervous and other tissues, and one should take precautions when performing a necropsy examination on suspect cases. Strict mosquito control and vaccination can prevent human and equine cases, and all equine cases should be reported to state health officials.[45,61]

10.13.1.2.9
Other Domestic Species

Calves may be affected naturally and experimentally with EEE.[62,63] Pigs may be affected by EEE[64] and VEE.[65] The signs of disease in these species are similar but are milder than disease in horses for the respective viruses. Burros and mules may contract all three diseases, and the disease is as severe as that identified in horses.[66]

10.13.1.3 PATHOGENESIS

After viruses are inoculated, they multiply in muscle, enter the lymphatic circulation, and localize in lymph nodes. Viruses replicate in macrophages and neutrophils and subsequently are shed in small numbers. Many of the viral particles are cleared at this time. If clearance mechanisms are successful, no further clinical signs develop. Neutralizing antibodies still will be produced. Several mechanisms of viral immunologic avoidance exist and include erythrocyte and leukocyte absorption. If viral elimination is not complete, the remaining viruses infect endothelial cells and concentrate in highly vascular organs such as the liver and spleen. Viral replication in these tissues subsequently is associated with circulating virus. The second viremic period often is associated with early clinical signs of disease. Infection of the central nervous system occurs within 3 to 5 days.[6,17,67,68]

10.13.1.4 CLINICAL SIGNS

Clinical signs are more profound in unvaccinated animals.[60,69] Acute clinical signs of EEE and WEE are nonspecific and include mild fever to severe pyrexia, anorexia, and stiffness. Viremia occurs during this period. After an experimental dose of EEE and WEE comes a 1- to 3-week incubation period. The incubation period is often shorter with EEE than WEE. Early signs of the diseases include fever and mild depression. This stage is transient and, presumably, often undetected because of lack of overt clinical signs. The acute signs may last for up to 5 days after they first manifest. Many cases of WEE do not progress beyond this point. 633
With EEE, progression is more common. Once nervous signs develop, the viremia is past, and animals are 634
unlikely to be able to amplify the disease. In progressive cases the fever may rise and fall sporadically. Cerebral signs may develop at any time but often occur a few days after infection. Acute signs often range from propulsive walking, depression, and somnolence to hyperesthesia, aggression, and excitability. Some horses may become frenzied after any sensory stimulation. Often conscious proprioceptive deficits are evident in the early stages. With progression, signs become less disparate and more consistent between EEE and WEE. The later signs are evidence of the dynamic nature of these conditions and increased severity of cerebral cortical and cranial nerve dysfunction. These signs include head pressing, propulsive walking, blindness, circling, head tilt, and facial and appendicular muscle fasciculations. Paralysis of the pharynx, larynx, and tongue is common. Death often is preceded by recumbency for 1 to 7 days. Comatose animals rarely survive. If animals are to survive, they show gradual improvement of function over weeks to months.[69–72]

VEE may have similar or different clinical presentations compared with WEE and EEE, which is most likely because of the difference in strain pathogenicity.[16,68] The pyrexia in cases of VEE peaks early and remains increased through the course of the disease. In experimental disease, endemic strains are associated with mild fever and leukopenia. Epidemic strains are associated with severe pyrexia and leukopenia.[6,68] Diarrhea, severe depression, recumbency, and death may be prominent before neurologic deficits are evident. Neurologic signs occur at approximately 4 days following infection. Other associated signs include abortion, oral ulceration, pulmonary hemorrhage, and epistaxis.[16,17]

10.13.1.5 DIAGNOSIS

The presumptive diagnosis is based on findings at clinical presentation and the presence of associated epidemiologic features. Serologic and necropsy evaluation provide a definitive determination.

10.13.1.5.1

Clinical Immunology and Virology

One usually identifies viral infections by complement fixation, hemagglutination inhibition, and cross-serum neutralization assays. A combination of these techniques increases the likelihood of a positive diagnosis.[73] A fourfold rise in antibody titer in convalescent sera commonly is recommended for diagnosis. However, one possibly may not detect a rise in titer. Viral antibodies are commonly present within 24 hours after the initial viremia, and their presence often precedes clinical encephalitis.[42] The concentration of antibodies increases rapidly and then decreases over 6 months.[74] An initial sample often is taken when encephalitic signs are present, which may be after titers have peaked. Therefore a second sample possibly may have a decreased titer compared with the initial sample. If increased titers exist for hemagglutination inhibition, complement fixation, and neutralizing antibody, then one can make a presumptive diagnosis on a single sample.[73] In the case of suspected VEE, an enzyme-linked immunosorbent assay can detect viral-specific immunoglobulin M antibodies to surface glycoprotein by 3 days after the onset of clinical signs. These antibodies are not produced in response to vaccine. The antibodies disappear by 21 days following infection.[74] One should use the assay for confirming acute VEE infection when one cannot collect convalescent serum samples. Viral cultures are unlikely to be fruitful, except in the case of acute VEE. One may isolate the virus from cerebrospinal fluid of horses with acute infections.[17] The usefulness of cerebrospinal fluid viral titers in light of a negative viral isolation is questionable. Fluorescent antibody, enzyme-linked immunosorbent assay, and viral isolation are useful in identifying virus in brain tissue.[75,76]

Colostral antibodies may interfere with diagnosis in foals. The antibody titers to VEE, WEE, and EEE viruses in the sera of 2- to 8-day-old foals are similar to those of dams. The serum half-life of maternal antibodies in foals is approximately 20 days.[77]

10.13.1.5.2

Clinical Pathology

The cerebrospinal fluid changes associated with togaviral infections are similar to those of other viral encephalitides and include increased cellularity (50 to 400 mononuclear cells per microliter) and protein concentration (100 to 200 mg/dl).

10.13.1.5.3

Necropsy Findings

For animals that die or are euthanized, one should perform necropsy and gross and histologic examinations with special reference to the central nervous system. The brain and spinal cord often have a normal gross appearance. In some cases vascular congestion and discoloration of the central nervous system is evident. Histologic findings include nonseptic mononuclear cell and neutrophilic inflammation of the entire brain.[16,78–80] Severe lesions are evident in the cerebral cortex, thalamus, and hypothalamus. Specific lesions include significant perivascular cuffing with mononuclear and neutrophil cell infiltration, gliosis, neuronal degeneration, and mononuclear cell meningeal inflammation. With VEE, liquefactive necrosis and hemorrhage of the cerebral cortex, atrophy of the pancreatic acinar cells, and hyperplasia of the pancreatic duct cells commonly occur.[79]

10.13.1.5.4

Differential Diagnosis

The differential diagnosis for EEE, WEE, and VEE should include other conditions associated with diffuse or multifocal neurologic deficits such as other togaviral encephalitides, trauma, hepatoencephalopathy, rabies, leukoencephalomalacia, bacterial meningoencephalitis, equine protozoal myeloencephalitis, and verminous encephalitis. 634 635

10.13.1.6

TREATMENT

No known effective, specific treatment of the viral encephalitides exists. Treatment is primarily supportive. Nonsteroidal antiinflammatory drugs (phenylbutazone, 4 mg/kg every 12 hours; flunixin meglumine, 1 mg/kg every 12 to 24 hours) control pyrexia, inflammation, and discomfort. Dimethyl sulfoxide given at 1 g/kg intravenously in a 20% solution may be useful in controlling inflammation and provides some analgesia and mild sedation. The use of corticosteroids is controversial because beneficial effects are short term and the risk of developing secondary bacterial infections increases. One may control convulsions with pentobarbital, 0.05 to 2.2 mg/kg diazepam administered intravenously, 2 mg/kg phenobarbital given orally, or 0.2 to 1.0 mg/kg phenytoin administered intravenously. If horses develop secondary bacterial infections, one should use appropriate antibiotic therapy. One should monitor hydration and administer balanced fluid solutions orally or intravenously as needed. Other supportive care should include dietary supplementation and administration of laxatives to minimize the risk of gastrointestinal impaction. If anorexia persists for more than 48 hours, one should use enteral or parenteral supplementation; one can use commercial formulations. For the short term, one may put pelleted feeds into suspension for oral administration. Protection from self-induced trauma may require protective leg wraps and head protection. If the horse is recumbent, one should attempt to provide support in a sling, and all animals should be bedded heavily.

10.13.1.7

PROGNOSIS

Complete recoveries from the neurologic deficits associated with these viruses are reported, but they are rare.[81] Animals that have recovered from EEE often have residual neurologic deficits that commonly include ataxia, depression, and abnormal behavior. Neurologic sequelae are similar but less common in horses that recover from WEE. For horses that develop neurologic disease, the mortality rate for EEE is 75% to 100%; for WEE, 20% to 50%; and for VEE, 40% to 80%.[16,42] If horses recover from any of the diseases, they seem to be protected variably for up to 2 years after infection. One would wisely assume that infection affords no protection.

10.13.1.8

PREVENTION

Prevention of these diseases should aim at reducing the concentration of insect vectors and implementing vaccination programs.[33–35,82–84] Most vaccines are killed (formalin-inactivated) viruses of chick tissue culture origin. Significant increases in antibody titer occur at 3 days after vaccination.[16,17,85–88] One should vaccinate susceptible horse populations with monovalent, divalent, or trivalent vaccines containing EEE, WEE, or VEE. Administration of trivalent vaccines increases specific antibody production to all viruses. Some cross-protection exists between EEE and WEE and between EEE and VEE, but none between WEE and VEE.[77,89,90] If one is to give VEE vaccine, simultaneous administration of all three vaccines is recommended.[91,92] The response to VEE vaccination alone is poorer in horses previously vaccinated against WEE and EEE.

[87,88,92,93] VEE vaccination does not seem to interfere with responses to EEE or WEE vaccination.[94] One should complete annual vaccinations in late spring or several months before the beginning of the encephalitis season. Adequate titers appear to last for 6 to 8 months. In areas in which the mosquito problem is prolonged or continuous, biannual or triannual vaccination is suggested. Vaccination of susceptible horses in the face of an outbreak is recommended. If vaccinated horses develop disease, the affected individuals are often young or old. Vaccination of mares 1 month before foaling enhances colostral antibody concentrations. Antibody concentrations in foals born to immunized mares appear by 3 hours after colostrum is fed and persist for 6 to 7 months.[77] Vaccination may begin at any age, but if they are vaccinated early, one should revaccinate foals at 6 months and 1 year to ensure adequate protection. Foals respond to vaccination with VEE in utero.[95]

Owners should use insecticides and repellents when possible and practical, should eliminate standing water, and in endemic areas or during an outbreak, should implement environmental insecticide application and should screen stalls. Horses with VEE can be persistently viremic and should be quarantined for 3 weeks after complete recovery. Cases of VEE must be reported in the United States. Other measures of disease control may be instituted by public health officials.

10.13.2 Flavivirus Encephalitis

Flavivirus species are associated sporadically with encephalitis in the United States and abroad. Seroconversion is often evident, but clinical disease is rare.

10.13.2.1 JAPANESE B ENCEPHALITIS

Sporadic cases of equine encephalitis have been observed, and serum neutralization tests have indicated the presence of Japanese encephalitis in affected horses.[96–102] In most instances, Japanese B encephalitis is a disease of human beings, and human beings are the source of infection for animals. 635

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The disease is distributed widely throughout the eastern Pacific Rim. As in the case of most togaviral encephalitides, mosquitoes are the natural vectors. The mosquitoes *Culex tritaeniorhyncus* and *Culex pipiens* are the principal vectors. The virus may overwinter in these mosquitoes.[103] In general, horses are dead-end hosts, but horse- to-horse transmission by mosquitoes is reported.[104] A cycle that involves human beings, mosquitoes, and pigs maintains the infection throughout the year. Wading birds such as egrets are the major sylvatic hosts.[105] In pigs, which are a major reservoir, the disease is associated with abortion and nonsuppurative encephalitis in animals less than 6 months of age.[106] Subclinical infection occurs in cattle, sheep, and goats.[107]

Clinical signs of the disease in horses vary widely in presentation and severity. Mild signs are pyrexia, depression, and icterus for a few days. More severe signs include severe pyrexia and depression, icterus, and petechiation of mucosal surfaces. Dysphagia and ataxia are common. Transient signs may include radial paralysis, blindness, hyperexcitability, profuse sweating, and muscle tremor. These severe signs are uncommon but when present often precede death. In most cases, complete recovery occurs in 5 to 10 days. The disease is differentiated from other virus-associated encephalitides of horses by serologic and virus isolation tests.[104]

Formalin-attenuated vaccines protect against natural and experimental encephalitis in pigs and horses.[108]

10.13.2.2 BORNA DISEASE

Borna disease, first identified in Germany, and Near Eastern equine encephalomyelitis, found in the Middle East, are indistinguishable structurally and antigenically. The virus has a strict tropism for neural tissues, where it can persist indefinitely. The method of viral transmission is unknown but is thought to be by inhalation,[109] ingestion, or tick (*Hyalomma anatolicum*) transmission.[110] Other vectors may exist. The infection of horses is often accidental and outside an apparent tick and wild bird cycle. Morbidity is low, but mortality is high. Borna disease in Germany may be associated with transfer of virus from the Near East to Europe by migratory birds.

The signs of disease are similar to other equine encephalitides. The incubation period is unusually long and can range from 4 weeks to 6 months. The long incubation is due to the time required for transport of the virus in dendrite and axonal processes from the site of inoculation to the hippocampus. Necropsy findings are similar to other viral encephalitides, but intranuclear inclusion bodies in nerve cells of the hippocampus and olfactory lobes of the cerebral cortex are characteristic.[109,111]

The disease has zoonotic potential.[110,112,113] A vaccine is available that appears to be protective for horses.[110,114]

10.13.2.3 OTHER TOGAVIRIDAE

The following viruses have been classified as *Flavivirus* species. Some have been classified into other groups of viruses from time to time.

California encephalitis is caused by a group of closely related viruses. California serogroup viruses often are classified as Bunyaviridae, as well. The important vectors seem to be *Aedes dorsalis, A. triseriatus,* and *Culex tarsalis,* and they can be associated with encephalitis in horses (snowshoe hare virus).[115] Seroconversion without clinical disease is widespread.[43,60,87,116–121] In the rare instance when horses develop clinical disease, they often recover completely within 7 to 10 days. Pyrexia and depression appear to be common signs.[116,120–122]

St. Louis virus is associated most commonly with encephalitis in human beings and may be involved in equine disease. Experimental inoculation in horses produces viremia but no clinical signs. Neutralizing antibody is often present. *Culex pipiens* and *C. tarsalis* are the major vectors. Wild birds seem to be the primary reservoir.[26,123–125]

West Nile virus meningoencephalitis occurs in horses in the French Mediterranean. Seroconversion without evidence of disease occurs in human beings, sheep, pigs, and birds. *C. molestus* appears to be the important vector.[126–128]

Powassan virus has been associated with nonsuppurative, focal necrotizing meningoencephalitis in horses. Antibodies for Powassan virus commonly are identified in Ontario and the eastern United States. *Ixodes cookei, I. marxi,* and *Dermacentor andersoni* appear to be important vectors, with snowshoe hares and striped skunks as major reservoirs. Zoonoses occur after bites by infected ticks. Approximately 13% of horses sampled across Ontario in 1983 were serologically positive for the virus.[129] Experimental infection with Powassan virus strain M794 in horses was associated with neurologic deficits within 8 days. A nonsuppurative

encephalomyelitis, neuronal necrosis, and focal parenchymal necrosis occur. Signs include tremors of the head and neck, ptyalism, myalgia, ataxia, and recumbency. No clinical signs were identified in inoculated rabbits, but widespread encephalitis characterized by lymphoid perivascular cuffing, lymphocytic meningitis, and lymphocytic choroiditis occurred.[43,129]

Main Drain virus was isolated from the brain of a horse with encephalitis in Sacramento County, California. Signs included incoordination, ataxia, stiffness of the neck, head pressing, dysphagia, pyrexia, and tachycardia. The major vector is *Culicoides varipennis,* which transmits the virus from infected rabbits and rodents.[122]

Horses have been identified with titers for Ross River and Murray Valley viruses. Experimental infection with either virus produced transient pyrexia, myalgia, and ataxia. Horses are unlikely to be efficient amplifiers of either virus or important pathogens.[130–133] In Australia, significant titers for Murray Valley encephalitis virus have been identified. The virus is more commonly a disease of human beings. An epidemic in human beings was associated with significant titers in horses. Some horses with clinical signs, significant titers, and histologic evidence of viral encephalitis were identified.[130,133] 636 637

In Michigan, evidence of Cache Valley and Jamestown Canyon virus were identified in clinically normal horses.[125] Other viruses, identified in areas around the world, that have been implicated in equine encephalitis or that are associated with encephalitis in other species and for which significant titers have been identified in horses include the louping ill,[134,135] Maguari,[26,117,136] Ross River,[133] Aura,[26,136] Una,[26,136,137] Highlands J,[48,125,138] Semliki forest,[139] and Getah viruses.[102]

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10.14

10.14—West Nile Virus Encephalitis

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West Nile virus had never been seen in the Western Hemisphere before 1999. The virus has a geographic range greater than any other known arbovirus and is found throughout Africa, northern to central Europe, and eastern Asia. The virus was isolated first from the blood of a woman in Uganda in 1937. The earliest reported epidemic of West Nile encephalitis occurred in Israel in 1957 but was recognized retrospectively. That outbreak involved more than 500 hospitalized patients.[1] The largest epidemic of West Nile encephalitis on record occurred in South Africa during 1974. This epidemic involved an area of about 2500 km^2 in the Karoo and southern Cape provinces and resulted in thousands of human infections. More recently, epidemics of West Nile encephalitis have occurred in Romania (1996 to 1997), Czechland (1997), Italy (1998), and Russia (1999) so that the virus is considered a reemerging mosquito-borne disease in Europe.[2] How or when West Nile virus was introduced into the United States is unknown. Speculation places the introduction through the importation of birds or possibly a mosquito transported into the United States by ship or airplane.

West Nile virus is a member of the Japanese encephalitis virus complex of the genus *Flavivirus,* family Flaviviridae. This genus of viruses is found in several countries around the world, and some of the common members of this serocomplex include the viruses for Japanese encephalitis, Murray Valley encephalitis, St. Louis encephalitis, and West Nile encephalitis and Kunjin virus.[2] Up until 1999 the only members of the *Flavivirus* detected in North America were those causing Powassan and St. Louis encephalitides. The epidemiology of West Nile virus is nearly identical to that of St. Louis encephalitis. In both diseases the agents are transmitted principally

by species of *Culex* mosquitoes and have birds as the reservoir. The diseases do differ in that West Nile virus causes disease and mortality in wildlife (birds, particularly crows and blue jays) and domestic animals (particularly horses).[1] Although St. Louis encephalitis virus does not cause any remarkable disease in wildlife or domestic animals, it and West Nile virus can cause disease in human beings.

10.14.1 Epidemiology

Early outbreaks of West Nile virus encephalitis in horses occurred in 1962 in France, 1963 in Egypt, 1996 in Morocco, 1998 in Italy, and now 1999 to 2002 in the United States.[2,3] Other outbreaks of West Nile virus occurred in the Camargue region of France and in Israel in 2000.[4,5] In the outbreak in France in the early 1960s, researchers recorded a 10% morbidity and 30% mortality rates.[6] In 1959 in Egypt, researchers reported seroprevalence of antibodies to West Nile virus of 14% to 89%, depending on the area tested. However, morbidity and mortality data were not available.[7] In Morocco the case fatality rate was 44.7% (42 of 94), and in Italy was 6 of 14 (43%).[2,8] In the outbreak in France in 2000, the fatality rate was 34 % with an overall seroprevalence of infection of 8.5%.[4] In contrast, the case fatality rate in Israel was much lower (19.7%).[5] In the United States in 1999, 9 of 25 (36%) horses with clinical signs of the disease died or were euthanized. Sixty cases of West Nile virus were reported in horses in 2000 from seven states with a case fatality rate of 38% (23 of 60). Almost 80% of the cases were from New York and New Jersey (46 of 60, or 77%), with 7 cases from Connecticut, 4 from Delaware, and 1 each from Massachusetts, Pennsylvania, and Rhode Island. The cases of equine West Nile virus in 2000 started in mid-August and ended in October. The age range of the horses was from 4 months to 38 years with a mean age of 14 years. More males were affected (36 of 60) than females, and a breed predilection was not apparent because 11 breeds were affected.[3] In 2001, 738 equine cases of West Nile virus were confirmed in the United States in 19 states, with an estimated case fatality rate of 25%. The first equine case in 2001 occurred in June, which is different from the previous 2 years in the United States. Rapid spread of the virus in 2002 in the United States resulted in 14,717 confirmed cases of West Nile virus and likely many unconfirmed cases. The number of cases in horses is spread over 40 states and several provinces in Canada, and the U.S. Department of Agriculture has estimated a 28% case fatality rate for 2002.

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10.14.2 Pathogenesis

Following the bite of a West Nile virus–infected mosquito, one can detect the resulting viremia in some horses but not in others. A study of experimental infections performed in the early 1960s demonstrated viremias in two of six donkeys and none of three horses inoculated. The viremia in the two donkeys lasted for 1 day.[7] In a recent study performed in the United States, infection with West Nile virus via *Aedes albopictus* mosquitoes resulted in viremias in seven of eight horses.[9] None of the viremias lasted more than 6 days, and the highest viremia in one horse was 460 Vero cell plaque forming units (PFUs) per milliliter.[9,10] After infection of the eight horses in the U.S. study, naïve mosquitoes were allowed to feed on all viremic horses. None of the mosquitoes allowed to feed on infected horses became infected.[10] The viremias in horses appear to be of low magnitude and short duration; therefore horses are unlikely to serve as important amplifying hosts in nature.[7,10] Neutralizing antibody titers developed within 7 to 11 days after infection in an experimental trial.[10] Recent studies in human beings infected with the West Nile virus have demonstrated neutralizing antibody titers for greater than 2 years. Some horses infected during the 1999 outbreak that have been tested subsequently for neutralizing antibody still have titers after 15 months,[3] which suggests that protection from a natural infection may be long term.

10.14.3 Clinical Signs

Not all horses infected with the West Nile Virus develop clinical signs of the disease whether naturally or experimentally infected.[3,5,7–9] The incubation period for development of clinical signs is difficult to establish; however, experimental infection resulted in development of clinical signs of encephalitis 8 days after inoculation in one horse.[9] All horses examined in an outbreak of the disease in Italy exhibited varying degrees of ataxia and weakness in the pelvic limbs. Asymmetric weakness was detectable in the rear limbs of some horses and involved one or both thoracic limbs in some horses. In six horses, clinical signs progressed with ascending paresis leading to tetraplegia and recumbency within 9 days. Depressed mental state and tremors were notable in a few cases; however, no behavioral or head posture abnormalities or signs of cranial nerve involvement were apparent.[8] In the United States, most of the horses with encephalitis were ataxic (85%), almost half were weak (48%), many were recumbent or had difficulty rising or both (45%), muscle fasciculations were common (40%), less than one fourth of the horses had fevers (23%), and less than one fifth had paralyzed or droopy lip (18%), a twitching face or muzzle (13%), teeth grinding (7%), and blindness (5%).[3] Interestingly, the equine cases in Israel were similar to those in the United States. Behavioral and cranial nerve abnormalities occurred in some horses in Israel that may be related to the strain of virus (Is98), which is similar to the strain found in the United States (NY99).[5]

10.14.4 Differential Diagnosis

One would have to consider rabies, botulism, equine protozoal myeloencephalitis, cervical vertebral myelopathy, equine herpes virus meylopathy, equine degenerative myelopathy, and other encephalitides such as western equine encephalitis, eastern equine encephalitis, and Venezuelan equine encephalitis in the differential diagnosis.
Behavioral abnormalities and ascending paralysis make rabies a primary differential diagnosis. In addition, many 641
cases of West Nile virus exhibit mild to severe muscle fasciculations, making botulism another primary 642
differential diagnosis. Many cases also are similar to commonly diagnosed neurologic diseases, particularly equine protozoal myeloencephalitis. Therefore diagnostic rule-outs are necessary.

10.14.5 Pathologic Findings

No gross pathologic lesions were detected in the Italian cases but did occur in some cases in the United States. Histologically, all animals exhibited slight to moderate nonsuppurative encephalomyelitis, primarily in the spinal cord and lower brainstem affecting gray and white matter. In some cases, moderate to severe hemorrhage was notable in the spinal cord. The most severe lesions were in the thoracic and lumbar spinal cord.[11] The lesions in the United States cases in which postmortems were performed were similar to those found in the Italian cases, except the brainstem lesions were more severe in the United States cases.[12] Unlike encephalitis caused by alphaviruses such as western equine encephalitis and eastern equine encephalitis, significant changes to the cerebral and cerebellar cortices were not apparent.[13]

10.14.6 Diagnosis

Routine complete blood counts and serum biochemical assays resulted in finding all values within their normal ranges in the few horses tested. Similarly, cytologic examination of the cerebral spinal fluid (CSF) was normal in all but one horse with a low white blood cell count. The total protein was normal in CSF of all horses except one

that had a high total protein with xanthochromia.[12] In a more recent study of 30 cases, cytologic findings of the CSF and protein concentration in only 8 of 30 horses were within normal ranges. Of the 22 horses with abnormal CSF results, 16 had a mononuclear pleocytosis with normal protein and the other 6 horses had normal cytologic findings with an increased protein concentration.[14]

Several serologic tests have been used to diagnose West Nile virus disease, including plaque reduction neutralization, hemagglutination inhibition, complement fixation, enzyme-linked immunosorbent assay (ELISA), and antigen capture ELISA. One also may attempt virus isolation from whole blood, serum, CSF, and brain or spinal cord tissues. Other diagnostics include reverse transcriptase polymerase chain reaction testing of central nervous system (CNS) tissues and immunohistochemistry.[1] In veterinary diagnostic laboratories, hemagglutination inhibition has been the most common test and has demonstrated greater sensitivity than complement fixation.[13] The plaque reduction neutralization test requires BSL3 laboratory capabilities; therefore, few laboratories are equipped to perform this test. In the United States, most of the samples have been tested by the National Veterinary Services Laboratory in Ames, Iowa. In the acute cases of West Nile virus encephalitis in horses, antigen capture ELISA has been used to detect West Nile virus–specific immunoglobulin M (IgM). In experimentally and naturally infected horses, IgM antibodies were detectable within 8 to 10 days and persisted for less than 2 months. Immunoglobulin M antibodies have not been detected in vaccinated horses; therefore, this test is useful even if the disease becomes endemic. In the study by Ostlund, Crom, Pederson, et al., the authors were able to isolate West Nile virus from the CNS tissues in some (7 of 10) horses and detect West Nile virus RNA using reverse transcriptase polymerase chain reaction testing in all 10 horses from which brain tissue was submitted.[3] Most state diagnostic laboratories have the capability of performing two ELISA tests for West Nile virus–specific IgM and IgG. Veterinarians should consult with their state departments of agriculture and departments of health to determine sampling required and where samples are to be sent.

10.14.7 Treatment

Supportive care is the only treatment available. Antiinflammatory medications such as nonsteroidal antiinflammatory drugs, steroids, and dimethyl sulfoxide (10%) may help to alleviate central nervous system inflammation and pain. One may treat CNS edema using mannitol (0.25 to 2 g/kg intravenously), and vitamin E (5000 to 8000 IU/day orally) may help to alleviate oxidant injury. Several other treatments have been attempted, such as ribavirin and interferon, as antiviral agents; however, the efficacy of these medications is unknown. Additionally, some clinicians are using hyperimmune plasma based on limited use in human beings. Whether these novel treatments are helpful remains to be determined. Some horses may require intravenous fluids and possible nutrient support. Some horses that are recumbent and unable to rise require support by slings as well.[13] For horses unable or reluctant to receive oral medications, one may insert a feeding tube (Mila International Inc., Florence, Kentucky).

10.14.8 Prevention

Two methods exist for preventing or controlling West Nile virus infections in horses and both are important: integrated mosquito control and vaccination. The integrated mosquito control includes reduction of mosquito breeding sites and the use of larvicides and adulticides. The more breeding sites are controlled, the less pesticide is required. Owners should house horses indoors during peak periods of mosquito activity (dusk and dawn), 642
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should avoid turning on lights inside the stable during the evening and overnight, because mosquitoes are attracted to lights, and should place incandescent bulbs around the perimeter of the stable to attract mosquitoes away from the horses. Black lights do not attract mosquitoes well. Owners should remove all birds that are in or

close to the stable to reduce the potential reservoirs and should eliminate areas of standing water on their property. Shallow standing water, used tires, manure storage pits, and drainage areas with stagnant water are ideal mosquito breeding places. Topical preparations containing mosquito repellents are available for horses. One should read the product label before using and should follow all instructions. Fans blowing on the horses in the stable help deter mosquitoes. Owners should fog the stable premises with a pesticide in the evening to reduce the number of mosquitoes and should read directions carefully beforehand. Local mosquito control authorities may be able to help in assessing the mosquito breeding risks associated with a client's property.

The second method of controlling West Nile virus infections in horses is vaccination. A vaccine was developed using killed virus and has been sold under a conditional licence since August 2001. Some recent data from the company developing the vaccine suggest that the vaccine is efficacious. The study used 19 vaccinated horses and 11 controls. Horses were vaccinated 12 months before challenge. Although 9 of 11 controls developed viremia, only 1 of 19 of the vaccinated horses became viremic, suggesting that vaccination reduces viral replication. In addition, none of the study horses developed a fever.[15] More recent data, based on follow-up of West Nile virus cases from Ohio, suggest the vaccine is effective in reducing the risk of death from West Nile virus infection. The data indicate that 5.9% of horses died after receiving 2 or more vaccinations, which is similar to the percentage of horses in the experimental challenge that developed viremia (5.3%).

No efficacy data are available at this time regarding protection during natural infections; however, the efficacy is expected to be similar to that of the killed virus vaccine for Japanese encephalitis. Japanese encephalitis virus belongs in the same serocomplex as West Nile virus. The Japanese encephalitis vaccine was developed in Japan in the early to mid-1940s, and the Japanese Ministry of Agriculture studied the efficacy of the vaccine in 1948. The ministry found the clinical encephalitis cases among horses that had received two vaccinations before the epidemic season was 0.05% (51.6 per 100,000), whereas the clinical encephalitis cases among nonvaccinated horses was 0.44% (443.5 per 100,000).[16] Therefore the relative risk for Japanese encephalitis in nonvaccinated horses in Japan was greater than 8 times higher than in vaccinated horses. Further evidence that the vaccine has been effective in Japanese horses is the reduction in clinical encephalitis cases in horses in Hokkaido, Japan, from 337.1 per 100,000 horses in 1948 to none in 1967. Similar results were apparent after the initiation of vaccination in Singapore.[17] A vaccine also has been developed in China, and current efficacy data demonstrate that the risk of clinical Japanese encephalitis in unvaccinated horses (172.1 per 100,000) was 7.5 times higher than in horses that had been vaccinated (23 per 100,000).[18] A new vaccine is being developed in the United States to prevent West Nile virus encephalitis in horses. The new vaccine is a recombinant DNA vaccine that includes a Japanese encephalitis virus signal sequence in the plasmid. This DNA vaccine provided 100% protection in mice and horses from West Nile virus–infected mosquitoes.[9] Therefore because West Nile virus and Japanese encephalitis are in the same serocomplex, the successful reduction of clinical cases of Japanese encephalitis in several countries using the killed Japanese encephalitis vaccine, construction of the new West Nile virus DNA vaccine using Japanese encephalitis virus sequences, the efficacy data of the challenge trial and the Ohio data, the West Nile virus vaccine is expected to be reasonably efficacious. The author recommends administering the West Nile virus vaccine initially in two doses 3 to 6 weeks apart and then yearly afterward. The second dose should be given at least 4 weeks before the beginning of the mosquito season. If the horses are show horses or racehorses that are being transported frequently (highly stressed), the author suggests administering an additional booster in the last week of July or the first week in August.

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10.15

10.15—Rabies

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10.15.1 Epidemiology and Pathogenesis 644

Rabies is an uncommon disease in the equine, but because of its zoonotic potential, one should consider it in the differential diagnosis in horses showing neurologic signs of less than 10 days' duration. In the United States during 1993, 606 cases of rabies were reported in domestic animals, with 0.5% of these being in horses and mules.[1]

The rabies virus is a large, cylindric, bullet-shaped neurotropic rhabdovirus (genus *Lyssavirus,* family Rhabdoviridae).[2] Rhabdoviruses are enveloped with single-stranded RNA. They are heat-labile and are susceptible to degradation by radiation, strong acids, alkalis, most disinfectants, lipid solvents, and anionic solvents.[2,3] Rabies virus is transmitted by saliva-contaminated wounds. In the horse the most common method of infection is a bite wound from a wild carnivore or insectivorous bat carrying the virus.[4] The most common reservoir hosts in the United States are skunks, raccoons, and the red fox.[2] However, domestic dogs, cats, and other horses may transmit rabies to horses by bite wounds. Furthermore, rabies virus can be transmitted by droplet inhalation, orally, or transplacentally. Droplet transmission has been reported to have occurred in foxes, coyotes, opossums, and raccoons in a bat cave in Texas. In that report the virus was isolated from the air in the cave.[5] Aerosolization of the virus also caused an outbreak of rabies in a laboratory. Transplacental transmission of the virus has occurred in naturally infected cattle and experimentally infected mice and bats.[2]

Rabies virus infects and replicates in myocytes at the inoculation site and may remain undetectable for weeks or months before moving centrally. The virus infects peripheral nerves by traversing neuromuscular and neurotendinous spindles. Progression along the nerve is thought to occur in the tissue spaces of the nerve fasciculus.[5] After progressing centripetally up the peripheral nerve by axoplasmic flow, the virus replicates in spinal and dorsal root ganglia of the corresponding peripheral nerve. Once the virus reaches the central nervous system (CNS), spread occurs rapidly through multiplication in neurons of the brain, spinal cord, sympathetic trunk, and glial cells. Spread of rabies virus also can occur through passive transport within cerebrospinal fluid or blood.[2–4] Finally, the virus reaches tissues outside the CNS via centrifugal movement of the virus along nerve axons.[2,3]

The incubation period for rabies varies from 9 days to 1 year in horses. The incubation period can be affected by the virus strain, host species, inoculum size, and proximity of the inoculation site to the CNS.[2,4] Retention of virus in myocytes at the inoculation site may be a mechanism for variation in the incubation period. A shorter incubation period also may be explained by the virus entering peripheral nerves soon after exposure and rapidly migrating centripetally to the CNS without replication in nonneural tissue.[2]

10.15.2 Clinical Signs

No signs are pathognomonic for rabies infection in horses. Clinical signs on presentation vary and range from lameness to sudden death.[6–9] Hyperesthesia, ataxia, behavior change, anorexia, paralysis or paresis, and colic have been reported as initial clinical signs.[6–11] One rarely finds a bite wound, and the horse may or may not be febrile. The site of inoculation and its proximity to the CNS influence what clinical signs one observes.[2,7,8] The 644
neurologic signs exhibited in rabies-infected horses can be classified into three forms depending on the 645
neuroanatomic location in the CNS infected by the virus. First, in the cerebral or furious form, one may see aggressive behavior, photophobia, hydrophobia, hyperesthesia, straining, muscular tremors, and convulsions.[12]

Second, in the brainstem or dumb form, one commonly sees depression, anorexia, head tilt, circling, ataxia, dementia, excess salivation, facial and pharyngeal paralysis, blindness, flaccid tail and anus, urinary incontinence, and self-mutilation.[10,12,13] Finally, in the paralytic or spinal form, one sees progressing ascending paralysis, ataxia, or shifting lameness with hyperesthesia, and self-mutilation of an extremity.[11–14] Most affected animals with the paralytic form become recumbent in 3 to 5 days, with normal eating and drinking often remaining.[13] The neurologic signs may vary as the virus spreads to other portions of the CNS. Thus horses may have clinical signs of two or all forms of rabies. Disease progresses rapidly, and death is usually inevitable regardless of the clinical manifestation. Antiinflammatory therapy can delay virus progression,[12] but death usually occurs within 5 to 10 days after onset of clinical signs.[9,13]

10.15.3 Diagnosis

Antemortem diagnosis of rabies is difficult, but one should consider the disease in horses showing rapidly progressing or diffuse neurologic signs. Other diseases that one should consider include hepatoencephalopathy, togaviral encephalitis, protozoal encephalomyelitis, nigropallidal encephalomalacia, botulism, lead poisoning, cauda equina neuritis, meningitis, space-occupying mass, trauma to the brain or spinal cord, and esophageal obstruction.[3,5,13] Clinical laboratory data of body fluids are nonspecific. Cerebrospinal fluid may be within normal reference range or may show a moderate increase in total protein concentration (60 to 200 mg/dl) and a pleocytosis (5 to 200/dl).[3,5,13] Fluorescent antibody testing of tactile hair follicles of facial skin taken on biopsy or corneal epithelium may help diagnose rabies ante mortem. The fluorescent antibody technique detects the rabies virus antigen in these tissues. Unfortunately, a negative test does not exclude rabies as a differential diagnosis.[9] One can achieve definitive postmortem diagnosis by submitting half the brain in 10% formaldehyde for histologic examination and the other half frozen to a public health diagnostic laboratory for immunofluorescent antibody tests, mouse inoculation, and monoclonal antibody techniques. The whole brain may be shipped unfrozen on ice for further rabies evaluation and testing. One should examine the rest of the carcass only with careful precautions against transmission of the virus, if present, by wearing gloves, caps, and masks until a negative rabies diagnosis is made.

Common histopathologic changes are a mild, nonsuppurative encephalomyelitis, perivascular cuffing by mononuclear cells, gliosis, glial nodules, and neuronal degeneration.[3,13] These lesions occur most commonly in the hippocampus, brainstem, cerebellum, and gray matter of the spinal cord. Large intracytoplasmic eosinophilic inclusions within neurons and ganglion cells, known as Negri bodies, are pathognomonic for rabies.[2,3,13] However, in 15% to 30% of confirmed rabies cases, Negri bodies are not present in histopathologic sections, especially if the animal died or was euthanized early in the disease process.[2] The most commonly used and fastest diagnostic test for rabies is the fluorescent antibody test. This technique may identify 98% of infected brain specimens. The mouse inoculation test is the most accurate method for diagnosing rabies virus infection but requires 5 to 6 days to complete. The mouse inoculation test involves the injection of suspect brain or salivary gland tissue homogenates intracerebrally in mice and observation of clinical and neurologic signs or death.[2,3] The monoclonal antibody test has been used most recently for rabies diagnosis in horses. The test can differentiate specific street, fixed, or vaccinal strains of rabies virus by their glycoprotein or nucleocapsid antigens.[2] This is important in postexposure vaccination of human beings and animals when using the specific strain of virus.

10.15.4 Treatment and Prevention

No specific treatment exists for rabies in horses. Symptomatic treatment and supportive care may help prolong the disease course to complete diagnostics and to rule out other diseases with similar signs. Prolonging the course, however, creates a risk of exposure to handlers and other animals. Therefore one should isolate any animal suspected of having rabies and should handle it as little as possible. Horses that are known to have been exposed to rabies should have all wounds cleaned and lavaged with iodine or quaternary ammonium disinfectant, and rabies antiserum, if available, infiltrated around the bite wound.[3,13] No postexposure protocol exists for unvaccinated or vaccinated horses. However, exposed horses should be quarantined for 6 months and observed for the occurrence of neurologic signs. Unvaccinated horses should not receive postexposure prophylaxis until after the 6 months of quarantine. Two inactivated diploid vaccines (Imrab-1, Pitman-Moore, Inc., Terre Haute, Indiana; and Rabguard, Norden Laboratories Inc., Lincoln, Nebraska) are approved for use in horses. These vaccines are recommended to be given annually in high-risk areas to horses older than 3 months of age.[12,13] The dosage is 2 ml administered in the semimembranosus or semitendinosus muscle group. The manufacturer does not recommend administration into the neck muscles.[4] 645

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Several arguments are ongoing concerning vaccination of horses against rabies. The potential for a rare vaccine reaction—illness with neurologic signs following the use of a modified live virus (SAD strain) rabies vaccine—has been reported in the United States.[6] The risk also exists that vaccinated horses bitten by a rabid animal will confer a silent or attenuated form of the disease, and the animal will shed the virus.[12] This theory, however, is not proven. Public concern and safety outweigh the potential risks of vaccinating horses. Therefore one should follow recommendations to vaccinate in endemic areas. One should report any animal positively diagnosed with rabies to public health officials.

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10.16

10.16—Equine Motor Neuron Disease

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Equine motor neuron disease (EMND) is an acquired neurodegenerative disease of adult horses that has been reported in North and South America, Japan, and Europe.[1–9] Lower motor neuron disease was recognized in 1988 based on histologic changes in skeletal muscle,[10] and EMND was first described in 1990.[1] In the 1990s, EMND was recognized worldwide in an apparently increasing frequency[3,11]; however, recently the number of EMND cases appears to be decreasing.[9] The decrease may be caused by preventive management measures taken for horses at risk of developing the disease.

The disease affects primarily the motor neurons in the spinal cord ventral horn cells and brainstem and leads to characteristic clinical signs including generalized neuromuscular weakness and neurogenic muscle atrophy.[1,2,5,12] Equine motor neuron disease closely resembles human motor neuron disease, which is known as amyotrophic lateral sclerosis (ALS) or Lou Gehrig disease.[1,13] However, EMND only affects the lower motor neurons, which is not the case in ALS. A chronic lack of antioxidants is implicated in the pathogenesis of this disease.

10.16.1

Clinical Signs

EMND occurs in adult horses with a mean age of onset of clinical signs of 9 years (range of 2 to 23 years).[14] The risk for EMND increases with age, peaking at around 15 years.[15] Quarter Horses appear at increased risk for developing EMND; however, this may reflect management factors.[2,15] No gender predilection is apparent.

Clinical signs vary depending on the stage or duration of the disease. Currently, a subacute and a chronic form are recognized.[9]

10.16.1.1

SUBACUTE FORM

During the early phase of EMND, trembling, muscle fasciculations, base-narrow stance, shifting of weight in the rear limbs, abnormal sweating, excessive recumbency, muscle atrophy, and weight loss despite a normal or even ravenous appetite are the most characteristic clinical signs (Figure 10.16-1).[1,2,6,8,9] Ataxia is not a clinical sign of EMND. A horse with EMND moves better than it stands.[2,8] Muscle wasting is most noticeable in the quadriceps, triceps, and gluteal areas. In more than 50% of cases horses may display a lower than normal head carriage with muscle wasting of the cervical musculature (hangdog appearance).[8] Onset of 646
muscle wasting may occur for approximately 1 month before the acute onset of clinical signs. 647

Figure 10.16-1 Characteristic appearance of a horse with equine motor neuron disease, including muscle wasting, sweating, base-narrow stance, low head carriage (hangdog appearance), and tail head elevation.

10.16.1.2 CHRONIC FORM

Owners may present horses for poor performance, gait abnormalities (stringhalt-like movement), and failure to gain weight. Trembling and muscle fasciculations are not pronounced in the chronic form of EMND. Horses with the chronic form of EMND are often horses that have stabilized from the subacute form; however, some horses develop the chronic form without experiencing the subacute form. Muscle atrophy varies from mild to severe, and some horses may appear emaciated.[1,8,9]

10.16.1.3 SUBCLINICAL FORM

Experimental evidence indicates that horses may develop a subclinical form of EMND, which may affect performance and safety.[8,9]

In the subacute and chronic forms of EMND the tail head often is elevated because of denervation atrophy and fibrotic contracture of the sacrococcygeus dorsalis medialis muscle.[6,8,9] In approximately 30% of cases, ophthalmoscopic examination may reveal a distinctive retinopathy with a mosaic pattern of brown pigment deposition in the fundus. These horses are not necessarily visually impaired.[16,17]

10.16.2 Pathologic Findings

Gross necropsy findings in EMND include diffuse muscle atrophy and pallor of especially the intermediate vastus and medial head of the triceps muscles. The central nervous system and peripheral nerves are grossly normal. Histologically, one may detect noninflammatory neuronal degeneration and neuronal loss at all levels of the spinal cord, but such loss is most obvious in the cervical and lumbar intumescence. One finds the lesions in the ventral horn cells (lower motor neurons) of the spinal cord gray matter; the nuclei of cranial nerves V, VII, and XII in the brainstem; and the nucleus ambiguus. Secondary axonal degeneration with loss of myelinated fibers because of dysfunction or death of these motor neurons is present in the ventral spinal roots; spinal nerves; cranial nerves V, VII, IX (sometimes), and XII; and peripheral motor nerves.[1,12] Peripheral motor nerve degeneration also may be evident on antemortem muscle biopsy.[18]

Skeletal muscle changes include nonspecific myopathic changes such as excessive fiber size variation, internal nuclei, and cytoarchitectural alterations. Scattered fiber degeneration and necrosis is a consistent finding in EMND. Atrophy of type I and type II muscle fibers occurs in severely affected muscles in EMND and is pathognomonic of denervation atrophy.[1,12,13] EMND predominantly affects type I fibers, in contrast to denervating diseases in other species, and has not been reported in ALS. Motor neurons supplying the type I fibers have a higher oxidative activity and thus may be more susceptible to oxidative injury.[12] Similar to ALS, where at least 30% of motor axons must be destroyed before clinical evidence of atrophy occurs, in horses with muscle wasting caused by EMND, a mean motor neuron loss of 31% was recorded.[19]

A pigment retinopathy may be visible, as well as deposition of lipopigment in the endothelial capillaries of the spinal cord. These findings have been reported in other animals with vitamin E deficiency.[16,17] Besides the abnormalities in the neuromuscular system, no other pathologic findings have been found consistently in horses with EMND.

10.16.3 Pathophysiology

The clinical signs of neuromuscular weakness result from the generalized denervation muscle atrophy found in horses with EMND. The pathogenesis of the neuron damage and death in EMND is not understood fully but is thought to result from free radical damage. Equine motor neuron disease occurs sporadically and affects horses of all ages and breeds. Therefore infectious agents or environmental toxins are less likely to be a cause of the disease.[2]

A chronic vitamin E deficiency is thought to be the most important factor in the cause of EMND based on the fact that affected horses consistently have low plasma vitamin E concentrations and have not had access to pasture or green forages for a prolonged time.[2,11,15,20] Vitamin E concentrations in the central nervous system, 647

648 peripheral nerves, muscle, liver, and adipose tissue have been found to be correlated to blood concentrations.[9] Horses generally have been on the same premises for more than 2 years before developing clinical signs.[15] The type I muscle fiber atrophy and the lipopigment deposition in the capillaries of the spinal cord are pathologic findings suggesting an oxidative type of injury as a cause of the neuronal death.[13] The pigment retinopathy found in approximately 30% of cases and the lipopigment deposition in the vasculature of the spinal cord occurs in other species and is related to a vitamin E deficiency.[8,16]

Equine degenerative myeloencephalopathy, another neurodegenerative disease in horses, also is associated with a vitamin E deficiency; however the central nervous system lesions in equine degenerative myeloencephalopathy are different from those in EMND. Vitamin E deficient adult horses have been suggested to develop EMND after a transitory period of triggering events such as exposure to neurotoxins or excessive amounts of prooxidants.[2,9] So far a neurotoxin has not been identified. The presence of prooxidants such as copper and iron in spinal cord, liver, and plasma has been investigated but has not been confirmed to play a role in the development of EMND.[9,21]

Although EMND is the only naturally occurring model for ALS[13] and oxidative injury is implicated in the cause of both diseases, some important differences exist between the two.[6,12,13] In contrast to EMND in which the disease process is limited to the lower motor neurons, ALS affects upper and lower motor neurons. Only mild degeneration of the pyramidal tracts is present in horses with EMND, but the pyramidal tracts in horses are less extensive and poorly developed compared with human beings. ALS is familial in approximately 10% of cases, and 20% of patients with familial ALS carry a mutation of the Cu/Zn superoxide dismutase 1 gene.[22] This metalloenzyme is one of the principal oxygen-derived free radical scavengers and protectants against oxidant injury to the nervous system. The mutation of this gene leads to an altered form of the enzyme that is actively toxic to cells.[23] In these patients the quantity of oxidants is increased, whereas in horses with EMND a lack of antioxidants is the presumed cause of neuronal degeneration and death.[2,24] Mutations in the Cu/Zn superoxide dismutase 1 gene have not been found in EMND.[25] The cause of spontaneously occurring ALS is unknown; however, the similarities between familial ALS and spontaneous ALS suggest a common pathway for neuronal death.[13] The question of why the disease affects motor neurons so selectively has not been answered thus far. Motor neurons are particularly susceptible to oxidative injury by having high energy requirements associated with the maintenance of long axons. The high concentration of polyunsaturated fatty acids in neuronal cell membranes make these cells particularly susceptible to lipid peroxidation.[26] A recent investigation has shown that chronic or episodic deficits in oxygen and glucose, through an altered function of vascular endothelial cell growth factor, result in a failure of the motor neurons to meet their metabolic requirements. This altered function of vascular endothelial cell growth factor resulted in ALS-like symptoms and neuropathy.[27] If a relationship between superoxide dismutase 1 and altered vascular endothelial cell growth factor function exists, it remains to be investigated.

10.16.4 Diagnosis

Clinical signs and a history of other EMND cases in the stable and absence of pasture or green hay may lead to a tentative diagnosis of EMND. Ophthalmoscopic examination may reveal fundic lesions in approximately 30% of cases. Serum enzyme activities of aspartate aminotransferase and creatine kinase are generally mildly to moderately increased. The plasma vitamin E concentration is consistently low (less than 1 μg/ml). Cerebrospinal fluid analysis has been performed in horses with EMND and demonstrated elevated immunoglobulin G concentrations in approximately 50% of cases. Intrathecal production of immunoglobulin G also occurs in ALS; however, in both diseases this is considered a secondary effect of the disease process rather than a cause. The albumin quotient was normal in most horses examined, indicative of normal blood-brain barrier function.[2]

Electromyography of cervical, facial, triceps, rear limb, and tail head muscles may be useful in acute cases.[1,28] To eliminate motion artifacts, one can sedate horses or place them under general or caudal epidural anesthesia.[28]

However, the electromyographic changes, which include positive sharp waves and fibrillation potentials, may be difficult to evaluate because they are expected in any peripheral nerve disease, myopathy, or myositis.[1,6,28]

In some horses with EMND the glucose absorption test has been found to be abnormal; however, histopathologic examination of the small intestine has failed to identify lesions. The plasma glucose concentration increases only by 40% following 1 g/kg of 20% glucose administered orally, but relatively normal peak concentrations occur after 0.5 g/kg xylose administration (18 to 25 mg/dl). An abnormality in intestinal glucose transport has been identified in vitro.[6,8,9] The clinical significance of this finding is unknown.

More invasive diagnostic tests to confirm the tentative ante mortem diagnosis of EMND are the examination of muscle and nerve biopsies. A biopsy of the sacrocaudalis dorsalis muscle is easy to obtain in a standing horse, and microscopic examination of this muscle may reveal changes consistent with denervation muscle atrophy and 648
scattered muscle necrosis. This test has a sensitivity of approximately 90%. One should place the muscle biopsy 649
sample on a tongue depressor in 10% formalin to prevent contracture artifact.[18] Examination of a biopsy of the ventral branch of the spinal accessory nerve may be more sensitive in chronic cases. Horses generally need to be anesthetized for one to obtain a biopsy from this nerve. Similar as with muscle biopsies, nerve biopsies must be placed on a tongue depressor and in 10% formalin or another fixative suitable for electron microscopy. An experienced neuropathologist should examine nerve biopsies carefully; samples may reveal only evidence of smaller Büngner's bands (columns of proliferated Schwann cells).[12,29]

The definitive diagnosis is based on postmortem examination of spinal cord, brainstem, nucleus ambiguus, and skeletal muscle. The most important differential diagnoses that one should consider are laminitis, rhabdomyolysis, and colic. Other diseases that may cause similar signs are botulism, equine protozoal myeloencephalitis, polysaccharide storage myopathy, iliac thrombosis, equine grass sickness, and lead toxicosis.[8,9,30]

10.16.5 Treatment and Prevention

Currently, no treatment for EMND has been proved efficacious. In acute cases an antiinflammatory dose of corticosteroids or nonspecific antioxidant treatment with dimethyl sulfoxide may be beneficial. The only recommended treatment is based on the idea that this disorder is caused by oxidative injury and on the fact that horses with EMND consistently have low plasma vitamin E concentrations.

Treatment with vitamin E (5000 to 7000 IU/horse/day) results in an increase of plasma vitamin E concentrations to 2.0 μg/ml or greater after 4 to 6 weeks.[9] This treatment may be beneficial; however, full recovery is unlikely because neuronal death is irreversible.[6,8,9] Treatment with vitamin E has been associated with improvement of clinical signs; however, no published studies examining the effect of treatment exist at this time. Response to treatment is thought to depend on the number of neurons that are damaged versus those that are dead. Currently, no diagnostic modality exists to examine this in live horses.

One should give vitamin E supplements to horses that have limited or no access to green grass or hay for prolonged periods. The recommended dose for supplementation is 2000 IU/day of *dl*-α-tocopherol acetate. Not all commercial feeds or supplements contain required amounts of vitamin E for deficient horses. Periodic monitoring of plasma vitamin E concentrations is recommended in horses that are at risk for developing EMND.[8,9]

10.16.6 Prognosis

The prognosis is poor for return to performance and guarded for life. Although no published investigations exist regarding the survival rate and follow-up of horses with EMND, horses with EMND generally have been shown to follow one of three possible clinical courses.[8,9] Approximately 20% of horses continue to deteriorate, and the severe weakness and excessive recumbency necessitate euthanasia. In approximately 40% of horses, clinical signs appear to stabilize; however, these horses do not regain muscle mass and may develop severe gait abnormalities. Continued clinical abnormalities frequently lead to euthanasia within 1 year of onset of clinical signs. The third group of horses (approximately 40%) show dramatic improvement following treatment with vitamin E, and many may regain a normal muscle mass. These horses may remain stabilized, that is, appear normal, for 1 to 6 years or more; however, many relapse, resulting in euthanasia. In a small number of human beings with ALS, a similar scenario occurs when clinical signs stabilize without progression for years.[13] In horses with EMND the relapse appears associated with return to exercise and may be caused by exercise-induced premature death of remaining neurons.[8,9]

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10.17

10.17—Botulism

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Botulism is a caused by a gram-positive, spore-forming, toxin-producing obligate anaerobic bacteria known as *Clostridium botulinum*.[1–5] This bacteria can survive for prolonged periods because of its ability to form spores. The bacteria are distributed widely in nature and can be found in soil, agricultural products, and marine environments.

The bacteria produce several types of antigenically distinct toxins known as A, B, C1, C2, D, E, F, G, and H. The types A, B, E, F, and G are found in the environment, whereas types C and D are found within the intestinal tract of animals and birds.[3,5,6] The molecular weight of the clostridium toxins is about 150 kd.[3] Botulism is a severe neuroparalytic disease caused by the botulinum toxin. Human disease is caused by types A, B, E, and rarely F.[1]

Several diseases result from botulism and can present in a variety of ways. Forage poisoning usually occurs in adult horses following the ingestion of preformed toxin found in feed material that has been contaminated by bacteria. The toxin is absorbed from the intestine or produced in an infected wound and disseminates by the systemic circulation until it binds to specific receptors where it blocks the release of acetylcholine. The result is usually a descending symmetric paralysis. The contaminated feed material may be grain or roughage (usual). In this form of the disease, B or C toxin has been isolated, although a majority of cases result from type B forage poisoning. In a 1997 paper, a majority of horses were demonstrated to be affected because of ingestion of feed material containing decaying vegetable matter with bacterial proliferation and toxin production rather than because of contamination by decomposing animal carcasses in the feed material.[5] In horses that had disease resulting from type C toxin, the cause was the ingestion of decomposing tissue of animals that contained the bacteria within the lumen of the intestinal tract.[7] A report describes type C1 and C2 toxins resulting in signs of neurotoxicity in one horse.[7,8] The same feed given to horses and cattle has been shown to result in disease in the horses while not affecting the cattle, suggesting a species difference in susceptibility to these toxins.[6,7]

Wound botulism has been demonstrated in horses that resulted from type B *C. botulinum* infection in a wound.[2] The bacteria sporulates under anaerobic conditions, resulting in production and release of the toxin. Published reports have described this type of infection following castration, an injection abscess, an infected umbilicus, and other contaminated wounds.[2,4,5,9] This problem is unique to horses, although a few cases have been described in other species; for example, in human beings following sharing of needles and illicit drug abuse.

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In foals a syndrome referred to as toxicoinfectious botulism, or shaker foal syndrome, occurs.[5,6,10] The disease appears to be a sporadic condition affecting foals from a few days to several months of life[10] and appears to result from type B toxin, which has been found in spores contaminating the soil and which can survive for years. One study demonstrated that a high percentage (94%) of soil samples collected from farms that previously have had affected foals contained type B neurotoxin gene.[11] The foals on these farms are suspected to have ingested contaminated soils, and this may have led to the proliferation of spores within the intestinal tract, toxin production and absorption from the intestinal tract, and disease. A similar condition has been described in human infants fed honey or other food contaminated with botulism spores. In adult horses the intestinal flora appears to have a protective eaffect against the proliferation and absorption of spores and toxin. Diagnosis is difficult and most often is based on typical clinical signs; however, in about 30% of affected foals the toxin may be identified in the feces during an acute clinical episode.[5,11,12]

Most cases of botulism in horses result from type B botulism.[2,3,5–10] Less frequently, cases of type C and A have been recognized. Cases of type D botulism in horses have been suspected in the United States, but no cases have been confirmed at this time. In the United States, type B spores are the type most often found in soils in the mid-Atlantic states, whereas type A spores are identified more typically on the West Coast. One report describes an outbreak of type C botulism resulting from contamination of 200,000 tons of hay cubes by decaying rodent carcasses. Whenever more than one horse on a premise demonstrates clinical signs compatible with botulism, quick analyis of the entire ration and the feeding methodology to determine if contamination has occurred is important. This aggressive approach may prevent additional horses or other animals on the farm from becoming infected. Beginning the investigation with the feed, especially ensilage, is always best because this is the most common

source of toxin. Conditions may be optimal for proliferation of botulism spores and toxin production in improperly prepared hay or hay stored in plastic bags.[7]

10.17.1 Clinical Signs

The clinical signs of botulism in horses are often the same regardless of the source of contamination or the type of toxin, even though one may see subtle differences between toxin types A, B, and C.[5,6,9,10] The onset of sign varies but is usually within 24 hours of exposure, although onset can occur in as short as 12 hours or as long as several days.[1] The incubation period maybe associated with inoculum size, which suggests that the shorter the incubation, the more severe the disease. The disease usually affects motor nerves with high efferent traffic, resulting in weakness, dysphagia, and poor muscle tone. One can detect the lack of normal muscle clinically by evaluating eyelid tone, which often is weak. Affected horses appear to maintain coordination and therefore appear weak but not ataxic. Early in some cases the owners will note prolongation of the time the horse takes to eat its grain or note abdominal discomfort and colic. The time to ingest a small quantity (usually a cup) of grain can be useful as a predictor of whether the horse has botulism. Some horses may have colic, decreased salivation, and occasionally urinary retention resulting from involvement of the cholinergic autonomic nervous system.

Over time horses demonstrate muscle fasciculations, often first in the triceps region, that eventually progress to the entire body and result in recumbency. At first the horse appears to gain strength after lying down for a short time and may return to standing spontaneously or with coaxing. Eventually the horse is unable to rise. Clinical evaluation should include careful examination of the tongue, eyelid and tail tone, and the pupillary light response, which appears sluggish. One will note ptosis of the eyelids. In horses affected for a longer time one may note an abnormal pattern of respiration. Death often results from respiratory failure.[5,6,8]

10.17.2 Differential Diagnosis

The differential diagnosis for horses affected with botulism can be any cause of weakness and dysphagia in horses and should include protozoal myeloencephalitis; equine motor neuron disease; equine herpesvirus (type 1) myeloencephalopathy; rabies; several toxins such as lead, yew, and yellow star thistle; low blood calcium; and hyperkalemic periodic paralysis.[5,6,10] One also should consider less common clinical diseases such as tick paralysis vascular accident or even unusual examples of exercise intolerance.

The diagnosis of botulism is difficult and frequently is based on clinical signs and history. To confirm the diagnosis, one must detect the toxin in feed, serum, gastrointestinal contents, feces, or debris collected from a wound.[4,5,10–13] The toxin is stable in frozen tissues or plasma and can be stored at −20° C for several weeks. The presence of botulism spores in intestinal contents or feed or the presence of an antibody response to *C. botulinum* in a recovered patient are also useful to confirm the diagnosis of botulism in horses.

Methods for detecting botulism toxin include mouse bioassay, enzyme-linked immunosorbent assay (ELISA), 651
polymerase chain reaction test, and use of an optical-based biosenser.[4,8,11–13] The mouse bioassay is sensitive 652
but requires several days to complete and uses many mice. If serum has been collected from a horse in the acute stage of infection, this assay is most accurate. One of the problems associated with this test in serum of horses is that because these animals are so sensitive to botulinum toxin, clinical signs may appear before the toxin reaches threshold levels required for detection. One must add specific antiserum to the test to detect the exact toxin type. Other tests are available, although these are less sensitive than mouse inoculation. The ELISA may be less

specific because of cross-reactivity with other clostridial toxins, but it does have a turnaround time of 24 hours. The polymerase chain reaction test was used to identify an outbreak in Australia caused by type B botulism.[11] The fastest but least sensitive is the optical fiber–based biosenser.[12] The detection of botulism spores from the feces of infected horses has been useful in some outbreaks.[5,8] Compared with normal horses, the detection of spores in the feces is a rare occurrence (16 out of 507 samples). The use of ELISA for the detection of antibody in the serum of horses has been helpful to detect type C and D toxin in horses and cattle.[5]

Another test that may be of benefit is electromyography to identify a decremental response following repetitive stimulation. Although this test has been helpful in human beings, it is rarely helpful in horses. One should suspect a diagnosis of botulism in horses with acute onset of flaccid paralysis, weak or poor eyelid tone, poor tail tone, slow or difficult eating, and dysphagia.

10.17.3 Treatment

Treatment of horses with botulism is usually expensive, time consuming, and often unrewarding. If the disease is recognized early, the prompt use of specific or multivalent antiserum may be helpful. If the disease is slowly progressive, administering 200 ml of antiserum (30,000 IU) to foals or 500 ml (70,000 IU) to an adult horse may be beneficial. Otherwise, nonspecific therapy such as nursing care, tube feeding, sedation to reduce muscle activity, antimicrobial agents, and laxatives may help. One possibly may assist horses with difficulty breathing by using mechanical ventilation.[2,5,6,8,10]

Prognosis is poor with botulism, although for horses that are still standing and can be given antiserum, the prognosis may approach 70%.[4] The shorter the time the horse is recumbent and the sooner it can swallow, the better the prognosis. In horses that do survive, the recovery is complete. The disease is preventable by vaccination using type B toxoid. This vaccine was developed to prevent disease in newborn foals and is given to pregnant mares. The usual schedule is three doses at 1-month intervals with revaccination 6 weeks before foaling annually.[5,6,10]

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10.18

10.18—Equine Grass Sickness (Equine Dysautonomia)

Yvette S. Nout

Equine grass sickness (EGS) is an acquired degenerative polyneuropathy that predominantly affects the neurons of 652
the autonomic and enteric nervous system. The disease was recognized first in Scotland in 1909[1] and since then has 653
been reported in other parts of England, continental Europe, and Australia.[2,3] A clinically and histopathologically similar disease, *mal seco,* occurs in Argentina, southern Chile, and the Falkland Islands.[4] So far the disease has not been recognized in northern America.

The disease affects postganglionic parasympathetic and sympathetic neurons and is characterized as a dysautonomia. Clinical signs vary, and the severity of EGS is suggested to be related to the extent of neuronal damage. The disease occurs sporadically and is often fatal. Although EGS has been studied extensively, the cause of the disease remains unknown.

10.18.1

Clinical Signs

Equine grass sickness has been reported in other equids such as the Przewalski's horse, zebra, donkey, and pony but primarily occurs in young, mature horses that have access to pasture. Horses with EGS are generally between 2 and 12 years of age and horses between the ages of 3 and 5 years are at greatest risk for developing the disease. No breed or gender predilection to the disease is apparent.[3]

Acute, subacute, and chronic forms of EGS are recognized, of which clinical signs overlap from one form to the other.[5,6] Most horses are depressed and dysphagic. The dysphagia is caused by cranial nerve involvement and is recognized by drooling of saliva, feed material in the nares, impacted feed material in the buccal pouches, and difficulty drinking.

10.18.1.1

ACUTE FORM

Clinical signs are related to acute onset of gastrointestinal ileus. The course of disease is generally less than 48 hours. Signs of abdominal pain may be severe and are caused by gastric and small intestinal distention. Affected horses are hypovolemic, and the reduced circulating volume may cause death from cardiac failure. Other clinical signs are generalized or patchy sweating, fine muscular fasciculations, pyrexia, severe dysphagia, and bilateral ptosis. Examination per rectum reveals small intestinal distention and often a mild

secondary impaction of the large colon. One can obtain a large quantity of reflux following nasogastric intubation.

10.18.1.2 SUBACUTE FORM

The course of disease is 3 to 7 days, and clinical signs are less severe than in acute EGS. Horses with subacute EGS do not develop gastric or small intestinal distention but develop large colon impactions. These horses have a characteristic “tucked-up” stance. Clinical signs include intermittent colic and patchy sweating. These horses often have rhinitis sicca.

10.18.1.3 CHRONIC FORM

The course of the disease is weeks to months. Cachexia is the most prominent clinical abnormality in horses with chronic EGS. The entire gastrointestinal tract is empty in these horses, and they usually have rhinitis sicca characterized by accumulation of mucopurulent material in the nasal passages. Other clinical signs in animals with chronic EGS are sweating, muscle tremors, gait abnormalities (short-strided), leaning against walls, base-narrow stance, snoring, pica, and penile prolapse. In a later stage, one may observe coat abnormalities such as areas of piloerection, growth of a long coat, and pallor of the coat.

10.18.2 Pathologic Findings

Gross necropsy findings in acute EGS are consistent with gastrointestinal ileus. A large fluid-filled stomach and distended small intestine are present. Secondary impacted ingesta is often present in the large colon and cecum. Splenomegaly and erosions of the esophageal mucosa are other findings in acute EGS. Gross necropsy findings in subacute EGS are less severe and include colonic impaction.[2,5] One may find inspissated mucous in the lumen of the small colon and rectum and evidence of intraluminal hemorrhage such as inspissated blood or black feces. In horses with subacute and chronic EGS, one may find rhinitis sicca.

Neuronal lesions are most severe in the autonomic ganglia (cranial cervical, stellate, celiacomesenteric) and enteric nerves. One finds less severe lesions in some brainstem nuclei and parts of the spinal cord.[2,5–7] Changes detected in affected neurons are degenerative changes and include chromatolysis, loss of Nissl's substance, loss of the nucleus, spheroid formation, neuronal swelling, and an increase in the number of lysosomes and mitochondria.[2,5,6] Loss of a recognizable Golgi apparatus appears to be an early neuropathologic event in EGS.[5]

A recent study has demonstrated a reduction of neuropeptide expression in nasal mucosal innervation in horses with EGS.[8] This is suggested to underlie the presentation of rhinitis sicca seen in this disorder.

Similarly to other neurodegenerative diseases, one finds a specific anatomic distribution of neuropathologic conditions in EGS, which supports the hypothesis of a common factor sensitizing the neurons to specific insults. As in EGS, multiple system involvement is a common feature in human neurodegenerative disorders. The central pathologic condition in EGS appears to be specific and repeatable, consistent with findings in primary dysautonomias in other species. However, in EGS, one does not see the increasing severity of pathologic findings and severity of clinical signs with increased duration of disease consistently as is seen in classical 653
neurodegenerative diseases in EGS. Moreover, the decrease in severity of pathologic conditions of the brain with 654
increased duration of disease may suggest a healing process from the initial insult. The association between central neuronal pathologic findings and severity of clinical disease is unclear. In one study, increased neuronal

pathologic findings were observed in milder clinical cases (chronic EGS),[7] which contrasts to another study that demonstrated increased neuronal pathologic findings in acute cases of EGS.[9]

10.18.3 Pathophysiology

Clinical signs are related to the damage of the autonomic nervous system. The most severe lesions are found in the myenteric and submucosal plexuses of the ileum and less severe changes occur in the celiacomesenteric ganglion. The altered autonomic activity results in cessation or decrease of intestinal peristalsis and subsequently leads to the development of ileus and colonic impaction. The dysphagia in most cases of EGS is caused by cranial nerve or brainstem involvement.[5] Based on epidemiologic and pathologic findings in horses with EGS, an unidentified neurotoxin is implicated as the causative agent of the disease; however, because of the complexity of the disease, multiple factors are suspected to be involved in the pathogenesis of EGS.[2,3,5]

Multiple experimental studies have failed to identify the causative agent(s) of EGS. Risk factors for developing the disease have been identified, however. Young horses between 3 and 5 years of age are at greatest risk for developing EGS.[3] Older horses have been suggested to be resistant to EGS, perhaps following nonfatal exposure to the causative agent. Moreover, horses that had been in contact with cases of EGS were 10 times less likely to develop the disease.[10] Similarly, transfer of maternal antibodies against this causative agent may explain the fact that EGS has not been reported in horses younger than 6 months. Horses that are kept solely at pasture are at increased risk of developing EGS; however, the disease has been reported in stabled horses and in horses that had no access to grass at all. The type of pasture or the type of supplementary feeds do not appear to be associated with the development of EGS. Horses that have been on the premises for less than 2 months and horses that have been moved recently to a different pasture are at increased risk of developing EGS. Horses are more likely to be affected with EGS when they are housed on premises that have experienced the disease previously. Although cases of EGS can occur year-round, the incidence of EGS consistently has been found to be greatest during the spring months, particularly if a period of warm weather is followed by a period of wet weather.

Paravertebral ganglionic damage exceeds and occurs earlier than prevertebral ganglionic damage. The putative neurotoxin is suggested to be ingested and initiates damage in the enteric plexuses, which leads to immediate functional deficits seen in the acute form of EGS.[5] Functional lesions precede structural lesions caused by secondary retrograde axonal degeneration. In chronic EGS, less initial enteric nerve damage occurs, and subsequently fewer secondary prevertebral ganglionic lesions occur. Consequently, the body has more time to develop functional and structural compensatory mechanisms. The less severe damage to the celiacomesenteric ganglion compared with the damage in the paravertebral ganglia is suggested to be caused by hematogenous spread of an ingested neurotoxin.

Currently, toxicoinfection with *Clostidium botulinum* type C is being investigated as a cause of the disease.[11–13] This organism grows and produces toxin in the gastrointestinal tract. The role of this organism in the cause of EGS is supported by the fact that type C botulinum neurotoxin has been detected in the gastrointestinal tract of 67% of horses with acute and 74% of horses with chronic EGS compared with 10% of control horses.[14] Moreover, horses with EGS were found to have lower immunoglobulin G concentrations to the surface antigens of *C. botulinum* type C and to type C botulinum neurotoxin. An association between antibody concentration and form of EGS was not demonstrated, but horses that had been exposed to other horses with EGS or that had been grazing on pasture where the disease had occurred previously had significantly higher antibody concentrations to these antigens.[11] These findings may indicate the development of a protective immune response against the

putative neurotoxin. More recently it has been demonstrated that fresh grass may contain botulinum neurotoxin, and this supports the hypothesis that EGS may be a form of soil-borne botulism in horses.[12] It is suggested that natural-occurring biofilms may protect bacteria and chance their survival; however, further research in the areas of etiology, pathogenesis, treatment, and prevention of EGS is necessary.

The sulfur amino acid depletion in horses with EGS has been suggested to contribute to the pathogenesis of this disease through the increase of oxidative neuronal death; however, this has not been proven.[15] A further study found no evidence of systemic macromolecular oxidative damage in horses with EGS.[16] Mycotoxin toxicity also has been implicated as a cause for EGS,[13] and low sulfur amino acid concentrations may enhance the toxicity of mycotoxins.

10.18.4 Diagnosis

No noninvasive definitive test exists to obtain an antemortem diagnosis of EGS. Serum biochemical and hematologic examinations do not reveal specific changes in horses with EGS. Plasma amino acid analysis in 654
horses with acute EGS demonstrated a significant increase in plasma taurine concentration and a significant 655
decrease in plasma cysteine, arginine, citrulline, histidine, isoleucine, leucine, methionine, threonine, tyrosine, and valine concentrations. In horses with subacute and chronic EGS, these changes were less significant and the plasma amino acid profile normalized. Although one cannot use the amino acid analysis as a definitive test for EGS because of overlap in individual amino acid concentrations for control horses, horses with EGS, and horses grazed with EGS-affected horses, the test may have value as an additional diagnostic aid, for adequate plasma cysteine concentrations (greater than 17 μmol/L) may exclude EGS.[15] Most horses with EGS and healthy co-grazed horses had significantly decreased plasma sulfur amino acid (cysteine and methionine) concentrations compared with healthy control animals.

When watching a horse eat, one may notice signs of abnormal esophageal function such as esophageal spasm and reverse peristalsis. Contrast radiography and endoscopy of the esophagus may reveal abnormalities such as megaesophagus and esophageal erosions.[6]

Examination of an ileal biopsy, collected via laparotomy, is the only method to confirm EGS ante mortem.[18] The neuronal degeneration is present in the enteric plexuses from the esophagus to the rectum, but the most severe changes are present in the terminal ileum. Examination of a rectal mucosal biopsy is specific but not sensitive for EGS.

The most important differential diagnoses for acute EGS are a small intestinal strangulating lesion and duodenitis–proximal jejunitis. Clinical signs and neuronal lesions in chronic equine motor neuron disease and chronic EGS may be similar, and these similarities have led to the speculation that these two diseases are related.[19] Further examination, however, reveals important differences such as the fact that equine motor neuron disease occurs in older horses that have not had access to pasture, and EGS occurs in young horses with access to pasture. Equine grass sickness sometimes occurs along with equine motor neuron disease.[2,19]

10.18.5 Treatment and Prevention

No effective cure exists for EGS. Horses with acute EGS may respond initially to gastric decompression and intravenous fluid therapy. Management of horses with chronic EGS includes nursing care and the use of the prokinetic drug cisapride and the appetite stimulant diazepam.[6]

10.18.6 Prognosis

Equine grass sickness is usually fatal. The case fatality rate for acute and subacute cases is 100%. If horses with acute EGS are not euthanatized within 48 hours, they die from circulatory failure or gastric rupture. Horses with subacute grass sickness usually are euthanatized within 7 days.[7] Horses with chronic EGS often are euthanatized because of weakness and emaciation; however, when given appropriate care at a referral hospital, approximately 50% of horse may survive.[6] Survivors may return to work, but some display residual abnormalities such as mild dysphagia, sweating, and coat changes.

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10.19 10.19—Lyme Disease in Horses

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Lyme disease is the most common vector-borne infectious disease in human beings in the United States. Initially recognized in the mid-1970s in Lyme, Connecticut, as the cause of unexplained rheumatoid arthritis in children, the causative agent of Lyme disease was discovered to be a spirochete, *Borrelia burgdorferi*. In addition to human beings, Lyme disease affects domestic animals such as dogs, cats, cattle, and horses.[1–3]

Human beings and animals acquire Lyme disease by transmission of *B. burgdorferi* through the bite of an infected tick. In the eastern and midwestern United States the vector is the black-legged tick or deer tick, *Ixodes scapularis* (formerly known as *I. dammini*), whereas in the western United States the vector is the western black-legged tick, *I. pacificus*. Not all ticks are infected with the spirochete, and infection varies by tick species and geographic region. These ticks have a 2-year, three-stage life cycle and feed once during each stage.[4] The larvae hatch in the spring and summer and are usually noninfective because transovarial transmission is rare.[5] The tick may become infected at any stage of the life cycle by feeding on small mammalian hosts, typically the white-footed mouse *(Peromyscus leucopus),* which is a natural host for the spirochete. The nymphal stage emerges the next spring and is most likely to transmit the disease because it is small, difficult to see, and engorges faster; engorgement is necessary to transmit *B. burgdorferi*. Once the tick has engorged, *B. burgdorferi* is transmitted to the host via lymphatics or blood. The stage of the life cycle responsible for transmitting Lyme disease in horses is unknown.

10.19.1 Epidemiology

In endemic areas of the northeast and midwestern United States, approximately 20% of nymphal stages and 30% to 40% of adult stages of *I. scapularis* are infected with *B. burgdorferi*. In contrast, *I. pacificus* often feeds on lizards that are poor reservoirs for *B. burgdorferi,* and only 1% to 3% of these ticks, including the nymphal stages, are infected with the spirochete.[5] This difference in the number of infected ticks may explain the difference in the prevalence of Lyme borreliosis between eastern and western United States. Seroprevalence is high in the northeastern United States but is not known for other parts of the country.[3] The apparent increasing incidence of disease in human beings and animals may result from an increased deer population, increased number of ixodid ticks, expansion of human and horse populations into previously rural woodland areas, or increased recognition of the disease manifestations.

The disease has a seasonal prevalence and is most common in spring, summer, and fall with a peak incidence in June and July. In some climates such as California, ticks may be active year-round. The organism is maintained in a complex life cycle of small wild mammals and immature stages of the black-legged tick. Larval and

nymphal stages of the tick acquire the infection when they feed on infected mice. Deer are not a reservoir for Lyme disease but rather a host for the adult stages of the tick.

10.19.2 Clinical Signs

Clinical signs associated with *B. burgdorferi* in horses, as in human beings, are often nonspecific and involve multiple body systems. In human beings, clinical signs frequently include annular rash, myalgia, aseptic meningitis, arthritis, and seventh cranial nerve palsy.[5] The disease usually begins as a skin rash and often progresses to involve joints and the nervous and cardiac systems but may involve other body systems as well. In human beings the skin rash may be slowly progressive and sometimes may take up to 1 month before becoming apparent.

In horses, typical clinical signs of Lyme borreliosis include chronic weight loss, sporadic lameness, laminitis, low-grade fever, swollen joints, muscle tenderness, and anterior uveitis. Some horses may demonstrate clinical illness and depression and may go off feed in a short time. The bacteria may enter the central nervous system within a short time following exposure. Chronic arthritis may develop as a result of autoimmune mechanisms, although this is not fully proved.

10.19.3 Diagnosis

The diagnosis of Lyme disease is often difficult. History of tick exposure or living in a Lyme endemic area are helpful and when combined with the identification of clinical signs along with elimination of other diseases allows a presumptive diagnosis. Examination of blood work for other diseases may be more beneficial than a positive blood test for Lyme, although one should complete such a test as well. In human beings an early increase in serum immunoglobulin M often occurs. Response to therapy also helps to support a presumptive diagnosis of Lyme disease. Diagnosis of Lyme disease in horses is difficult,[6] and in many cases one bases a
presumptive diagnosis on history, clinical signs, and response to antibiotic therapy in an animal with probable 656
cause to be infected (i.e., possible exposure). In human beings the skin lesions are obvious. Blood tests are of 657
limited value, although enzyme-linked immunosorbent assay and Western blot testing on blood from suspect animals has been reported.[1,2] One may test samples of joint fluid, cerebrospinal fluid, and tissues from affected patients for presence of organisms.

10.19.4 Pathogenesis

B. burgdorferi organisms are capable of nonspecifically activating monocytes, macrophages, and synovial lining cells, as well as natural killer cells, B cells, and complement leading to production of host proinflammatory mediators. These proinflammatory mediators seem to localize in joints, leading to a chronic arthritis and lameness. In addition, *B. burgdorferi* has developed strategies to interact with the mammalian host, including adhesion to host cells and components of the extracellular matrix such as fibrinectin, β_3 integrins, and glycosaminoglycans.

10.19.5 Treatment and Prevention

Many horses are treated annually for a presumptive diagnosis of Lyme disease. Treatment can be a prolonged course of antibiotic treatment with tetracycline, doxycycline, and ceftiofur. One may give medications by oral,

intravenous, or intramuscular routes and expect a response within 2 to 4 days. In some horses the clinical signs may show an initial worsening as a response to toxins released following death of the organisms.

Currently, no licensed vaccine is available to prevent Lyme disease in horses, although several vaccines are on the market at this time and a human vaccine is being developed. Aids to prevention include daily grooming with removal of ticks, along with the use of tick repellents that contain permethrin. One should apply tick repellents to the head, neck, legs, abdomen, and under the tail. Keeping pastures mowed and removing brush and woodpiles makes the environment less hospitable for rodents, which in turn decreases the tick population.

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10.20 10.20—Head Shaking

Robert H. Mealey

10.20.1 Clinical Signs

Head shaking is a widely recognized disorder characterized by persistent or intermittent, spontaneous, and frequently repetitive vertical, horizontal, or rotary movements of the head and neck.[1] The affected horse shakes, jerks, or flicks its head uncontrollably in the absence of obvious physical stimuli, and the condition can be severe enough to render the horse unusable and dangerous.[2–5] Head shaking often is accompanied by snorting and sneezing, and many horses rub their noses on stationary objects or on the ground while moving. In addition to shaking the head up and down, flipping the upper lip, or shaking the head side to side, horses also may act as if a bee has flown up the nostril or may strike at the face with the forelimbs.[2,6,7] Head-shaking horses also may exhibit avoidance behavior, with low head carriage, corner seeking, and nostril clamping.[1,8] To characterize the severity of clinical signs, a grading system of 1 to 5 has been described, with grade 1 being a rideable horse with intermittent and mild signs (facial muscle twitching) and grade 5 being an unrideable, uncontrollable, dangerous horse with bizarre behavior patterns.[1] Clinical signs are usually worse during exercise; however, head shaking can affect horses at rest.[1–5,7] Mean age of onset is typically 7 to 9 years, and Thoroughbreds and geldings appear to be overrepresented in some studies.[2,3,6] Head shaking can be seasonal or nonseasonal. Early literature suggested an increased incidence of head shaking during the warmer months of the year,[5] and a later study 657

indicated that the peak periods of onset were spring and early summer.[3] Recent studies have found that 64%[6] and 59%[2] of head-shaking horses are affected seasonally, with the majority developing signs in the spring or early summer. 658

10.20.2 Pathogenesis

Early work suggested 58 possible causes for head shaking, including 2 that have received considerable attention recently: photophobia and trigeminal neuralgia.[9] Other reported and suggested causes include behavioral resentment to rider-induced head and neck flexion, exercise-induced hypoxia, ear mites, cranial nerve dysfunction, otitis media or otitis interna, cervical injury, ocular disease, guttural pouch mycosis, dental periapical osteitis, vasomotor rhinitis, allergic rhinitis, *Trombicula autumnalis* (harvest mite) larval infestation, maxillary osteoma, and equine protozoal myeloencephalitis.[3,4,9–14] A study of 100 head-shaking horses revealed a potential specific cause in only 11 horses, and elimination of the abnormality resulted in resolution of the head shaking in only 2 of them.[3] Idiopathic head shaking has been used to describe the majority of cases in which no specific underlying cause is found, and necropsy results in these horses reveal no lesions.

The clinical signs exhibited by most head-shaking horses now generally are thought to result from sharp, electric-like, burning pain involving the trigeminal nerve.[1,2] Trigeminal nerve involvement is supported by the observations that some horses improve after blocking the infraorbital nerve (part of the maxillary branch),[15,16] and that most horses improve after blocking the posterior ethmoidal nerve (part of the ophthalmic branch).[1] Horses that head shake in response to light stimulation have been described as photic head shakers.[8] Most of these horses are affected seasonally (spring and summer), improve at night or when brought indoors, and improve when blindfolded or when gray lenses are placed over their eyes. Photic head shaking is hypothesized to be caused by optic-trigeminal summation (stimulation of the optic nerve that results in referred sensation to parts of the nose innervated by the trigeminal nerve), a mechanism similar to that proposed for photic sneezing in human beings.[2,8] Many features of head shaking in the horse are similar to trigeminal neuralgia in human beings, a severe chronic pain syndrome characterized by dramatic, brief stabbing or electric shock–like pain paroxysms felt in one or more divisions of the trigeminal distribution, spontaneously or on gentle tactile stimulation of a trigger point on the face or oral cavity. The disease is associated with microvascular compression and pathologic changes in the trigeminal root and trigeminal ganglion.[17] The basic underlying cause of head shaking in the horse may be trigeminal neuralgia,[1] and recent work suggests a degenerative disorder of the brainstem nucleus of the trigeminal nerve (D.C. Knottenbelt, personal communication). The trigeminal nerve is postulated to be hypersensitive and to fire in response to a variety of trigger factors including wind, airway turbulence, increased blood flow, pollen, dust, warmth, cold, insects, allergies, or other irritations. These trigger factors appear to act intranasally in many horses but could act in any trigeminal sensory region.[1]

10.20.3 Diagnosis and Treatment

One should perform a complete physical examination, including neurologic, dental, ophthalmic, and otoscopic examinations to rule out potential underlying causes. Other diagnostics could include endoscopy of the nasal passages, pharynx, and guttural pouches; radiography of the skull and cervical spine; complete blood count; and serum biochemistry profile. These examinations reveal no abnormalities in most cases. A recent survey suggests that one should consider idiopathic head shaking if two of the following three clinical signs are present: vertical flipping of the head, acting as if an insect has flown up the nostril, or rubbing the muzzle on objects.[2] For photic

head-shaking horses, clinical signs should improve following blindfolding or after placement of a mask shielding the eyes from the sun.

Cyproheptadine, an antihistamine (histamine$_1$) and serotonin antagonist with anticholinergic effects, has been used (0.3 mg/kg orally b.i.d.) to treat head-shaking horses, resulting in improvement in 5 of 7 horses in one study[8] and 43 of 61 horses in another.[2] Horses that respond do so within 1 week, and some may respond within 24 hours. Although the mechanism for efficacy of cyproheptadine in these cases is unknown, blocking of serotonin-mediated pain sensation could be involved.[8] Other investigators have found cyproheptadine alone to be ineffective, but the addition of carbamazepine (4 to 8 mg/kg orally t.i.d. or q.i.d.) resulted in 80% to 100% improvement in seven of nine cases, with horses responding within 3 to 4 days.[1] Carbamazepine is a sodium channel-blocking antiepileptic drug and is the drug of choice for treating trigeminal neuralgia in human beings.[18] Carbamazepine alone was reported to be effective in head shaking horses, but results were unpredictable.[1] The elimination half-life of carbamazepine in the horse is less than 2 hours, making sustaining therapeutic concentrations difficult; the drug is therefore of more benefit diagnostically than therapeutically in head-shaking horses (D.C. Knottenbelt, personal communication). Other medications and therapies including nonsteroidal antiinflammatory drugs, corticosteroids, antihistamines, acupuncture, chiropractic manipulation, homeopathy, and feed supplements are generally ineffective in most horses.[2,19] 658

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Diagnostic blockade of trigeminal nerve branches can be useful for identifying trigger points in head-shaking horses. Blockade of the infraorbital nerve may improve some horses and might identify candidates for infraorbital neurectomy.[15,16] However, results of infraorbital blockade and infraorbital neurectomy do not correlate, and because of neuroma formation, self-trauma, and low success rate, infraorbital neurectomy is not a recommended procedure.[15] A high percentage of horses improve after blockade of the posterior ethmoidal nerve, and sclerosis of this nerve results in temporary improvement in some horses.[1]

Therapies aimed at decreasing the response to trigger factors can be effective and include tinted contact lenses; face masks or hoods that block sunlight, wind, insects, dust, etc., with mesh or dark eye cups; and nose nets.[1,2,8,19,20] A recent owner survey found that nose nets that cover the nostrils with mesh and include a draw string or elastic band that applies pressure to the upper lip resulted in some improvement in 70% of head-shaking horses, and that 70% or more improvement occurred in about 30% of the horses.[20] Finally, permanent tracheostomy is effective in some horses,[1,4] presumably because airflow then bypasses the nasal cavity where the majority of trigger factors are thought to act.[1]

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10.21

10.21—Verminous Encephalomyelitis

Eduard Jose-Cunilleras

Aberrant migration of helminth and fly larvae through the central nervous system (CNS) of horses and donkeys is a rare but important cause of severe neurologic disease. Parasites that have been reported to affect the brain and spinal cord of equids include rhabditid nematodes (*Halicephalobus [Micronema] deletrix* [synonym *H. gingivalis*]), strongyloid nematodes (*Strongylus vulgaris, S. equinus,* and *Angiostrongylus cantonensis)*, spiruroid nematodes *(Draschia megastoma),* filarid nematodes (*Setaria digitata* and other *Setaria* spp.), and warble fly larvae (*Hypoderma* spp.).

Antemortem diagnosis is often impossible; however, a high index of suspicion may be warranted for certain clinical signs (acute onset or rapidly progressive asymmetric, focal, or multifocal brain or spinal cord signs) and changes in cerebrospinal fluid (CSF) analysis; in which case the treatment regimen should include specific

antiparasitic drugs (e.g., fenbendazole) in addition to the more routinely used symptomatic and antiinflammatory treatments (nonsteroidal antiinflammatory drugs, dimethyl sulfoxide, corticosteroids, antiprotozoal drugs, etc.). 659

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10.21.1 Causes

10.21.1.1 *HALICEPHALOBUS (MICRONEMA) DELETRIX (H. GINGIVALIS)*

Halicephalobus deletrix is synonymous with *H. gingivalis,* the latter being the most recent terminology.[1] The parasite was referred to as *Micronema deletrix* until 1970s and 1980s.

The life cycle of *Halicephalobus* nematodes is unknown. This small roundworm generally is considered a saprophytic organism that occasionally acts as a facultative parasite in horses and human beings. The likely route of entry is through nasal and oral mucosa followed by possible hematogenous spread to organs with high vascularization such as the brain, spinal cord, and kidneys. *H. deletrix* recently has been shown to be transmitted from a mare to her foal because of mammary infestation.[2]

Organs affected by migration of *Halicephalobus* include the brain, spinal cord, nasal and oral cavities, pituitary gland, and kidneys and less commonly the lymph nodes, heart, lungs, stomach, liver, and bones.[3,4] Organisms affecting the CNS have been reported to have a predilection for the basilar, pituitary region of the brainstem.[5] Characteristic histopathologic lesions in the CNS include malacia, granulomatous and lymphohistiocytic inflammatory infiltration, meningitis, and vasculitis in addition to identification of the nematodes. Other clinical signs include oral and nasal granulomata, renal involvement (*H. deletrix* is visible in the urine), granulomatous osteomyelitis,[6] and granulomatous chorioretinitis.[7]

Most of the cases of *Halicephalobus* encephalomyelitis in adult horses reported in the literature during the last 30 years describe simultaneous renal granulomatous lesions encapsulating the nematodes (Table 10.21-1). Granulomata within or adjacent to the renal parenchyma were observed on postmortem examination in 13 of 16 horses with neurologic disease, and of the other 3, in 1 case lesions were confined to the sacral spinal cord and cauda equina and in another case only the skull and brain was examined at postmortem. In contrast, all 3 cases reported to date in foals showed no renal involvement but did show pulmonary granulomata.[2,5]

10.21.1.2 *STRONGYLUS VULGARIS, S. EQUINUS*, AND *ANGIOSTRONGYLUS CANTONENSIS*

Aberrant strongyle larval migration is a much less common cause of neurologic signs because of routine broad-spectrum antiparasitic treatment with ivermectin and moxidectin. The pathogenic mechanisms described for strongyle encephalomyelitis include aberrantly migrating fourth- and fifth-stage larvae in the intima of the aorta or left ventricle, which causes endothelial damage, stimulates the clotting cascade, and results in formation of a thrombus that often contains the parasitic larva.[20] Lesions are generally asymmetric because of random migration in the brain, although migration along the spinal cord has been reported in a donkey.[21]

10.21.1.3 *DRASCHIA MEGASTOMA*

Adult *D. megastoma* worms are found in the stomach of equids, and they cause mucosal granulomatous masses and mild chronic gastritis. The life cycle of *Draschia* is indirect because the organism uses flies as the intermediate host. Eggs and larvae are released into the gastric lumen, and the first larval stage passes in the feces and is ingested by fly larvae of the genus *Musca* in which *Draschia* organisms develop to infective larvae. Finally, horses become infected when the third larval stage is deposited on the host by adult flies. Infective larvae that are ingested and reach the stomach develop into adults and complete the life cycle. Larvae deposited in damaged skin result in local inflammation and development of extensive granulation tissue, which is the typical lesion of cutaneous habronemiasis.

Stray *D. megastoma* larvae may be found anywhere throughout the body, and a case has been reported of *D. megastoma* migration in the brainstem of a horse in southern United States resulting in asymmetric brainstem disease.[22]

10.21.1.4 *SETARIA* SPP.

Infestation with the filarial nematode *Setaria* is common in the abdominal cavity of ungulates where the organism does not cause significant clinical effects. *S. labiato-papillosa* is found in cattle, and *S. equina* is found in horses. Microfilariae gain access to peripheral circulation and then are transmitted to other potential hosts via mosquitoes. In a postmortem examination study of 305 horses in Japan, *S. equina* was recovered from 66 of those horses (~22%).

The cattle parasite *S. digitata* occurs only in Asia, and infestation in unnatural hosts (horses, sheep, goats, camel, and human beings) has been associated with cerebrospinal nematodiasis. This condition can be enzootic in India, Burma, and Sri Lanka, and is called “Kumri” (weak back) and has a seasonal occurrence, usually during the fly season (late summer and fall).[27]

Two cases of aberrant *S. digitata* CNS migration have been reported in Japanese racehorses, and one case of *Setaria* larval migration in the brainstem and cervical spinal cord has been reported in a 12-year-old Quarter Horse mare in the midwestern United States.[19,28]

10.21.1.5 *ANGIOSTRONGYLUS CANTONENSIS*

Adult parasites are found in the right ventricle and pulmonary artery of rats. The pulmonary circulation carries 660
eggs released by adult worms to the alveoli, where the first larval stage develops and migrates up the trachea, 662
is swallowed, and passes in feces. Snails and slugs are intermediate hosts, and ingestion of the snail results in ingestion of the infective larvae that migrate through CNS, and finally, the larvae reach the heart via the circulation.

TABLE 10.21-1 Signalment, Organism Identified, Clinical Signs, and Organs Affected in Cases of Verminous Encephalomyelitis

SIGNALMENT	ORGANISM	CLINICAL SIGNS	ORGANS AFFECTED	AFFECTED AREAS IN CENTRAL NERVOUS SYSTEM	REFERENCE
19-year-old Saddlebred gelding	*Halicephalobus*	Stranguria Ataxia Blindness Fever Recumbency	Kidneys Brain	Cerebrum Thalamus Hypothalamus	30
12-year-old pony gelding	*Halicephalobus*	Incoordination Voracious appetite Fever Recumbency	Kidneys Brain	Cerebrum Cerebellum	8
6-year-old pony gelding	*Halicephalobus*	Ataxia Recumbency	Kidneys Brain	Thalamus Cerebellum Pons and medulla	3
8-year-old Arabian female	*Halicephalobus*	Ataxia Mandibular swelling	Mandible Brain	Thalamus Midbrain	9
18-year-old Quarter Horse	*Halicephalobus*	Ataxia Recumbency	Kidney Brain	—	10
4-year-old Appaloosa gelding	*Halicephalobus*	Mandibular mass	Mandible	—	11
13-year-old Pasofino male	*Halicephalobus*	Ataxia Head pressing Recumbency	Kidneys Brain	Cerebellum Hypocampus	12
19-year-old Tennessee gelding	*Halicephalobus*	Ataxia Head pressing Decreased PLR Nystagmus	Kidney Brain	Thalamus Midbrain Meninges	12

12-year-old female	*Halicephalobus*	Ataxia	Brain	Meninges	13
		Recumbency			
14-year-old Paint gelding	*Halicephalobus*	Ataxia	Cauda equina	Cauda equina	14
		Urinary incontinence	Sacral spinal cord and sacral rootlets		
		Cauda equina syndrome	Sacral rootlets		
		Recumbency			
23-year-old	*Halicephalobus*	Fever	Brain	Cerebrum	15
Saddlebred gelding		Ataxia	Eye	Cerebellum	
		Blindness		Brainstem	
				Optic nerve and retina	
6-year-old Quarter	*Halicephalobus*	Ataxia	Brain	Midbrain	16
Horse female		Recumbency		Cerebrum	
				Cerebellum	
16-year-old	*Halicephalobus*	Renal disease	Kidney	Optic nerve	4
Holsteiner male		Blindness in right eye	Eye	Cerebellum	
		Seizures	Brain	Hippocampus	
		Comatose			
5-year-old Miniature male	*Halicephalobus*	Ataxia	Kidneys	Cerebral cortex	4
		Uveitis	Brain		
		Testicular enlargement	Testicles		
17-year-old	*Halicephalobus*	Ataxia	Brain	Cerebrum	17
Tennessee male		Hyperesthesia		Cerebellum	
		Opisthotonus		Brainstem	
		Nystagmus		Cervical spinal cord	
10-year-old	*Halicephalobus*	Uveitis	Eyes	Hypothalamus	7
Thoroughbred gelding		Head pressing	Brain	Thalamus	
		Aggressive behavior	Kidneys	Brainstem	
12-year-old	*Halicephalobus*	Weakness	Kidneys	Brainstem	18

Thoroughbred male		Ataxia	Brain	Cerebellum Meninges	
13-year-old Quarter Horse gelding	*Halicephalobus*	Draining tract in right side of mandible Ataxia Fever	Mandible Kidneys Brain	—	6
18-day-old male	*Halicephalobus*	Weakness; inability to stand	Lungs Brain	Neurohypophysis Hypothalamus	5
7-week-old male	*Halicephalobus*	Ataxia Convulsions Blindness	Lungs Brain	Cerebellum Cerebellar peduncles Meninges	5
3-week-old Thoroughbred male	*Halicephalobus*	Seizures Fever Ataxia	Lungs Brain	Cerebellum	2
12-year-old Quarter Horse female	*Setaria*	Ataxia Recumbency Urinary incontinence Decreased tail tone	Brain	C1 spinal cord	19
Group of 8 ponies	*Strongylus*	Visual deficits Incoordination Convulsions	Brain	Cerebral cortex Thalamus Mesencephalon	20
2-year-old donkey male	*Strongylus*	Paraparesis Tetraparesis	Lumbar spinal cord	—	21
10-year-old Paint male	*Draschia*	Circling to left Tetraparesis Blindness in left eye Facial nerve paralysis	Brain	Midbrain Pons Temporal cortex Globus pallidus	22
9-month-old Appaloosa gelding	*Angiostrongylus*	Tetraparesis	Brainstem Spinal cord	—	23

4-month-old Thoroughbred male	*Angiostrongylus*	Tetraparesis	Spinal cord	—	23
14-year-old Quarter Horse gelding	*Hypoderma*	Ataxia Circling Right facial nerve paralysis Blindness in right eye	Brain	Midbrain Pons	24
	Hypoderma	—	Brain	Left corpus striatum	25
	Hypoderma	—	Brain	Brainstem	26

PLR, Pulpillary light response.

Aberrant CNS migration of *A. cantonensis* larvae has been reported in two foals with tetraparesis in Australia,[23] and aberrant migration is a recognized cause of eosinophilic meningoencephalitis in human beings and dogs.[29]

10.21.1.6 *HYPODERMA* SPP.

Cattle are normal hosts of *Hypoderma* spp., but horses are accidental hosts of warble fly larvae. In cattle, 662

Hypoderma larvae penetrate the skin after hatching from eggs attached to hair in the rump and lower legs. 663 These larvae migrate through connective tissues to reach the esophagus, in the case of *H. lineatum*, or to the vertebral canal around epidural fat, in the case of *H. bovis*. Finally, the larvae migrate back to the skin over the back where they create a breathing hole. Cutaneous myiasis is uncommon in horses, and warble fly larvae occasionally may migrate aberrantly into the brain, and tissue damage causing necrosis and hemorrhage is extensive because of the big size of the instars.[24] Instars can enter through large natural foramina such as the foramen magnum, optic foramen, and occasionally intervertebral foramina.

10.21.2 Clinical Signs

Severity and duration of clinical signs vary from mild, transient, and insidious to severe and fatal. In some cases, clinical signs progress over 2 to 4 months with periods of improvement or stabilization.[4,22,30] Variability of clinical signs depends on the number of parasites (thromboembolic shower of *Strongylus vulgaris*), on the size of the migrating organism (*Hypoderma* spp. larvae are large and cause severe necrosis and hemorrhage as they migrate in the CNS parenchyma), and neuroanatomic localization of the lesions. Horses suffering from larval migrations in the brain (*S. vulgaris, Halicephalobus deletrix, D. megastoma,* and *Hypoderma* spp.) may display head tilt, circling, recumbency, blindness, hyperesthesia, stiff neck, ataxia, head pressing, recumbency, seizures, and coma. In those cases in which larvae cause lesions in the spinal cord (*S. vulgaris, Halicephalobus deletrix, Setaria* spp., *A. cantonensis*), clinical signs may include focal or multifocal asymmetric ataxia, weakness, dog-sitting posture caused by paraparesis, increased patellar reflexes, atonic bladder, poor tail tone, and poor rectal tone with impaction of feces (see Table 10.21-1). Recently, a case of cauda equine neuritis has been reported that was caused by *H. gingivalis* migration in the sacral spinal cord and cauda equina nerves.[14]

10.21.2.1 Differential Diagnosis

One should consider verminous encephalomyelitis in all cases of acute or progressive asymmetric disease of the spinal cord, cerebrum, cerebellum, or brainstem. If brain involvement is evident without other localizing signs, the differential diagnosis list may include equine togaviral encephalomyelitis, rabies, equine protozoal myelitis (EPM), trauma, cerebral abscess or basilar epidural empyema, bacterial meningitis, hepatic encephalopathy, neoplasia, and leukoencephalomalacia. If the neurologic signs are limited to spinal cord involvement, other diseases to include in the differential diagnosis list may be cervical stenotic myelopathy, EPM, equine herpesvirus 1 myeloencephalopathy, trauma, West Nile virus meningoencephalitis, equine degenerative myeloencephalopathy, trauma, spinal osteomyelitis or discospondylitis, vertebral fracture, and neoplasia. If the only signs present are related to cauda equina syndrome, other conditions to consider are polyneuritis equi, sacral or coccygeal fracture, EPM, equine herpesvirus 1 myeloencephalopathy, sorghum or Sudan grass toxicity, epidural abscess from tail blocking, and neoplasia.

Initial diagnostics should include complete blood count, serum chemistry profile, urinalysis, and CSF collection for cytologic evaluation. In addition, serologic examination and viral isolation and CSF Western blot for EPM may help eliminate several more common viral and protozoal myeloencephalitides. Radiographs of the cervical or lumbosacral spine and myelography may be required to rule out more common causes of spinal cord disease (cervical stenotic myelopathy, trauma, fractures). Although only routinely available at referral hospitals, advanced imaging techniques (e.g., computerized axial tomography and magnetic resonance imaging) may be useful in diagnosing parasitic encephalomyelitis or other conditions with similar clinical signs.

Cerebrospinal fluid analysis in cases of parasitic encephalomyelitis can be normal; however, CSF changes are common and include xanthochromia, increased protein, and neutrophilic and mononuclear pleocytosis, but eosinophils rarely occur (Table 10.21-2). Only in case of *A. cantonensis* were eosinophils the predominant cell type on CSF cytologic examination. In addition, *H. deletrix* eggs are visible on microscopic examination of a CSF sample subjected to cytocentrifugation.[5] Similarly, *H. deletrix* larvae may be visible on microscopic examination of urine sediment or semen in cases of renal and testicular involvement.[4]

Antemortem diagnosis may be possible in those cases in which renal or bony involvement is detected and nematodes are identified in biopsies of the affected tissues.[6] As described previously, renal involvement with granulomatous lesions encapsulating *H. deletrix* worms is observed in most cases of *H. deletrix* encephalomyelitis. Therefore transabdominal renal ultrasound examination and ultrasound-guided biopsy (in those cases with renal lesions present), as well as microscopic examination of urine sediment, may prove useful in cases of acute onset progressive asymmetric neurologic disease, especially if one suspects cerebrospinal nematodiasis caused by *H. deletrix*.

Additionally, a polymerase chain reaction–based diagnostic test has been developed for confirmation of *Setaria* encephalomyelitis in goats, sheep, and horses.[31] This test is based on specific amplification of *Setaria* filarial DNA from a blood sample from the host. 663

TABLE 10.21-2 Cerebrospinal Fluid (CSF) Analysis in Verminous Encephalomyelitis

ORGANISM	WHITE BLOOD CELLS IN CSF	PROTEIN IN CSF	CELLS IN CSF	LARVAE PRESENT IN URINE	REFERENCE
Halicephalobus	2030 cells/μl	89 mg/dl	Mostly PMNs[*]	—	9
Halicephalobus	25 cells/μl	69 mg/dl	15% N, 56% L, 22% M, 5% E, 2% B	—	12
Halicephalobus	81 cells/μl	114 mg/dl	9% N, 41% L, 50% M	—	12
Halicephalobus	60 cells/μl	710 mg/dl	—	—	14
Halicephalobus	495 cells/μl	112 mg/dl	34% N; 37% L; 29% M	No	15
Halicephalobus	—	—	—	Yes	4
Halicephalobus	—	—	—	No	18
Halicephalobus	179 cells/μl	71 mg/dl	Predominantly N; few L, M, and E	No	6
Halicephalobus	35 cells/μl	100 mg/dl	25% N, 58% M, 17% E, 2% L	No	5
Halicephalobus	16 cells/μl	76 mg/dl	31% N, 22% L, 47% M	—	2
Setaria	Increased	Increased	—	—	19
Strongylus	Two of 8 ponies had increased white blood cell counts: 42 cells/μl and 1080 cells/μl.	One of 8 ponies had high protein at 175 mg/dl.	—	20	
Strongylus	9988 cells/μl	550 mg/dl	72% N, 14% L, 12% M, 2% E	—	21
Draschia	Normal	Normal	—	—	22
Angiostrongylus	1560 cells/μl	—	1% N, 8% L, 14% M, 77% E	No	23

* PMN, Polymorphonuclear neutrophil leukocytes; N, neutrophils; L, small lymphocytes; M, large mononuclear cells; E, eosinophils; B, basophils.

10.21.3 Pathologic Findings

The gross and histopathologic lesions depend on the parasite involved. Thorough postmortem examination with sectioning and histopathologic examination of all areas of the CNS relevant to the antemortem clinical signs are important to identify migrating parasites. *Strongylus vulgaris* and *Hypoderma* spp. larvae are easy to see, but other nematodes are only visible on microscopic examination. Some reports describe how one may recover and examine whole, fixed nematodes for distinctive morphologic features by centrifugation of formalin solution in which the brain had been fixed.[8,17] Gross examination of other tissues for evidence of *S. vulgaris* thrombi or presence of *Halicephalobus deletrix* granulomatous lesions may help establish a postmortem diagnosis.

On histopathologic examination, one generally sees extensive tissue necrosis with mixed inflammatory response. *H. deletrix* migration in the CNS typically results in histiolymphocytic infiltrates, malacia, glial proliferation, and perivascular lymphocytic cuffing. Migrations of warble fly and *S. vulgaris* larvae result in severe hemorrhage, large malacic tracts, and edema caused by their relative larger size.

10.21.4 Treatment

Treatment of verminous encephalomyelitis in horses is often unrewarding. None of the cases reported in the literature have responded favorably to antiinflammatory drugs and antihelmintics. Only in one case of *H. deletrix* granuloma limited to the prepuce, therapy with ivermectin and diethylcarbamazine was successful.[32] However, numerous cases of cerebrospinal nematodiasis likely respond favorably to antiinflammatory drugs and antihelmintics, but one never reaches a definitive diagnosis because diagnosis requires a postmortem examination.

Therapeutic recommendations depend on the severity, progression, and localization of neurologic signs and on consideration of possible contraindications (e.g., if one suspects bacterial or viral cause, one should avoid using corticosteroids). In cases of acute neurologic disease use of the following antiinflammatory drugs may be warranted: nonsteroidal antiinflammatory drugs such as phenylbutazone (2.2 mg/kg twice daily), flunixin meglumine (1 mg/kg twice daily), or ketoprofen (2 mg/kg twice daily); intravenously administered dimethyl sulfoxide (1 g/kg as a 10% solution once daily for 3 to 5 days); and corticosteroids (dexamethasone at 0.1 to 0.25 mg/kg once daily or prednisolone at 0.2 to 4.4 mg/kg once daily). If one observes cerebral signs, one may administer mannitol (0.25 to 2 g/kg as a 20% solution once daily), or hypertonic saline may be warranted in an attempt to minimize cerebral edema. In countries in which equine protozoal myelitis is known to occur, antiprotozoal treatment is recommended with sulfadiazine (20 mg/kg by mouth twice daily) plus pyrimethamine (1 mg/kg by mouth once daily) or alternatively with ponazuril (5 mg/kg once daily) or diclazuril. 664

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Specific antiparasitics suggested for treating verminous encephalomyelitis include benzimidazole compounds (oxfendazole, thiabendazole, fenbendazole and mebendazole), diethylcarbamazine and ivermectin for the treatment of nematodes, and organophosphates (trichlorfon and dichlorvos) for the treatment of warble fly larvae. Although ivermectin is effective against most equine parasites, it may not be the best choice because of its delayed method of killing, which may take as long as 10 to 14 days. Some authors have suggested administration of fenbendazole at 50 mg/kg by mouth once daily for 3 days[33,34]; however, specific data on the efficacy of antihelmintics in treating nematode or warble fly larvae migration through the CNS are not available.

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11 CHAPTER 11 DISORDERS OF THE SKIN

Christine A. Rees[*]

11.1 Basic Structure and Function of the Skin

The skin is the largest and one of the most important organ systems within the body. Without skin, a human being or animal would die. The functions of the skin are these:

1. An enclosing barrier that prevents water, electrolytes, and macromolecules from being lost and provides internal support for various organs within the body.
2. A storage reservoir for vitamins, fats, carbohydrates, electrolytes, water, proteins, and other materials.
3. A protective barrier against chemicals and physical factors such as heat, cold, microbiologic organisms, and ultraviolet light (melanocytes).
4. A sensory organ for heat, cold, touch, pain, pressure, and itch.
5. A regulator of body temperature through hair coat.
6. A regulator of blood pressure by changes in peripheral vasculature.
7. A producer of keratinized structures such as hair, hooves, horn, and the uppermost layer of the epidermis.
8. A secretory and excretory organ for apocrine and sebaceous glands that includes antimicrobial factors.
9. A provider of flexibility, elasticity, or toughness to allow for shape and form.
10. An immunosurveillance organ through keratinocytes, lymphocytes, and Langerhan's cells.
11. An indicator of general health (e.g., icteric skin can indicate liver dysfunction).[1]

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11.2 Gross Anatomy

11.2.1 SKIN

Skin thickness varies from 1 mm to 5 mm depending on the body location. The skin thickness is greatest at the dorsum and the proximal extremities and gets thinner ventrally and distally down the extremities. Skin is thickest on the forehead, dorsal neck, dorsal thorax, and base of the tail. The skin is thinnest on the ears and in the axillary, inguinal, and perianal areas.[2,3]

The skin consists of two main layers: the epidermis and dermis. The epidermis consists of the basal layer, spinous layer, granular layer, clear layer, and horny layer. Each layer has different types of cells and differs in function. The basal layer consists of a single column of cells that are attached to the basement membrane.

Typical basal keratinocytes have an oval nucleus, prominent nucleolus or nucleoli, and little heterochromatin. The basal keratinocytes contain melanosomes; various metabolic and synthesizing organelles such as mitochondria, lysosomes, rough endoplasmic reticula, and Golgi complexes; and cytoskeletal structures such as keratin intermediate filaments, microfilaments, and microtubules. Structures that help form the attachment and help to shape the basal layer are also present; these cells are known as desmosomes and hemidesmosomes.[4,5]

The spinous layer of the epidermis comprises two to four layers. The cells become progressively differentiated as they move outward toward the skin surface. The spinous layer cells first appear polyhedral and then become 667

668 more flattened as they develop and move outward. The spinous cells contain cellular organelles and keratin filaments. The cells of the uppermost one to two layers of the granular layer contain small, oval, membrane-bound organelles (300 nm) with alternating lamellae called lamellar granules (membrane-coating granules, keratinosomes, Odland bodies) formed in the Golgi region. They contain polar lipids such as phospholipids, glycosphingolipids, free sterols, and so-called probarrier lipids and hydrolytic enzymes that convert the probarrier lipids into stacks of neutral lipid-rich lamellae that assemble in the intercellular space, coat the surfaces of the cornified cells, and provide a barrier to the permeation of the skin. The spinous cells have abundant desmosomes that consist of adhesion plaques and submembranous plaques involved in cell cohesion.[4,5]

The granular layer consists of highly differentiated cells that are flattened polyhedrons with keratohyalin granules. In this layer of the skin, dephosphorylation and proteinolysis activate filaggrin, which cross-links and bundles with keratin filaments into large macrofilaments. Filaggrin is broken down in the stratum corneum to free amino acids important for the proper hydration of the epidermis. Lamellar granules present in the granular layer aggregate beneath the plasma membrane and fuse with it, opening a channel for the release of granule contents such as polysaccharides, glycoproteins, acid hydrolases, acid lipase, and probarrier lipids into the intercellular spaces. After release, polar lipids are remodeled into hydrophobic, neutral, lipid-rich lamellae that form an effective barrier. A dense layer of highly cross-linked proteins is deposited inside the inner leaflet of the membrane. Together with the membrane, this boundary is referred to as the cornified cell envelope, a rigid structure that resists degradations and is rich is glutamyl-lysine isopeptide cross-links. At the uppermost aspect of this layer, substances such as keratins, filaggrin, and the cornified cell envelope are passed on to the cornified cell layer.[4,5]

The cornified layer contains the largest and most numerous cells of any zone in the epidermis. Cells are large, flattened, and polyhedral; overlap with the margins; and associate through interlocking ridges and modified desmosomes. The contents of the cell are limited primarily to disulfide-bonded keratin filaments, highly cross-linked bundles of macrofilaments, and filaggrin. The compact organization of the proteins becomes looser toward the outer epidermal surface. The breakdown of filaggrin to its constituent amino acids in the outer cell layers accounts for the greater water-holding capacity of the cells and the more diffuse organization of the filaments. Uricanic acid is degraded from filaggrin with histidase and absorbs ultraviolet light. Therefore uricanic acid is important in ultraviolet light protection. The pyrrolidone carboxylic acid derived from glutamine is hygroscopic and helps to keep the skin hydrated even in a dry environment. The preserved trilaminar plasma membrane outside the cornified cell envelope becomes discontinuous and desquamates toward the upper portion of the cornified layer, so that the envelope serves as the real cell membrane.[4,5]

The dermis contains a variety of cells that are essential for the skin to function normally. The dermis contains cellular elements such as fibroblasts, macrophages, histiocytes, eosinophils, and mast cells in the fibrous matrix and nonfibrous matrix. The fibrous matrix consists of collagen and elastic fibers. The nonfibrous matrix consists of glycosaminoglycans and proteoglycans. The blood vessels, nerves, lymphatics, hair, and sebaceous and

apocrine glands are found within the dermis. The sebaceous and apocrine glands in the horse are larger and more numerous compared with those in other large animals species.[4,5]

The area that separates the epidermis from the dermis is known as the basement membrane zone. This layer of the skin functions as a scaffold for organization (important for growth and differentiation of keratinocytes) and repair (speeds up reepithelization), acts to regulate selective permeability by only allowing passage of molecules of the correct charge and molecular size, provides a physical intercellular barrier (tumor containment), and links epithelia to their underlying matrices. The basement membrane consists of structures such as tonofilaments, hemidesmosomes, laminin, fibronectin, proteins such as collagen, laminin-like molecules such as entactin and nidogen, anionic glycosaminoglycans such as heparin sulfate, anchoring fibrils, oxytalan filaments, elaunin fibers, microthread-like filamentous threadwork, and large glycoproteins such as fibrillin.[5]

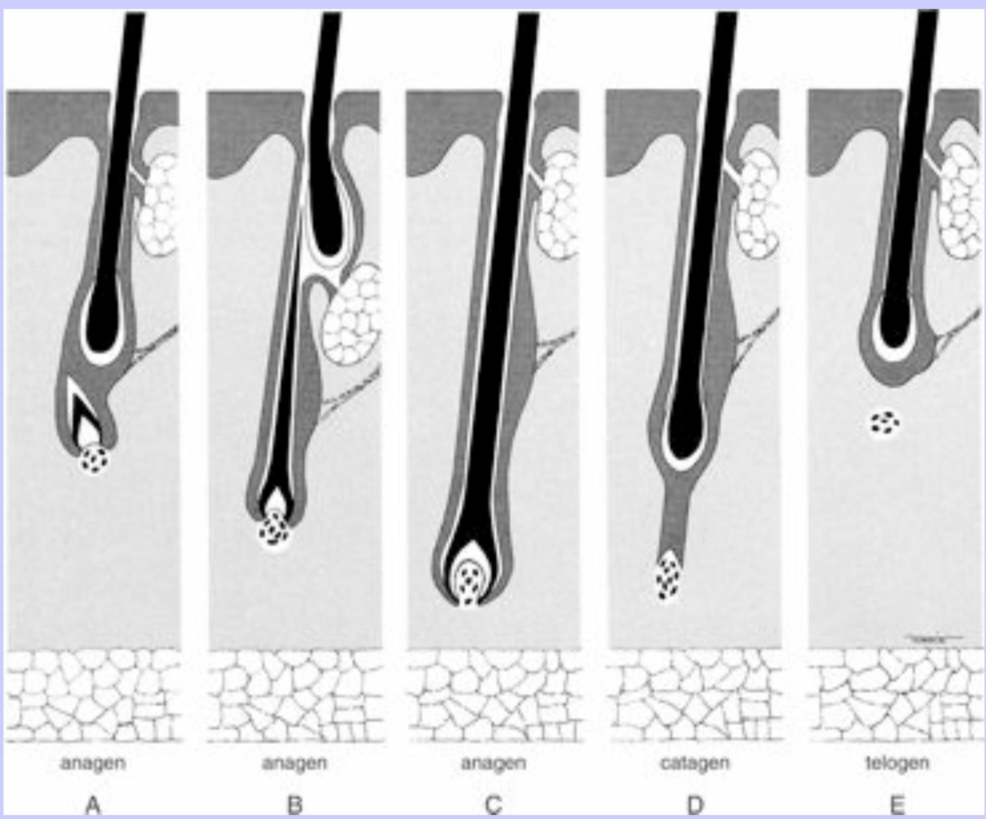
11.2.2 HAIR

The hair tends to cycle in a specific manner. Initially, hairs are in the growing or anagen phase, progressing through an intermediate or catagen phase into a resting or telogen phase (Figure 11-1). The period of time that hairs are in the catagen phase is short, and finding hairs in this stage of the cycle is difficult using a trichogram (plucking hairs and looking at them under the microscope). A variety of conditions including temperature, photoperiod, and hormones can affect the hair cycle.[1,6]

The hair follicles are located within the dermis and are associated with sebaceous and apocrine glands. Hairs are important in thermoregulation, protection of the skin from external factors, and as an ornament to attract other animals. The types of hairs found in equine skin are simple primary hairs. Horse hairs tend not to shed at once. Instead their hair sheds in patches or in a mosaic pattern.[1]

Ambient temperature affects the texture of the hairs. In warm temperatures the hair coat comprises thick, medullated hairs. Piloerection of hairs occurs to aid in cooling the body. In contrast, in cold temperatures the 668
coat comprises longer, finer, and poorly medullated fibers. This type of hair coat provides more insulation from 669
cold. The hairs of the fetlock, mane, and tail do not shed like the other hairs on the horse.[1]

Figure 11-1 Phases in the life cycle of a hair. **A,** Anagen begins with the renewal of the intimate relationship between the papilla and the undifferentiated cells that partially enclose it. **B,** As anagen proceeds, matrix cells generate a new hair that pushes upward toward the surface and in the process dislodges the old club hair. **C**, Mature anagen hair follicle consists of infundibular, isthmus, and inferior segments. **D,** During catagen, the entire inferior segment of the follicle shrivels upward as a thin cord of epithelial cells and is followed upward by the papilla. **E,** During telogen, the club hair rests in its cornified sac at the level of the hair erector muscle. (From Moschella SL, Herley HJ: *Dermatology*, ed 3, Philadelphia, 1992, WB Saunders.)



11.2.3 ADDITIONAL SKIN-RELATED STRUCTURES

The horse and other Equidae have skin structures unique to these species, including the ergot, chestnut, and hoof. The ergot is a small mass of cornified tissue located in a tuft of hair on the flexor surface of the fetlock. The

ergot is a vestige of the second and fourth digits of extinct Equidae. The chestnut refers to a mass of horny tissue on the medial surface of the radius and is believed to be a vestige of the first digit. The hoof is the horny covering over the distal end of the third digit. The hoof includes the frog, important for shock absorption and stimulation of blood flow to the foot, and other components that provide a firm structure to facilitate motion.

11.3 History

A patient history of the horse can provide the clinician with useful clues to help determine the underlying cause of a dermatologic problem. Figure 11-2 is an example of a form with different types of dermatologic questions. For example, most allergies manifest with the clinical sign of pruritus. The owner may notice the horse rubbing, scratching, licking, or chewing, or physical examination may reveal evidence of pruritus (i.e., broken hairs and 669
excoriations). Pruritus does not confirm a diagnosis of allergic disease. Other conditions such as parasitic 671
infestation or secondary bacterial or fungal skin infection may be pruritic. One may rule out these differential diagnoses by performing the diagnostic tests discussed later in this chapter.

Figure 11-2 Sample equine history form.


EQUINE DERMATOLOGY HISTORY FORM

Date ______

Age when purchased ______

What is this horse's use? ______

What is your complaint about the horse's skin? ______

Age of horse? ______ Age when skin problem started? ______

Where on the body did the problem start? ______

What did the skin problem look like initially? ______

How has it spread or changed? ______

Is the problem continual or intermittent? ______

What season did the problem start? ______

Is the problem seasonal or year-round? ______

If seasonal, what seasons is the disease present? ______

Does the horse itch? ______ If so, where? ______

Do any horses in contact with the affected horse have skin problems? ______

If so, are they similar or different from this horse's problem? ______

Do any people in contact with the horse have skin problems? ______

Do you use insect control? ______ If so, describe. ______

Do any relatives of this horse have skin problems? ______ If yes, explain. ______

Please list any injectable, oral, or topical medications that have been used to treat the problem (veterinary or "home remedies"): ______

Did any help the condition? ______ If yes, which ones? ______

Did any aggravate the condition? ______ If yes, which ones? ______

Describe the environment where the horse is kept: Indoors ______

______ Outdoors ______

What is the horse fed? ______

What feed additives do you use? ______

What is your deworming schedule? ______

Did the horse receive ivermectin? ______ If so, when? ______

List any other major medical problems or drugs that the horse received: ______

List any additional information you feel is relevant to the skin disease: ______

Insect allergies and atopy frequently recur seasonally. However, some horses may have year-round or nonseasonal pruritus with insect allergies or atopy, which is more common in warm climates in the United States. One also should include food allergy on the list of differential diagnoses for horses with nonseasonal pruritus.

Questions as to whether other horses in the stabling or pasture facility have dermatologic lesions may be important. Knowing if grooming aids (brushes, combs, etc.) are used on more than one horse is important. Pruritus that affects multiple animals may indicate the possibility of a parasitic or infectious process.

Response to previous therapy is another useful historical clue. One should determine the dose and frequency of medication administration. If the appropriate drug or drug dose is not used, then the patient may not respond.

Other factors to consider include feed, stabling environment, and supplements or other medications (wormers, sprays, shampoos, etc.) used regularly. If any of this changed before the development of the dermatologic lesions, the change may be related to the problem in the horse.

11.4 Physical Examination

One should consider the type of skin lesions and lesion distribution when performing a physical examination on the equine patient with dermatologic disease. Pustules and vesicles are fragile skin lesions that are not found easily and are most common in bacterial or autoimmune skin diseases. Pustules and vesicles are considered primary lesions. Other examples of primary lesions include papules, wheals, erythema, and nodules. Secondary lesions commonly observed in horses may include scale, crusts, excoriations, fistulae, ulcers, necrosis, hyperpigmentation, hypopigmentation, lichenification, hyperhidrosis, and scars.

The location of lesions is important because certain dermatologic conditions tend to occur on certain areas of the body. This tendency is especially true with parasitic hypersensitivity reactions because certain parasites have a predilection for certain areas of the body.[7] For example, pinworms tend to cause intense pruritus of the tailhead, and black flies tend to have a predilection for the head, ears, and ventral abdomen. More detailed information concerning insect feeding preferences is covered in the insect hypersensitivity section. One should examine the horse for evidence of external parasites such as ticks or flies.

The clinician always should perform a thorough physical examination, including determining the temperature, heart rate, and respiratory rate. If abnormalities in any of these parameters exist, the horse may have a systemic disease or more than one medical problem. For the health of the animal, exploring and treating all possible medical problems is important.

11.5 Common Causes of Pruritic Dermatoses

Pruritus is the sensation to rub, lick, scratch, or chew.[8] Horses most commonly rub the skin and hair coat when they are pruritic (Figure 11-3). Clinical evidence that a horse is pruritic may include the presence of broken hairs, excoriations, hemorrhagic crust, alopecia, lichenification (thickening of the skin), and hyperpigmentation. Lichenification and hyperpigmentation occur most commonly following chronic inflammation and pruritus.

Conditions that tend to be pruritic include parasite infestations such as lice, mites, ticks, *Onchocerca, Habronema,* and pinworms or allergic reactions such as insect hypersensitivity, food allergy, contact allergy, and atopy. Occasionally, pruritus occurs with bacterial or fungal infections.

11.5.1 PARASITIC SKIN DISEASES

11.5.1.1 Lice (Pediculosis)

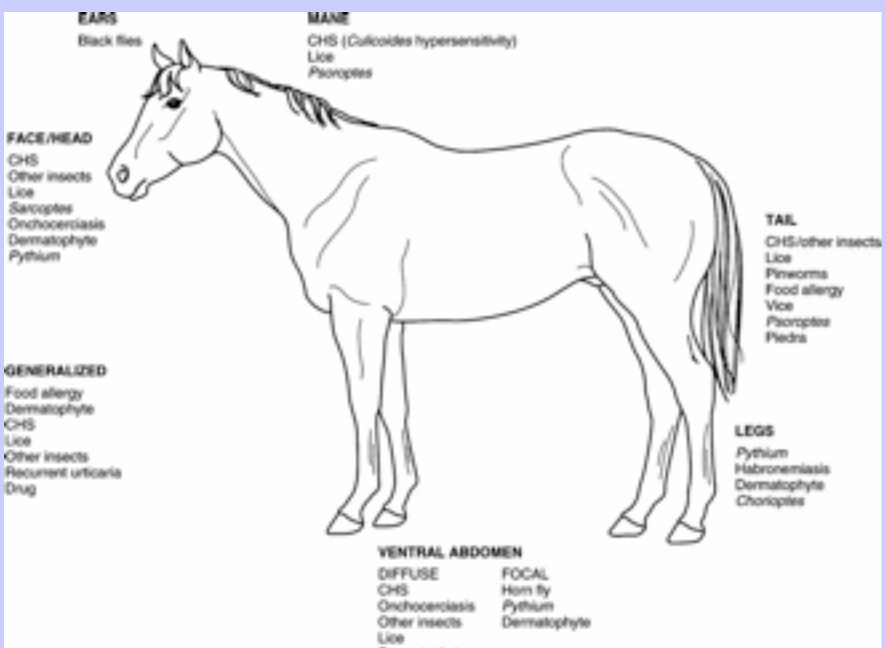
Two types of lice may be found on horses. Biting lice feed on epidermal debris, whereas sucking lice feed on blood and lymph. *Haematopinus asini,* the sucking louse of horses, most commonly infests the mane and tail and the hairs behind the fetlock and pastern. The biting louse, *Damalinia equi,* tends to feed on the dorsolateral trunk.

Lice are host-specific and complete their entire life cycle on the host. Female lice attach their eggs to hairs. In cool moist environments, lice can live for 2 to 3 weeks; they only live a few days off of the host. Lice infestations are transmitted by direct or indirect contact. Brushes, combs, and tack serve as fomites for transmission of lice. Adult horses may act as asymptomatic reservoirs for infection.

Lice infestations may occur year-round, but they are more common in the winter in northern climates. Cooler skin and hair coat temperatures are more favorable for development of eggs within the female, oviposition, and egg development.[9] Skin temperatures on the legs of horses, with the possible exception of Draft Horses, are too cold for louse development.

Louse infestation has no age, breed, or sex predilection in horses. Affected horses tend to be restless and have a poor appetite. They have a dry, dull hair coat with patchy areas of hair loss and excoriation. The coat may 671
appear moist and have a sour or mousy odor. Skin fasciculation may be the only evidence of early infestations 672
in foals. Some horses may be asymptomatic carriers that are identified only when foals or other susceptible animals become infested. Heavy infestations of sucking lice may cause anemia or severe debilitation.

Figure 11-3 Regional pruritus: differential diagnosis of pruritic skin diseases.



Diagnosis of lice infestations is by demonstration of adults and/or eggs on the hairs. Sometimes one cannot visualize lice easily in direct light. In these circumstances, the veterinarian may need to comb the hair coat with a fine-toothed comb to find the lice. Skin biopsies may reveal a nonspecific superficial perivascular eosinophilic dermatitis with or without intraepidermal microabscesses.[10]

When dealing with a lice infestation, the veterinarian should treat all animals on the premises. Ivermectin at 200 μg/kg orally every 2 weeks for three treatments is effective for treating sucking lice. Biting lice do not respond to ivermectin treatment, and so one should treat affected horses with other medications, such as dips. Common types of dip that one may use to treat sucking and biting lice infestations include lime sulfur, pyrethrins, methoxychlor, malathion, coumaphos, crotoxyphos, pyrethroids, and lindane. Lime sulfur and pyrethrin dips have fewer associated adverse reactions in horses than do other dip treatments. One should take care to use any product according to label directions. None of these treatments affect lice eggs, and two to three repeat applications of the dip are recommended at 2-week intervals. For optimal efficacy, one should treat the whole body of the horse when applying the dips. Other precautions include cleaning and disinfecting all tack and grooming supplies (brushes and combs); decontaminating the stabling area using a commercial premise spray used to kill fleas, because lice may survive for days to weeks in the environment; and although reinfestation is difficult to prevent, treating animals returning from shows, breeding farms, or training facilities prophylactically. 672

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11.5.1.2 Mites (Mange)

A variety of mites may infest horses. *Sarcoptes scabiei* var. *equi* (scabies, head mange), *Chorioptes equi* (leg mange), *Psoroptes equi* (body mange), *Pyemotes tritici* (straw itch mite), and *Trombicula* and *Eutrombicula* species (chiggers) are species associated with equine pruritic skin disease.[7] The pruritus accompanying a mite infestation results from a combination of mechanical irritation and hypersensitivity to the mite and by-products of the mite (i.e., feces).[11,12] The primary skin lesion from a mite infestation is a maculopapular eruption. These skin lesions are often difficult to find with chronic infestations.

11.5.1.2.1 Sarcoptes scabiei

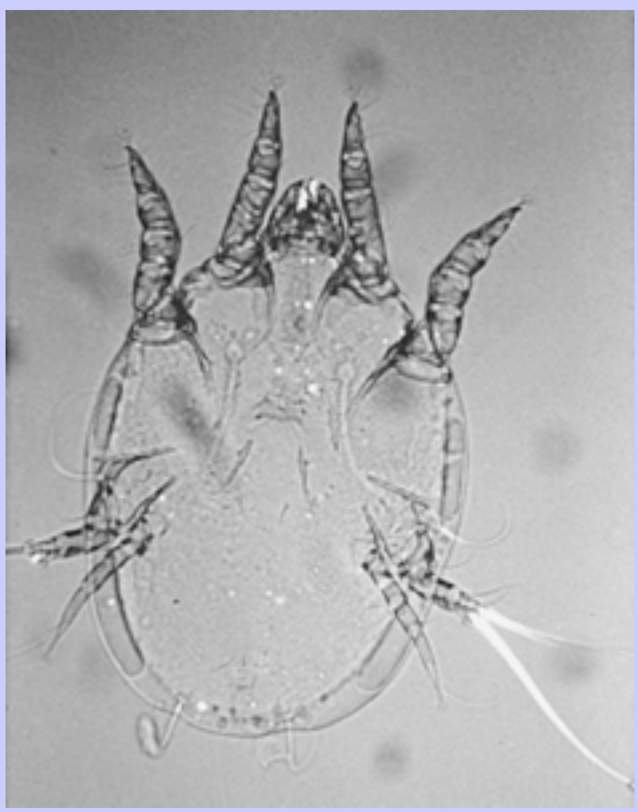
In the United States, *Sarcoptes scabiei* infestations in horses are a reportable disease.[7] This mite is highly contagious and infests horses and transiently infests human beings (self-limiting disease) in contact with parasitized horses. This parasite is capable of surviving off the host for up to 3 weeks, and transmission of scabies by fomites or in the environment is possible. Scabies mites burrow in the superficial epidermis and lay their eggs. In early infestations, mites are found in highest concentration around the head and neck. These mites seem to prefer the ears on horses. As disease progresses, mites usually spread over the entire body. A scabies infestation in a horse results in intense pruritus and generalized scaling and crusting with excoriations and lichenification. A secondary skin infection may result from the pruritus. One may elicit the itch-scratch reflex by scratching the horse over the withers, causing the horse to tuck its nose close to its chest and make smacking noises and exaggerated lip movements. This reflex suggests scabies but is not diagnostic, and one may elicit the reflex in some normal horses.[13] The scabies mite is circular with short legs, a terminal anus, and long unjointed pedicles. Unfortunately, as in other species, the scabies mites are difficult to find on skin scrapings. Therefore a negative skin scraping does not assure that the horse does not have scabies.

Ivermectin at a dose of 200 μg/kg orally is effective for treating horses with scabies. As in other species, one should repeat ivermectin treatment at 2-week intervals for a total of two to three treatments. Within 2 weeks the eggs hatch and new adult mites are present. Topical treatments such as lime sulfur dip, lindane, coumaphos, diazinon, malathion, or toxaphene also have been used. One should dip horses every 7 to 10 days for three to six treatments, treat all horses in contact with the infested horse, and decontaminate fomites (tack, brushes, etc.) and the environment.

11.5.1.2.2 Chorioptes equi

Chorioptes equi mites are host-specific and do not parasitize human beings (Figure 11-4). Unlike the scabies mite, infestation with *C. equi* is not a reportable condition. *C. equi* mites spend their entire life cycle on the host feeding on epidermal debris. Preferred feeding sites include the distal limbs and perineum. Infested horses exhibit intense pruritus with stomping of feet and rubbing of the perineal area. Skin lesions include alopecia, erythema, and crusting of the pasterns, fetlocks, or perineum. Draft Horses or other horses with feathered fetlocks are predisposed to *C. equi*. The mite tends to be more common in the winter months. One should include *C. equi* in the list of differential diagnoses for horses with pastern dermatitis or greasy heel. Treatment of this mite is the same as for *S. scabiei*. However, ivermectin-resistant *C. equi* mites have been reported, and topical dip therapy may be indicated if the horse does not respond to oral therapy. If one uses a topical medication, clipping the hair around the fetlocks and pasterns to ensure optimal contact of the dip with the skin and mites is advisable.

Figure 11-4 *Chorioptes* mite.



11.5.1.2.3
Psoroptes equi

Psoroptes equi mite infestations are a reportable condition in horses in the United States. *P. equi* mites are highly contagious to other horses but do not infest human beings. These mites do not burrow but live on the skin surface. They are biting mites that feed on serum and cellular components. Transmission of *P. equi* mites is by direct contact and exposure to fomites. Mite infestations tend to begin on the forelock, mane, and tail and spread to the trunk. These mites have been known to infest the ear canal and cause otitis 673
externa. Affected horses typically show head shaking or rubbing. As with many other mites, pruritus results 674
in alopecia, crusts, excoriations, and ulcers on the skin. The intensity of pruritus varies. History and clinical signs suggest the diagnosis of *P. equi* mange. One requires skin scrapings to diagnose this condition definitively. *P. equi* has an oval body with segmented pedicles. Treatment of *P. equi* is similar to that described for sarcoptic mange.

11.5.1.2.4
Trombiculidiasis

Trombiculidiasis (red bugs, harvest mites) is caused by an infestation with larvae of free-living adult mites (genus *Eutrombicula* or *Trombicula*). Larvae are most prevalent in grasses, forests, or swamps in late summer and fall. Small rodents are the natural host. Pathognomonic skin lesions are papules or wheals with a small orange, red, or yellow dot (trombiculid larvae) in the center. Areas of the body most commonly infested are the face, muzzle, distal limbs, ventral thorax, and abdomen. Diagnosis is by observation of the distinctive lesion with the larvae in the center. Trombiculidiasis is a self-limiting disease. However, some horses are uncomfortable with this infestation and should be treated. Recommended treatments include lime sulfur dip and pyrethrin sprays or dips as a one time treatment in association with prednisolone or prednisone at a dose of 0.5 mg/kg orally for 3 to 5 days.

11.5.1.2.5
Pyemotes tritici

Pyemotes tritici (straw itch mite) normally parasitizes the larvae of grain insects. Occasionally this mite parasitizes human beings and horses.[14] Infestation in horses occurs from contaminated hay fed in overhead racks. The mite produces a maculopapular crusted eruption on the head, neck, and trunk that is only occasionally pruritic. Diagnosis is based on history and clinical examination. This disease is self-limiting, and one should remove contaminated forage or feed the horse from the ground until all contaminated forage has been consumed. Infestations from fomites or hay fed on the ground have not been reported.

11.5.1.2.6
General Comments About Mites

Regardless of the type of mite present on the horse, skin scrapings should be part of the diagnostic workup. With the exception of lice and trombiculid larvae that one can see with the naked eye, one can diagnose mite infestations best by microscopic examination of skin scraping samples.

11.5.1.3
Ticks

Ticks are an important ectoparasite in the horse because of their potential role in disease transmission, including a variety of viral, protozoal, rickettsial, and bacterial infections. Tick infestations are most common in the spring and summer.

Argasid ticks lay eggs in cracks and crevices in the environment, and immature ticks infect hosts after hatching. Larvae and nymphs suck blood and lymph and then drop off to develop into adults. These ticks infest barns, sheds, and other areas where animals are found. In horses, *Otobius megnini* (spinose ear tick) tends to infest the ears and ear canal. Clinical signs of infestation include otitis externa, head tilt, head shaking, ear rubbing and occasionally aural hematomas.

Dermacentor, Ixodes, and *Amblyomma* species are the most common ixodid ticks of horses. These ticks are found outdoors and have complicated life cycles, and all stages of the life cycle are parasitic. The severity of clinical signs depends on the density of the infestation and whether the horse develops a hypersensitivity reaction to the bites. Infestations occur most commonly on the ears, face, neck, groin, distal limbs, and tail. Early lesions consist of papular to pustular eruptions that rapidly develop into crusts, erosions, ulcers, and hair loss. Hypersensitivity reactions may be local or general. Local responses appear as nodules that develop at the site of the tick bite. Although the pathogenesis is unknown, cutaneous basophil hypersensitivity is believed to be involved.[15] Systemic reactions are characterized by whole-body urticaria or multifocal urticarial plaques. In Australia, a hypersensitivity reaction to *Boophilus microplus* has been observed in sensitized horses.[16] Intense pruritus, papules, and wheals develop as soon as 30 minutes after ticks begin to feed.

Definitive diagnosis is by observation of ticks attached to the horse or in the ear canal. Treatment aims at killing ticks on the horse. One should apply pyrethrin or pyrethroid sponge-on dips to the body of the horse, taking extra care to soak skinfold areas. Usually only one treatment is necessary unless reinfestation occurs. Resistance to insecticides can occur rapidly, and knowledge of local resistance patterns is important. Infestations of *O. megnini* require mechanical removal of as many ticks as possible. One part rotenone and three parts mineral oil applied twice weekly is an effective otic parasiticide for horses. The author has used a commercially available pyrethrin otic preparation (Otomite Plus, Vibac, Fort Worth, Texas) for small animals in horse ears with success. Ivermectin also has been shown to be effective in treating ticks at a dose of 200 µg/kg orally.

11.5.1.3.1 Onchocerciasis

Onchocerciasis is a nonseasonal skin disease of the horse caused by the parasite *Onchocerca cervicalis*. This nematode lives in the ligamentum nuchae and produces microfilariae that migrate to the skin and are ingested by the intermediate host, *Culicoides* species. Microfilarial populations in the skin vary, and the highest concentrations occur in the dermis of the face, neck, and ventral midline, especially the umbilicus (Figure 11-5).[17] Microfilarial populations vary seasonally and are highest in the spring, which interestingly is the peak season for the *Culicoides* vector.[18] Microfilariae are more superficial in the dermis during the spring and summer months. Clinical signs of onchocerciasis are believed to be caused by an idiosyncratic 674
hypersensitivity reaction to microfilarial antigen(s) because many horses that have circulating microfilariae 675
do not have any gross skin or ocular lesions.[19] Whether the reaction is directed only at dying or dead microfilariae is unknown.

Figure 11-5 Ventral midline dermatitis caused by onchocerciasis.

Onchocerciasis has no breed or sex predilection and usually affects horses 4 years of age and older. Clinical signs are nonseasonal but may be worse in the spring and summer, most likely because of the added irritation from the vector. Lesions may occur on the face and neck, on the ventral chest and abdomen, or in all these areas.[20,21] Early lesions begin as a thinning hair coat. As the disease progresses, lesions may vary from focal to generalized areas of alopecia, scaling, crusting, and plaques. Affected areas may be excoriated severely, ulcerated, oozing, and lichenified. Annular lesions in the center of the forehead suggest the disease (Figure 11-6). Leukoderma usually develops at the site of lesions and is irreversible. Ocular lesions include sclerosing keratitis, vitiligo of the bulbar conjunctiva, white nodules in the pigmented conjunctiva, uveitis, and a crescent-shaped patch of depigmentation bordering the optic disk.[22]

One may make a presumptive diagnosis of onchocerciasis by demonstrating the microfilariae in the skin of animals with compatible historical and clinical findings. Skin scrapings and direct blood smears are often negative. One can demonstrate microfilariae most reliably with a mince preparation or via histologic examination of skin from a biopsy specimen.[21] Mince preparations require a 4- or 6-mm punch specimen of tissue obtained from the ventral abdomen. One places the tissue specimen in a Petri dish with a small amount of physiologic saline, minces it with a scalpel blade or razor, and incubates it at room temperature for 30 to 60 minutes. One then examines the specimen microscopically for evidence of the rapid motion of the microfilariae. Skin biopsies reveal a superficial perivascular eosinophilic dermatitis. Often microfilariae are visible in the superficial dermis.[7]

Figure 11-6 Equine onchocercosis.

Ivermectin at a dose of 200 μg/kg orally is the treatment of choice.[7,20,23] A single dose often produces remission of clinical signs within 2 to 3 weeks. However, some horses require two to three monthly treatments before clinical signs resolve. Approximately 25% of horses have an adverse reaction, such as ventral midline edema or pruritus, which occurs 1 to 10 days after treatment. In rare cases, severe umbilical and eyelid edema and fever may occur. In confirmed cases of onchocerciasis, one should perform a

thorough ocular examination to look for any signs of eye disease associated with onchocerciasis. Anecdotal reports suggest treatment may precipitate an episode of uveitis.

Prednisolone or prednisone at a dose of 0.5 mg/kg orally may be necessary in the first week of treatment to prevent exacerbation of skin and ocular lesions as a result of massive destruction of microfilariae but is not routinely recommended. Indications include the development of ventral midline edema, pruritus, and fever. None of the currently available anthelmintics kill adult parasites in the ligamentaum nuchae, and affected individuals require periodic retreatment with ivermectin when clinical signs recur. The prevalence of cutaneous onchocerciasis has decreased significantly with the advent of routine dewormings with ivermectin. 675

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11.5.1.3.2 Habronemiasis

Cutaneous habronemiasis is a common nodular skin disease caused by three species of nematodes: *Habronema muscae, H. majus (H. microstoma),* and *Draschia megastoma (H. megastoma)*. The house fly is an intermediate host for *H. muscae* and *D. megastoma,* whereas the stable fly is the intermediate host for *H. microstoma*. The normal life cycle for these parasites is similar. Adult nematodes live in the stomach and produce larvae. Larvae are passed in the feces and ingested by maggots of the foregoing intermediate hosts. The intermediate host deposits infective larvae near the mouth of the horse, which swallows them. Cutaneous habronemiasis occurs when intermediate hosts deposit infective larvae on skin, open wounds, or chronically wet areas.

A horse with cutaneous habronemiasis has ulcerative nodules in the spring and summer that partially or completely regress in the winter. No breed, sex, or age predilection exists. Some horses may be predisposed to cutaneous habronemiasis, exhibiting clinical signs each year, whereas other horses on the same premises never develop this condition.

Skin lesions of habronemiasis occur most commonly on the legs, urethral process of the penis, prepuce, medial canthus of the eye, or any area of trauma to the skin. Other areas that may be infected include the conjunctival sac, the lacrimal duct, and the third eyelid. Single or multiple nodules may be present. In most cases, pruritus is present, presumably following a hypersensitivity reaction to the parasite. The intensity of pruritus may vary from mild to severe. In severe cases in which ocular lesions are present, the horse can suffer from photophobia, epiphora, and chemosis. Dysuria can occur when lesions affect the urtheral process.

Lesions often are ulcerated and appear similar to exuberant granulation tissue. Yellow granules approximately 1 mm in diameter may be present.[24,25] Microscopic examination of these granules does not reveal branching hyphae such as one frequently observes with granules from pythiosis or zygomycosis lesions.

Differential diagnoses for cutaneous habronemiasis include bacterial granuloma, fungal granuloma, pythiosis, exuberant granulation tissue, squamous cell carcinoma, and equine sarcoid. Habronema may be present concurrently with other dermatologic conditions, and biopsy is important for a complete and definitive diagnosis.

Diagnosis is based on history, physical examination, cytologic examination, and biopsy of lesions. Cytologic examination of exudate sometimes reveals the presence of nematode larvae. These larvae are large (3 mm by 60 μm), with a spiny tail, and usually are motile. Skin biopsies reveal a nodular to diffuse

granulomatous dermatitis with large numbers of mast cells and eosinophils. Foci of coagulation necrosis are characteristic.[26,27] Sometimes this area of necrosis contains cross sections of larvae.

Treatment of cutaneous habronemiasis should include control of the associated hypersensitivity reaction and elimination of the parasite. Treating horses with ivermectin alone is not always effective. The author and co-workers examined 14 horses between 1988 and 1998 that had been treated with ivermectin in the preceding month and had intralesional habronema larvae present on biopsy. Similarly, 5 of 31 horses treated with ivermectin for habronemiasis required an additional treatment 2 to 4 weeks later.[28] One may treat the hypersensitivity reaction with systemic corticosteroids such as prednisone or prednisolone at a dosage of 1 mg/kg once daily for 10 to 14 days with a gradual decrease over an additional 2-week period. Corticosteroids and dimethyl sulfoxide (DMSO) often are combined with fenthion, thiabendazole, ronnel, or trichlorfon in a variety of topical preparations for treatment of habronemiasis.[27] Some more common topical lesional medications include (1) 1 oz dexamethasone (2 mg/ml) in 1 oz 90% DMSO, and 1 oz 20% fenthion; (2) 3 oz 90% DMSO in 15 ml dexamethasone (2 mg/ml), 40 ml nitrofurazone solution, and 1 oz trichlorfon powder; and (3) 30 ml 20% fenthion, ¾ lb petrolatum (heated), 10 mg triamcinolone acetonide powder, and 90 ml 90% DMSO. Ivermectin sometimes is used concurrently with these topical medications.

One may treat conjunctival habronemiasis with topical echothiophate drops 3 times a day to kill larvae in combination with an ophthalmic ointment that contains dexamethasone and an antibiotic.

Fly control is an essential part of treatment. Oil-based fly sprays tend to last longer than water-based fly sprays. Products that contain permethrin are effective.

Prognosis for horses with habronemiasis is good with appropriate and timely treatment. The veterinarian should warn the owner that habronemiasis may recur in subsequent years. Fly control is essential to preventing recurrence.

11.5.1.3.3 Pinworms

Equine pinworms, *Oxyuris equi,* cause intense anal pruritus. Adult pinworms are found in the colon. The female worm migrates down the gastrointestinal tract and deposits eggs around the anus. These eggs are cemented to the skin with a thick, sticky, yellow-gray substance. Horses develop perianal pruritus as a result of this sticky substance, which contains the eggs. In severe infestations horses may develop vague abdominal discomfort. This abdominal discomfort results directly from inflammation of the cecal and colonic mucosa in response to third- and fourth-stage pinworm larvae.[29]

Eggs develop to the infective stage within a 4- to 5-day period. At this time the cement substance cracks and dries and then detaches from the skin as flakes. These flakes contain a large number of eggs that adhere to walls, buckets, and other objects in the environment. 676

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Because one often does not observe ova of *O. equi* on routine fecal flotation, a diagnosis of pinworms is based on clinical signs and identification of pinworm eggs on cellophane tape preparations. One presses a piece of clear adhesive tape to the skin around the anus and removes the tape and places it on a slide for microscopic examination for oval ova with an operculum or plug at one end.

O. equi is treated easily with a variety of anthelmintic agents. Anecdotal reports exist of occasional resistance to ivermectin and pyrantel; these horses respond to treatment with fenbendazole. One may treat the horse once with fenbendazole and remove it from the environment for 1 month. (Pinworms can survive

in the environment under ideal conditions for up to 1 month.) Alternatively, one may treat horses with fenbendazole 3 times at 2- to 3-week intervals.

11.6 Allergies

11.6.1 INSECT HYPERSENSITIVITY

Insect hypersensitivity is a common cause of skin disease in the horse. Mosquitoes and species of *Culicoides* (biting midges, "sweet itch"), *Simulium* (black flies), *Tabanus* (horse flies), *Chrysops* (deer flies), *Stomoxys calcitrans* (stable flies), *Haematobia* (horn flies), *Musca* (house flies), bees, and wasps cause skin lesions in horses.[7]

Insect hypersensitivity reactions are seasonal in colder climates, can be nonseasonal in warmer climates, and may affect any age, sex, or breed of horse. This dermatologic condition may have an inherited component, but specifics of these claims have not been verified. The distribution of skin lesions varies and may depend on the type of insect involved. Certain insects have preferred feeding sites.[7,30] The black fly tends to feed on the head, ears, and ventral abdomen. Stable flies prefer feeding on the lower legs but also have been known to feed on the ventral abdomen, chest, and back. The location for *Culicoides* spp. feeding is species dependent. Classically, *Culicoides* spp. feed on the dorsal surface of the horse (especially the mane and tail areas), face, and ears (Figure 11-7). In Florida, some *Culicoides* spp. prefer feeding on the lower legs, ventral trunk and neck. Horn flies feed on the ventrum around the umbilicus (Figure 11-8). Mosquitoes prefer feeding on the lateral aspects of the body.

A few insects bite anywhere on the body and do not prefer a specific location. Examples include deer flies, horse flies, house flies, bees, and wasps.

Some insects require a specific environment to propagate. Knowledge of these requirements may be helpful in establishing insect control programs. *Culicoides* spp. and mosquitoes prefer free-standing stagnant water for propagation. In contrast, black flies prefer moving water such as a stream, brook, or river. Horn flies are obligate parasites of cattle that require fresh cattle feces for reproduction. Several species of insects, including stable flies and house flies, prefer decaying vegetation or manure for reproduction. They tend to be a bigger problem when sanitation practices at the stabling facility are problematic.

Figure 11-7 Two-year-old horse with *Culicoides* hypersensitivity.

The time of day that an insect prefers to feed also differs between species. Horseflies, stable flies, black flies, and horn flies are usually active during the daylight hours. Mosquitoes and most *Culicoides* spp. are active at night. Mosquitoes are most active at dusk and the first 2 hours after sunset. If the time of feeding is known for the potential offending insect, the horse may be stalled during peak feeding hours to limit exposure.

Types of dermatologic lesions seen with equine insect hypersensitivity include pruritus, alopecia, excoriations, and lichenification. A generalized papular-crusted eruption also may occur.

Diagnosis of insect hypersensitivity is based on a compatible history, physical findings, and elimination of other causes of pruritus in the horse. Skin scrapings show no abnormalities. Skin biopsies may reveal the presence of a superficial or deep perivascular eosinophilic dermatitis with epidermal spongiosis (intercellular edema of the epidermis), necrosis, and collagen degeneration. Intradermal skin testing is useful for diagnosing insect hypersensitivity.[31] Some insect allergens are inconsistently available (e.g., *Culicoides* spp.), and purchase of insect allergens for the nonspecialist veterinarian may be cost-prohibitive. Fortunately, therapy and environmental control measures are similar for most biting and flying insects, and therefore confirming the diagnosis by intradermal skin testing may not be crucial.

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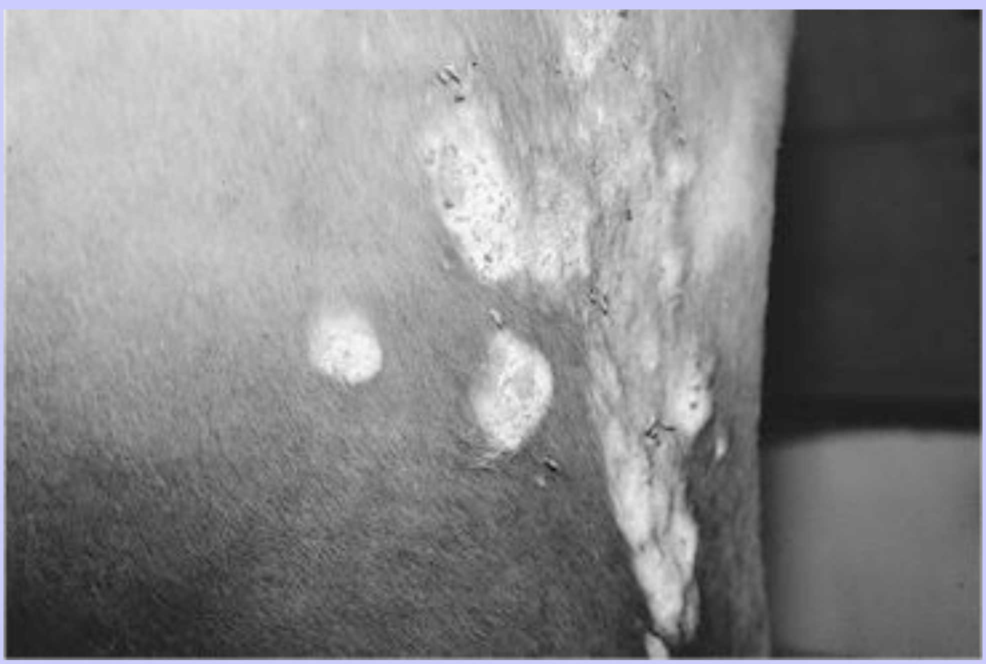
Figure 11-8 Alopecia with some crusting around the umbilicus on a horse with horn fly infestation.



Treatment of insect hypersensitivity involves insect control and judicious use of glucocorticoids.[31] Boxes 11-1 and 11-2 and Table 11-1 summarize recommendations. Ideally, one should stable horses during the target peak feeding period for insects and should screen windows with a small-meshed screen and spray with a residual parasiticide. If possible, one should eliminate bedding areas for flies (e.g., standing water and manure). Spraying horses with residual insecticides (e.g., pyrethrins or pyrethroids) is useful, and sprays containing permethrin and pyrproxifen (Knock-Out Spray, Vibrac, Fort Worth, Texas) are effective for treating horses with *Culicoides* spp.

hypersensitivity. Anecdotal reports suggest that weekly application of fipronil (Frontline, Merial, Iselin, New Jersey) may be useful for treating horses with *Culicoides* spp. hypersensitivity. Avon Skin So Soft bath oil (Avon Products, Inc., New York, New York) mixed in equal volumes with water has been used to repel insects in horses. However, one should use this product with care because contact dermatitis has been reported. One should follow label recommendations with changes depending on the response of the individual horse. Cattle tags impregnated with insecticide may be helpful if attached to manes, tails, or halters.

Anecdotal reports suggest the importance of applying repellents and insecticide sprays to the horse when the skin is cool and dry. This may minimize development of sensitivity reactions to products by limiting percutaneous absorption. Many horses are sensitive to petroleum-based products and develop erythematous skin and hair loss in areas where the insecticide, fly wipe, or bath oil has been applied to the skin. If a horse has a history of this type of reaction, performing an open patch test with any new product before applying the spray over the entire body is advisable. One applies a small amount of the test substance to an area of skin and observes the site for 72 hours for signs of erythema, swelling, and hair loss. Fly masks or fly sheets are useful adjunct therapy for control of insects.

In many cases, insect control alone is insufficient to alleviate discomfort and clinical signs. Hydroxyzine hydrochloride may be beneficial in some horses with insect hypersensitivity when administered orally at a dose of 200 to 400 mg every 8 to 12 hours.[32] This drug also has been useful in managing insect-induced urticaria. Side effects associated with hydroxyzine in horses include sedation or hyperactivity. If the horse does not respond after hydroxyzine administration, one may try systemic corticosteroids. The recommended dose for prednisone or prednisolone is 1 mg/kg orally administered daily until the pruritus is relieved (usually 1 to 2 weeks); one then tapers the dose to the lowest effective every-other-day dose.

11.6.1.1

BOX 11-1 PRIMARY MANAGEMENT STRATEGIES FOR COMMON EQUINE PESTS

11.6.1.1.1

1. Stabling

Tabanids: Diurnal and crepuscular periods

Black flies: Diurnal and crepuscular periods

Biting midges: Under fans; nocturnal and crepuscular periods

11.6.1.1.2

2. Exclusion Devices

Black flies: Ear nets

House flies: Face masks

Face flies: Face masks

11.6.1.1.3

3. Hay and Manure Management

Stable flies: Particularly hay in pastures

House flies: General sanitation

11.6.1.1.4 4. Cattle Management

Horn flies: Control pest on natural host

Face flies: Undisturbed cattle manure requisite for larval development

11.6.1.1.5 5. Water Management

Mosquitoes: Only certain species

Biting midges: Only certain species

11.6.1.1.6 6. Source Identification and Removal

Straw itch: Normally infested hay

Blister beetle: Normally products containing alfalfa

11.6.1.1.7 7. Restricted Grazing or Movement

Chiggers: Erratic distribution in spring or fall

Ticks: Mowing and understory control also helpful

Tabanids: Allow horses to escape from wooded areas

Poultry pests (sticktight fleas and lice): Separate horses from poultry

From Foil L, Foil C: Control of ectoparasites. In Robinson NE, editor: *Current therapy in equine medicine,* ed 3, Philadelphia, 1992, WB Saunders.

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11.6.1.2 BOX 11-2 GENERAL INSECTICIDE TYPES USED FOR EQUINE PESTS*

11.6.1.2.1 Insecticides

Face flies

Facultative myiasis

Horn flies

House flies

Lice

Mosquitoes

Sticktight fleas

Poultry lice

Ticks

11.6.1.2.2 Repellents

Black flies

Biting midges

Chiggers

Horseflies

11.6.1.2.3 Insecticides and Repellents

Biting midges

Black flies

Chiggers

Facultative myiasis

Mosquitoes

Stable flies

Ticks

11.6.1.2.4 Premise Treatment

House flies

Mosquitoes

Stable flies

* Categories may overlap because of differences in management systems or life cycles of different species.

Modified from Foil L, Foil C: Control of ectoparasites. In Robinson NE, editor: *Current therapy in equine medicine,* ed 3, Philadelphia, 1992, WB Saunders.

Anecdotal reports suggest that a DMSO dervivative, methylsulfonyl methane, may be effective as an aid to relieve pruritus associated with equine insect hypersensitivity. This medication is available as a powder to be sprinkled on food and should be used according to label directions.

Fatty acids have been recommended for control of pruritus associated with insect hypersensitivity.[33,34] The effectiveness of this therapy may depend on dose and fatty acid source. One study in Florida used flaxseed oil in

horses with *Culicoides* spp. hypersensitivity and found no benefit in decreasing pruritus.[33] A study in Canada demonstrated that flaxseed meal was beneficial in treating pruritus in horses with *Culicoides* spp. hypersensitivity.[34] These apparently disparate results may be explained by geographic factors or the source of flaxseed.

TABLE 11-1 Pesticides Recommended for External Parasite Control on Horses[*]

RESIDUAL INSECTICIDES	OTHER COMPOUNDS
PYRETHROIDS	**REPELLENTS**
Cypermethrin	MGK 326 *di-n-propyl isocinchomeronate*
Fenvalerate	Stabilene: butoxypolypropylene glycol
Permethrin	
Resmethrin	
Tetramethrin	
S-Bioallethrin	
Sumethrin	
ORGANOPHOSPHATES	**BOTANICALS**
Coumaphos	Pyrethrins (also insecticidal)
Dichlorvos	Synergists
Malathion	Piperonyl butoxide *5-[[2-(2-butoxyethoxy) ethoxy]methyl]-6-propyl-1, 3-benzodioxole*
Tetrachlorvinphos	MGK 264 *N-octyl bicycloheptene dicarboximide*
ORGANOCHLORINES	
Lindane	
Methoxychlor	

From Foil L, Foil C: Control of ectoparasites. In Robinson NE, editor: *Current therapy in equine medicine,* ed 3, Philadelphia, 1992, WB Saunders.

* Categories may overlap; for example, some pyrethrins are also insecticidal, and some pyrethroids are repellent. William B. Warner and Roger O. Drummond assisted in compiling this list.

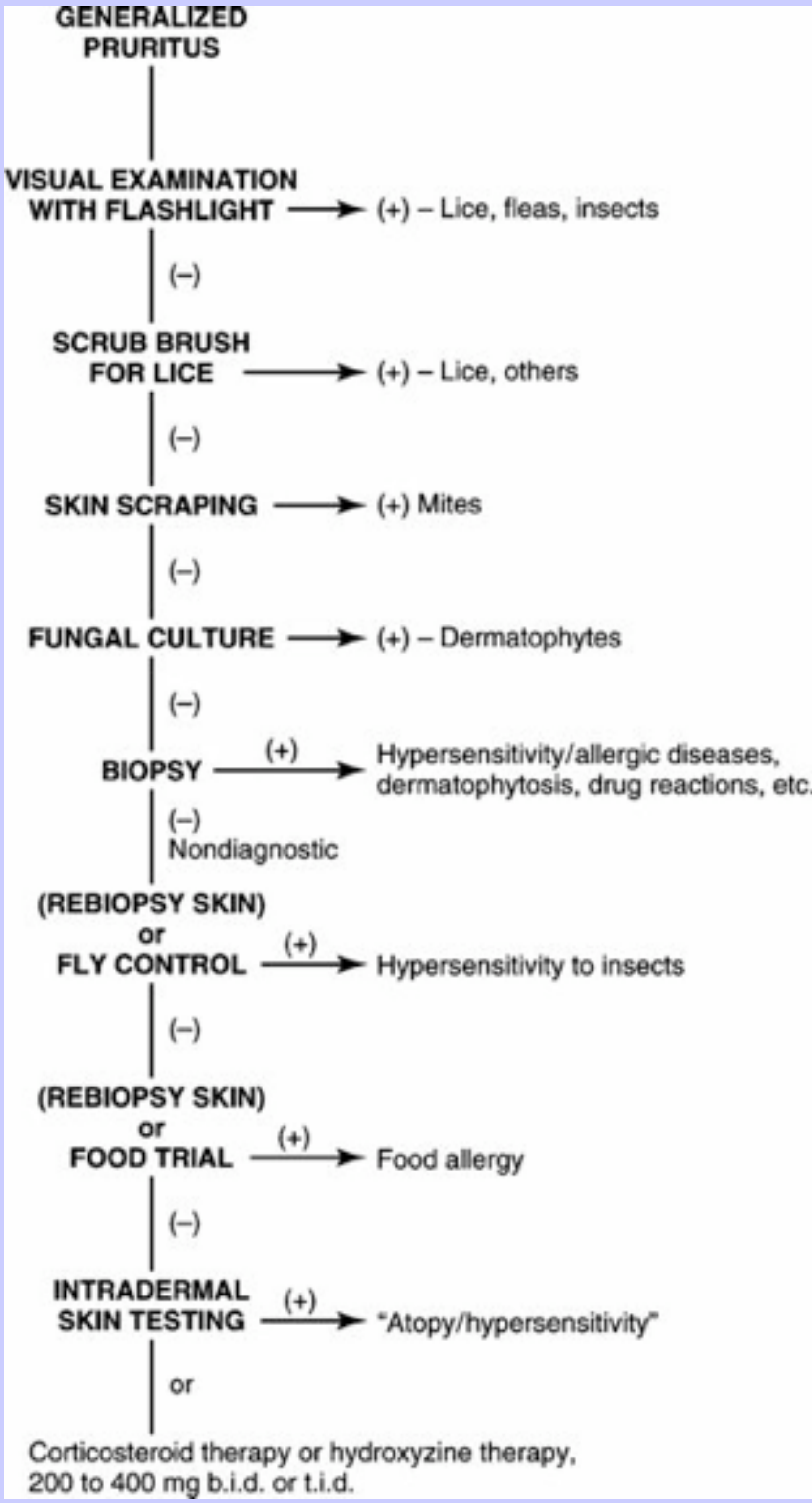
11.6.2 FOOD ALLERGY

Documented cases of feed-related hypersensitivity are rare in horses, and most information on this condition is based on anecdotal reports. Food hypersentitivity refers to an immune-mediated adverse reaction to a feedstuff unrelated to any physiologic effect of the feed. The pathogenesis of dietary hypersensitivity in horses is understood poorly but is believed to involve type I, type II, and type IV hypersensitivity reactions. The skin, respiratory tract, and gastrointestinal tract may be affected. The most commonly incriminated foods include potatoes, malt, beet pulp, buckwheat, fish meal, wheat, alfalfa, red and white clover, St. John's wort, chicory, glucose, barley, bran, oats, and tonics.[33]

Dermatologic manifestations of food allergy include generalized pruritus with or without papules, urticaria, and pruritus ani. Flatulence, loose stools, heaves, or asthma may accompany skin lesions.

Diagnosis is by eliminating the more common causes of pruritus in horses and by positive response to a food elimination trial (Figure 11-9). Hypoallergenic diets are individualized for each patient, and obtaining a thorough 679
dietary history from the owner is critical. One must identify any change in the diet (vitamin supplements, grains, 680
hays). A practical approach is to start by eliminating any supplements and concentrates from the diet. The trial should not include dusty feedstuffs because some horses can develop inhalant allergy to feedstuffs. One should feed oats and grass hay for 4 to 8 weeks and note whether a decrease in pruritus occurs. In the South, coastal Bermuda grass hay tends to be less allergenic than other types of hay. If the horse is notably less pruritic, one should reinstitute the previous diet to confirm that the pruritus is caused by a food allergy and that improvement was not coincidental. If food allergy is present, pruritus will return, and one should reinstitute the hypoallergenic diet until clinical signs subside. One identifies the offending substance by reintroducing one dietary item at a time each week and observing the horse for recurrence of clinical signs.

Figure 11-9 Flowchart for diagnosis of equine pruritic skin diseases.



11.6.3 CONTACT ALLERGY

True contact allergies are rare in horses, perhaps in part because the hair coat acts as a protective barrier. Irritant contact reactions are more common (see the following discussion). Contact allergies, when they occur, are type IV hypersensitivity reactions. Potential allergens are usually small molecules that penetrate the skin, bind to dermal collagen or carrier proteins, and are taken up by antigen-presenting cells (Langerhan's cells) in the dermis. On reexposure, an inflammatory response occurs that results in skin disease. Development of allergic contact reactions usually takes months to years.

Contact reactions most likely are related to pasture plants, changes in bedding material, insect repellents, topical medication, and tack items.[35,36] Parasiticides and bath oils applied as insect repellents are the most commonly identified causes of contact allergy. Repeated application of chemicals to the coat of sweating horses may predispose to development of a contact allergy because by compromising the protective barrier of the skin.

Clinical signs of contact allergy vary and depend on the duration of the allergic reaction. Erythema, swelling, oozing, pain, or pruritus develops within 2 to 3 days of application of the offending substance. If untreated, lesions often progress over weeks or months to alopecia, lichenification, and crusting. The distribution of the lesions may suggest the cause: legs (plants, bedding); face and muzzle (plants, bedding); face and trunk (tack); and face, ear, neck, and trunk (insect repellents).[36]

A definitive diagnosis is by provocative exposure or patch testing. Provocative exposure requires avoiding the suspect substance for 10 days or until lesions resolve, reexposing the horse to the substance, and noting recurrence of lesions or clinical signs over the next 7 to 10 days.[36] Provocative testing does not distinguish between an irritant reaction and an allergic reaction but does identify the offending substance. Patch testing requires the application of the suspect substance to an area of the skin for 48 to 72 hours. Plant material, bedding, or other particulate matter does not adhere well to the skin and is tested best under an occlusive dressing (closed patch test). For each test substance, one clips a 3-cm^2 area of hair on the thorax or dorsum, applies a small amount of the suspect substance to the skin, and covers the site with a gauze sponge bandage for 48 to 72 hours. One also should bandage a control site. On removing the bandages, one assesses the area for erythema, swelling, 680
induration, pain, and exudation. One should obtain biopsies of the control and test site(s) for histologic 681
confirmation of the diagnosis. One may apply liquid substances to the skin daily and not use an occlusive bandage.

Successful treatment requires accurate identification of the offending substance and avoidance. If this is not possible, one may prescribe short-term systemic corticosteroid therapy and mild cleansing shampoos.

11.7 Atopy

Atopy is an inherited dermatologic or respiratory condition in horses. Horses with atopy develop senstitizing antibodies, immunoglobulin E, to offending allergens. Allergens that have been implicated in this problem include molds, grasses, trees, fabrics, and dust. One or more of these allergens may be involved in an individual horse.[37]

Sensitizing antibodies cross-link mast cells, triggering release of inflammatory mediators with resultant inflammation in the affected target organ (i.e., skin or respiratory tract). In skin, pruritus is the end result.[32,37]

Arabian and Thoroughbred horses appear to be predisposed to develop atopy.[32] This breed predisposition suggests equine atopy may be an inherited condition. In one report a stallion and five of his offspring had atopy-induced urticaria.[38] The exact mode of inheritance is not known.

Atopy in horses is manifested as a seasonal to nonseasonal pruritic skin disease. Several types of skin lesions are observable, including alopecia, excoriations, crusts, scales, erythema, and hives (urticaria).[39] Urticaria is not always pruritic in horses. One can make a tentative diagnosis of atopy after eliminating other causes of pruritus and on positive response to exogenous corticosteroids.

The most effective way to confirm a diagnosis of equine atopy is by intradermal skin testing. One sedates the horse with xylazine at 0.05 mg/kg intravenously and clips a rectangular area on the lateral aspect of the neck. One then injects allergens known to cause allergic problems in a particular geographic area intradermally and denotes each site of allergen injection on the skin by a dot made with permanent marker. One reads the test at specific time intervals after injection: 15 minutes, 30 minutes, 4 to 6 hours, and 24 hours. One compares the negative (sterile saline) and positive (histamine 1:100,000 weight to volume) control reactions to reactions of the various allergens. The negative control is assigned an arbitrary grade of 0 and the positive control a grade of 4. One considers size of the wheal, firmness, redness, and shape when grading individual allergen reactions. Reactions greater than or equal to 2 are considered potentially significant. One must consider the history, clinical signs, and pollination times in the final determination of whether individual reactions are significant.

Certain allergens tend to cause many false-positive reactions, including alfalfa, corn, corn smut, grain dust, grain smut, black ants, mosquitoes, fire ants, *Rhizopus* spp., *Penicillium* spp., sheep wool epithelium, English plantain, red mulberry, black willow, mesquite, and dock sorrel. If a reaction occurs with one of these allergens and is inconsistent with the history and physical examination findings, it is probably an irritant reaction.

Horses with laminitis tend to have hyperresponsive immune systems and may be more likely to have false-positive skin test reactions than normal horses.[40] If the horse has had laminitis, one should interpret skin test results with care.

Drugs such as corticosteroids, antihistamines, fatty acids, and those that affect vasodilation such as acepromazine may interfere with skin test results and cause false-negative reactions. To avoid this situation, one should observe drug withdrawal times. Exact withdrawal times are extrapolated from small animal medicine and applied to horses. One should not administer long-acting injectable corticosteroids for 3 months before skin testing; oral or injectable dexamethasone, for at least 1 month before skin testing; and antihistamines, for 7 to 10 days before testing. A withdrawal time of 7 days is recommended for acepromazine.

Serum allergy testing has been recommended for diagnosis of atopy in horses. However, recent studies question the validity of this test. One study compared test results of three different serum allergy tests with intradermal skin test results in the same animal.[41] None of the three serum allergy tests reliably detected allergen hypersensitivity compared with results of the intradermal skin test. Serum allergy tests were not as sensitive as intradermal skin tests. At this time, in human and veterinary medicine, intradermal skin testing is considered the gold standard for confirming a diagnosis of atopy.[37]

Treatment of atopy should include hyposensitization whenever possible. One administers the offending allergens at a set amount and time to the horse. Many horses are treated with a maintenance schedule of 1 ml of a 20,000 protein nitrogen unit aqueous solution administered subcutaneously every 3 weeks. The exact mechanism of action for hyposensitization therapy is not known. Human beings with allergies tend to have a higher proportion of type 1

T helper cells compared with type 2 T helper cells.[42] After hyposensitization therapy the T helper cell populations shift toward normal. Other theories suggest that blocking antibodies are produced with hyposensitization. Regardless of the exact mechanism of action, hyposensitization therapy has been effective in human beings for more than 30 years.[43] The percentage of horses that respond to therapy varies and is thought to be similar to that in small animal medicine (59% to 86% effective).[43,44] Hyposensitization therapy may take 1 to 12 months (average 3 to 6 months) before it starts to work. One may use corticosteroids, antihistamines, and fatty acids in the period between initiation of hyposensitization therapy and expected response.

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Corticosteroids are a mainstay of therapy for allergies but have been associated with a variety of adverse effects including polyuria, polydipsia, increased suspectibility to infection, mood changes, elevations in liver enzymes, and laminitis.[45] The most commonly used corticosteroids in equine dermatology are prednisolone, prednisone, and dexamethasone. Prednisone is converted to prednisolone in the liver. The dose for either of these two drugs for treating atopy is 0.5 to 1.5 mg/kg daily as an induction dose (usually 4 to 14 days) tapered to 0.2 to 0.5 mg/kg every 48 hours for maintenance. However, evidence indicates that prednisone is absorbed poorly from the intestine of many horses after oral administration. Therefore failure to respond to oral prednisone therapy does not confirm that a disorder is steroid nonresponsive. The recommended dose for dexamethasone is 0.04 to 0.2 mg/kg administered orally, intramuscularly, or intravenously every 24 to 48 hours for 4 to 7 days, decreasing to a maintance dose of 0.01 to 0.02 mg/kg every 48 hours.

Several antihistamines have been recommended for treating equine atopy. Hydroxyzine is recommended at 200 to 400 mg orally, regardless of body mass, every 12 hours orally or 1.5 mg/kg orally every 8 to 12 hours. Other antihistamines that have been used include chlorpheniramine maleate (0.26 mg/kg every 12 hours orally), diphenhydramine (0.75 to 1 mg/kg every 12 hours orally), doxepin hydrochloride (300 to 400 mg every 12 hours or 0.5 to 0.75 mg/kg every 12 hours), and pyrilamine maleate (0.8 to 1.32 mg/kg intravenously, intramuscularly, or subcutaneously; may be repeated in 6 to 12 hours if needed). The author has used all of these medications in horses and believes that hydroxyzine and chlorpheniramine maleate work most consistently in horses. Side effects associated with antihistamine use include sedation and behavior changes.

Fatty acids can act synergistically with steroids and antihistamines to decrease inflammation. ω-3 and ω-6 fatty acids seem to be most beneficial. They shift products of the arachidonic acid cascade from more inflammatory to less inflammatory mediators.[46]

Many fatty acid supplements are currently available, including Derm Caps ES (DVM Pharmaceuticals, Miami, Florida), Derm Caps 100 (DVM Pharmaceuticals), and Glänzen 3 (Horse Tech, Lawrence, Iowa). The first two products are available as a capsule or liquid, whereas the latter product is a powder to be sprinkled on food.

Dosage varies according to the fatty acid product used. The dosage for Derm Caps ES is 5 capsules every 12 hours; the dosage for Derm Caps 100 is 2 to 3 capsules every 12 hours. The dosage for the Glanzen 3 is 2 to 6 oz powder sprinkled on the food per day (not to exceed 6 oz per day). This product also contains biotin and other vitamins and minerals.

Topical treatments may be a useful adjunct therapy for horses with atopy. Various shampoos, lotions, and sprays marketed for use in small animals may be of benefit in horses. Antipruritic ingredients may include oatmeal, aloe, pramoxine, and hydrocortisone. Combination products may be most effective. The author prefers the Relief products marketed by DVM Pharmaceuticals of Miami, Florida, that contain oatmeal and pramoxine. These products may be used daily or weekly.

11.8 Cutaneous Drug Reactions

Cutaneous drug reactions in horses are rare and can mimic any known skin disease. Drug reactions are believed to involve type I, II, III, or IV hypersensitivity reactions. Any therapeutic agent administered by any route may cause a drug reaction. The most commonly incriminated drugs are penicillin, streptomycin, oxytetracycline, neomycin, chloramphenicol, sulfonamides, phenothiazines, phenylbutazone, guaifenesin, aspirin, and glucocorticoids. Clinical signs vary, and pruritus may or may not be present. Urticaria is a commonly observed skin reaction. Less commonly, mucocutaneous ulceration or vesiculation may be present.

Diagnosis of a drug reaction depends on an accurate medication history. Skin biopsies may be helpful in supporting a clinical diagnosis. The most commonly reported patterns of inflammation are perivascular dermatitis and intraepidermal or subepidermal vesicular dermatitis.[36] One can confirm the diagnosis by provocative challenge, but this may lead to anaphylaxis or death and therefore is not recommended.

Treatment requires removal of the offending drug, symptomatic therapy, and avoiding related compounds. Drugs reactions, in general, do not respond well to glucocorticoids. A drug reaction usually subsides within 2 to 3 weeks but may last for months.

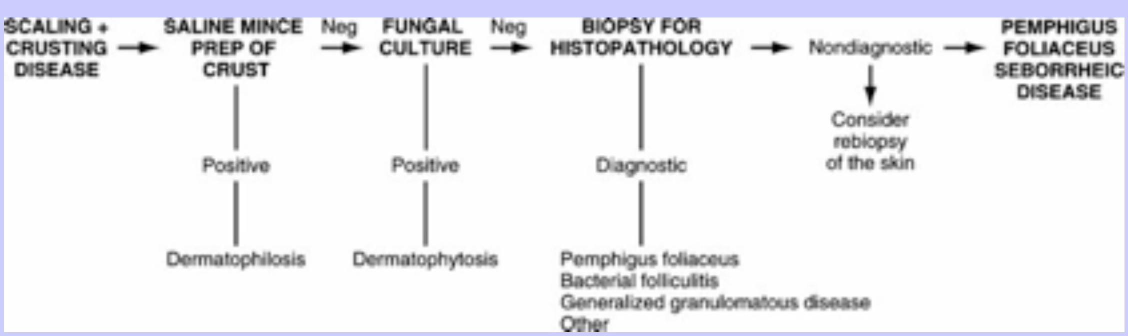
11.9 Causes Of Pruritus: Crusting And Exfoliative Dermatoses

Dermatophytosis and bacterial skin infections may cause pruritus in horses. They are recognized more commonly in the field as crusting or exfoliative dermatoses.

11.9.1 COMMON CAUSES OF SCALING AND CRUSTING DERMATOSES

Exfoliative dermatoses in horses have a wide range of causes. Historical and physical findings are important in differentiating these diseases. To make a diagnosis in difficult cases may require skin scrapings, fungal cultures, 682
bacterial cultures, mince preparations of crusts for cytologic examination of dermatophilosis, and skin biopsies. 683
If cost constraints are present, the most useful diagnostic test is a carefully obtained skin biopsy. Not scrubbing or preparing the skin in any way before obtaining skin biopsy specimens is critical. In addition, one should take extreme care to collect the crust when obtaining a skin biopsy from a horse with exfoliative skin disease. In many instances, one obtains the causative agent or evidence of a definitive diagnosis from histopathologic examination of the crust. Figure 11-10 shows a simplified approach to the diagnosis of exfoliative dermatoses.

Figure 11-10 Flowchart for diagnosis of equine scaling and crusting diseases.



11.9.2 INFECTIOUS CAUSES OF EXFOLIATION

11.9.2.1 Dermatophilosis (Rain Scald)

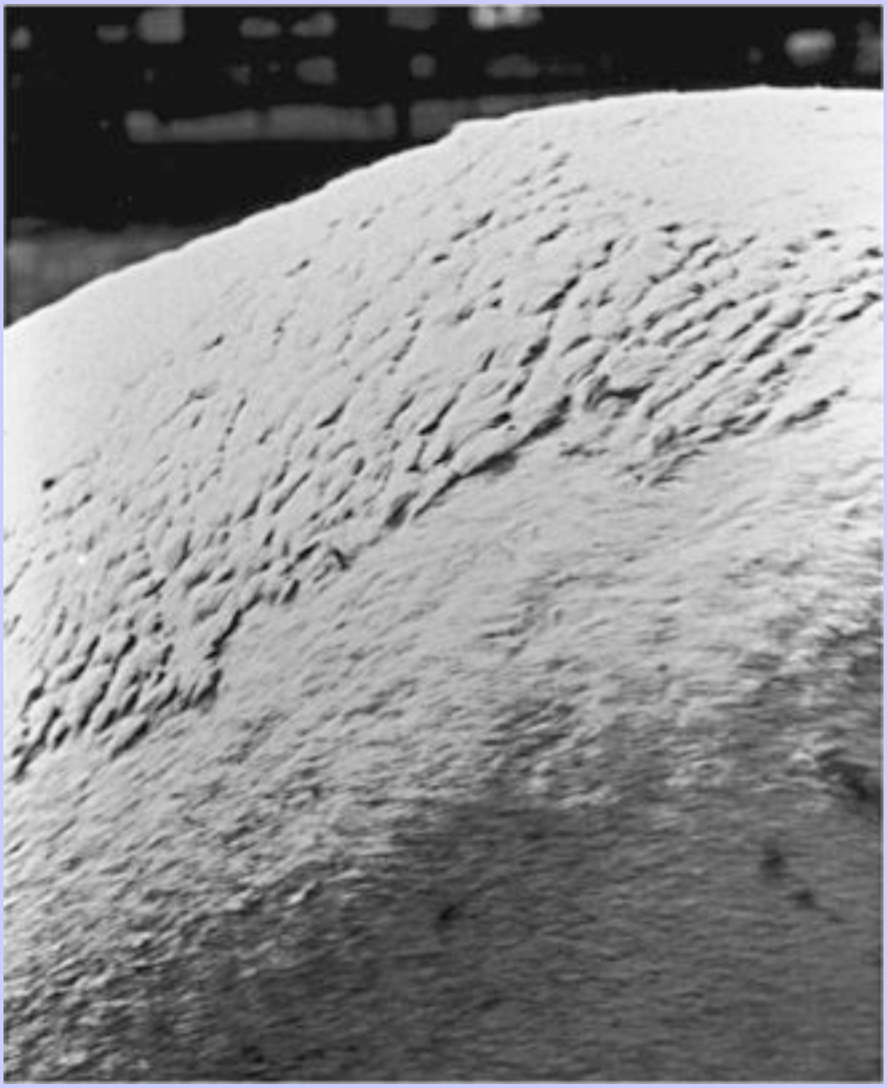
Dermatophilosis is a bacterial skin disease of horses and other large animals caused by the gram-positive, facultative anaerobic actinomycete *Dermatophilus congolensis*. The normal habitat of the organism is unknown, but the organism is thought to exist in a quiescent state on carrier animals until conditions are optimal for proliferation.[47] Whether carrier animals act as reservoirs of infection for other animals is unknown.

The development of lesions depends on chronic moisture and skin damage.[47,48] The organism cannot penetrate intact healthy skin, and moisture is required for release of zoospores. The skin can be damaged from chronic maceration caused by moisture, biting flies, vegetation, or underlying pruritic skin diseases. Chronic moisture is more conducive to the growth of the organism than intermittent but heavy rainfall. When these two prerequisites (moisture and trauma) are met, the organism multiplies. Moisture induces release of infective, motile, flagellated zoospores. These organisms are attracted to low concentrations of carbon dioxide and repelled by high concentrations of carbon dioxide. As the organism multiplies and inflammatory cells migrate into the area, the carbon dioxide concentration in the skin increases and zoospores migrate toward the surface of the skin in search of a more suitable environment. The organism again multiplies and numbers increase. This cycle repeats until layering, crusting, and matting of the hair coat results. Zoospores remain viable in crusts at ambient temperatures of 28° to 31° C for up to 42 months.[47]

Clinical signs of dermatophilosis can develop within 24 hours. The disease has a follicular orientation, and lesions appear as crusted, moist mats of hair that can resemble small paintbrushes. Under fresh crusts, the skin is soft, exudative, and yellow-tinged. Exudative crusted lesions tend to occur on the back, gluteal area, face, neck, and distal extremities (Figure 11-11). Lesions on the limbs may cause pain, swelling, and erythema, especially in white or lightly pigmented areas. Racehorses or horses in training commonly develop abrasions on the cranial surface of the hindlegs. These lesions are prime sites for infection. Severely affected horses may be febrile, depressed, lethargic, and anorectic and have a regional lymphadenopathy.

Dermatophilosis is diagnosed by demonstration of the organism by cytologic examination of the crust or by histologic examination of a skin biopsy specimen. One can make dermatophilosis preparations from dried or fresh crusts or from direct smears of exudate. One finely minces dried crusts in a few drops of sterile saline, allowing the preparation to dry for 45 minutes and then staining it. One may use a fast Giemsa, Diff Quik, or Gram stain for direct smears or dried mince preparations. The organism is visualized best under 1000× oil immersion and appears as fine-branching multiseptate hyphae with transverse and longitudinally arranged cocci (railroad track appearance; Figure 11-12). When one collects a skin biopsy from a horse with suspected dermatophilosis, submitting a specimen with the crust attached to the skin or hairs is important. Key histologic findings include folliculitis, intraepidermal pustules, intradermal edema, and alternating layers of parakeratotic (epidermal cells with retained nuclei) and orthokeratotic (keratinized epidermal cells without nuclei) hyperkeratosis with leukocyte debris.[48] The organism often is found only in crusts. 683

Figure 11-11 Horse with matting of coat caused by dermatophilosis.



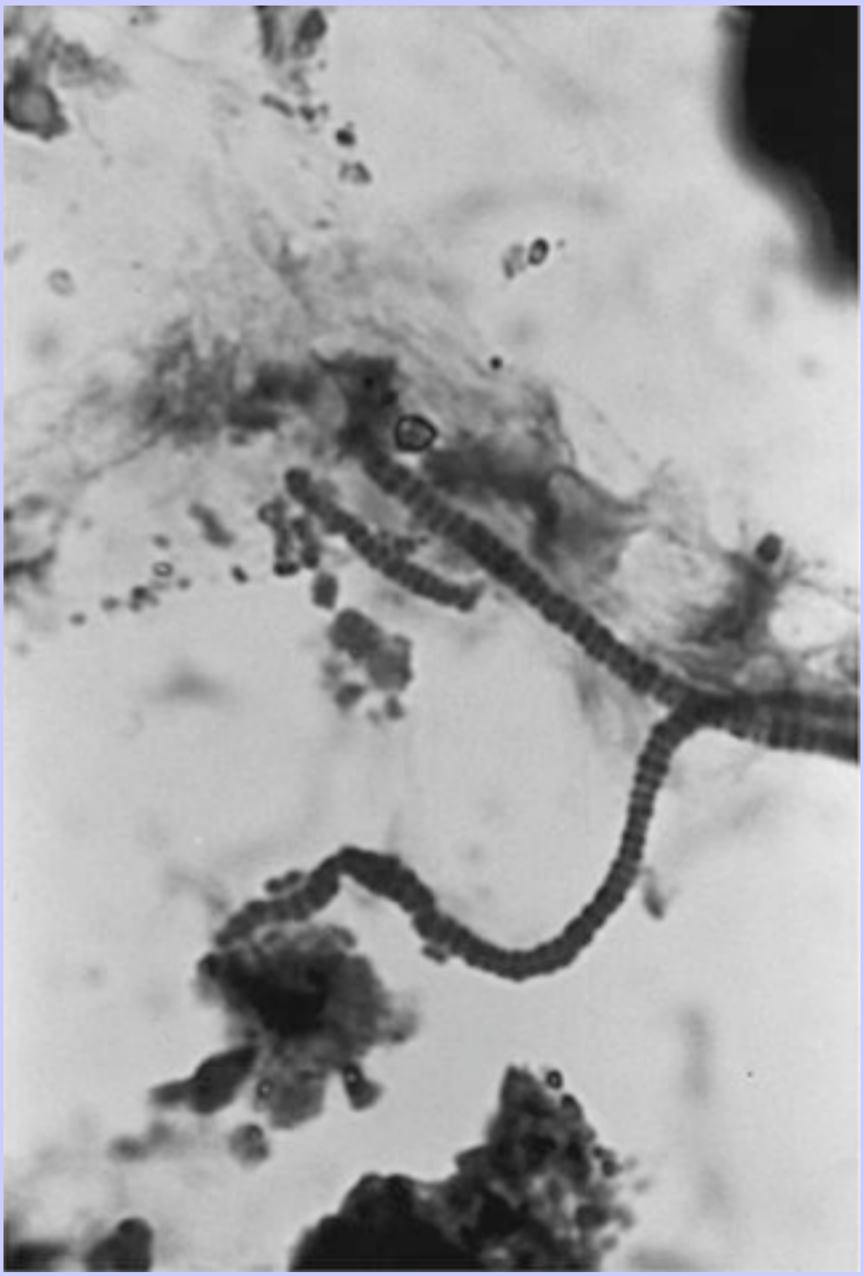
One may treat horses with mild infections with topical therapy alone. One gently should soak and remove crusts using a mild antibacterial shampoo such as chlorhexidine or benzoyl peroxide. Some horses for which this is difficult or painful may require sedation. One should dry the horse with towels and apply a topical antibacterial sponge-on dip (chlorhexidine 1:32 dilution of 2% stock solution, or lime sulfur 1:32 dilution of stock solution). Another option is a commercially prepared chlorhexidene 2% lotion (Resi-Chlor, Vibrac, Fort Worth, Texas) after bathing with an antibacterial shampoo. This formulation is a leave-on product with residual properties. Daily shampoo therapy should continue for 5 to 7 days until healing is evident, then twice weekly until all lesions have resolved. Horses with lesions on the muzzle may benefit from a topical cream with or without corticosteroids. Severely affected animals may benefit greatly from systemic antibiotics, such as procaine penicillin at 22,000 IU/kg intramuscularly twice daily for 5 to 7 days. Trimethoprim sulfa at 10 to 15 mg/kg orally twice daily for 5 to 7 days also may be effective. In either case, one should eliminate exposure to excessive moisture and skin trauma.

11.9.2.2

Dermatophytosis (Ringworm)

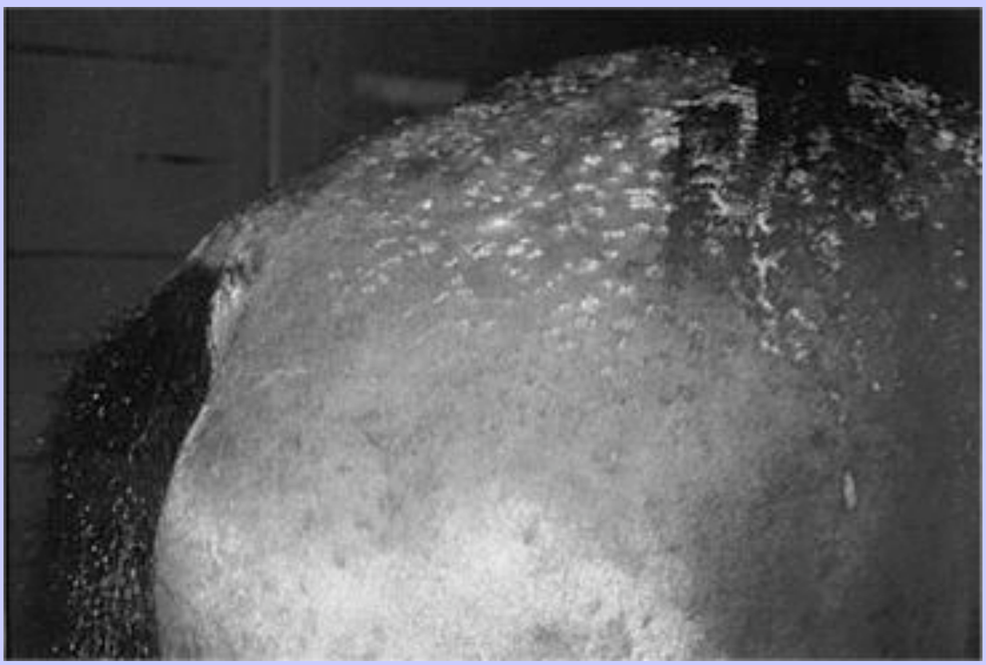
Dermatophytosis is the most common contagious skin disease in the horse. *Trichophyton equinum, Trichophyton mentagrophytes, Microsporum gypseum,* and *Microsporum canis* are the most frequent isolates. [49] The infection is self-limiting but may affect the function of the horse seriously and is of zoonotic importance.

Figure 11-12 Microscopic appearance of *Dermatophilus congolensis.*



Dermatophytes are transmitted by direct contact with an infected host or by indirect contact with contaminated fomites or the environment. Illness, poor nutrition, overcrowding, age (young or old immunosuppressed individuals), and stress predispose horses to infection. Chronic moisture from sweating or the environment damages the protective barrier of the skin, enhancing the opportunity for infection. When fungal spores contact the hair coat, the spores may be removed mechanically, may be unable to compete with the normal flora of the skin, or may remain on the coat in a dormant state until conditions are optimal for infection. Dermatophytes invade keratin of the epidermis and hair with the aid of enzymes that are allergenic to the host. Dermatophytosis is considered a biologic contact dermatitis.[49] The incubation period is usually several weeks. During this time, fungi invade the epidermal keratin, hair follicles, and the hair shaft itself. The epidermis responds to the intruder by increasing epidermal cell turnover in an effort to remove the fungi mechanically. 684 Clinically, the response is scaling. Hair shaft integrity is compromised, and hairs fall out or are fractured 685 easily, resulting in areas of alopecia characteristic of the disease. Infection is eliminated from a particular hair on shedding or as it enters the telogen stage, or the dermatophyte elicits an inflammatory response.

Figure 11-13 Dermatophytosis. The popular eruption on the advancing edge of lesions is notable.

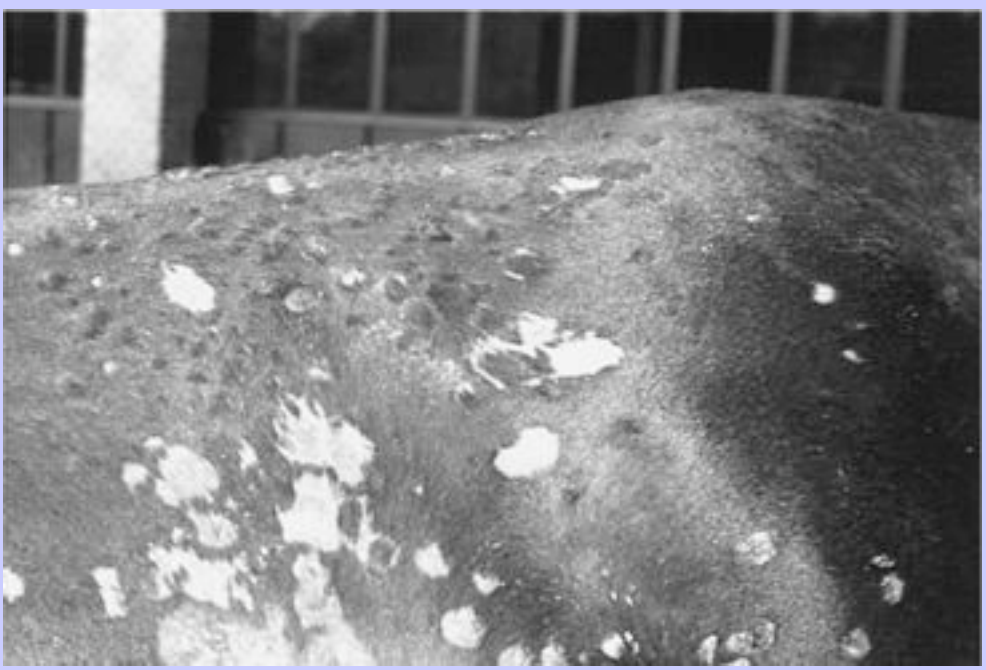


Clinical signs of dermatophytosis, including pain and pruritus, vary. The infection is follicular in distribution, and early lesions often begin as a papular eruption with erect hairs (Figure 11-13). Urticarial eruption may occur 24 to 72 hours before the owner notices the papules.[50] Lesions rapidly progress to crusted papules that spread circumferentially. The classic lesion is a circular patch of alopecia with stubbly hairs on the margin and variable amounts of scaling (Figure 11-14). Erythema and hyperpigmentation may be present. In rare instances, dermatophytosis may present as generalized scaling without significant hair loss. If the dermatophytosis causes follicular rupture, nodules, and ulcers, or a kerion reaction (inflammatory

dermatophyte infection that may resemble an abscess) may develop. Lesions are most common in areas where tack contacts the skin but may be limited to the posterior pastern.

Diagnosis is by demonstration and identification of the organism. Wood's lamp examinations are not useful for logistic reasons and, more importantly, because few cases of equine dermatophytosis are caused by *M. canis*. Direct examination of hair and scale is useful but requires special training. The most reliable tests are fungal culture and skin biopsy. The details of superficial fungal culture techniques have been reviewed elsewhere.[51,52] One should collect hairs for culture from the periphery of a newly developing lesion. Avoiding areas that may have been treated previously is critical. One should wipe the area gently with an alcohol swab before collecting hair samples to minimize growth of contaminants, should pluck hairs in the direction of growth, and transport them to the laboratory or clinic in a paper envelope for inoculation on dermatophyte test medium or Sab-Duets plates (Bacti-Labs, Mountain View, California), the commercial fungal culture media preferred by most veterinary dermatologists. Each plate contains Sabouraud's glucose medium and dermatophyte test medium. Using both media plates greatly enhances the culturing of suspect organisms. *Trichophyton* species isolation improves if one adds a few drops of B vitamin complex to the surface of the media and incubates the plate at 37° C. Because *T. equinum* is supposed to be one of the more common equine dermatophytes, one should add the B vitamin complex to all equine fungal cultures. One should select skin biopsy sites with care and should not scrub or wipe the area before sampling. Early lesions with crusts are ideal. Histologic findings include folliculitis, perifolliculitis, and furunculosis; superficial perivascular dermatitis with ortho- or parakeratosis; and intraepidermal vesicular or pustular dermatitis. Septate fungal hyphae and oval spores may be present in the superficial keratin or hair follicle.[51]

Figure 11-14 Classic signs of dermatophytosis (ringworm).



Dermatophytosis is a self-limiting disease, and most cases heal spontaneously within 1 to 6 months. Treatment strongly is advised to minimize contagion to other animals and human beings. In horses, antifungal shampoos and sponge-on dips are the treatment of choice. The author has found the most effective topical treatment

combination to be weekly bathing with a shampoo that contains 2% miconazole followed by a lime sulfur dip (Lym-Dyp, DVM Pharmaceuticals, Miami, Florida) for 4 to 8 weeks. Systemic antifungal drugs, such as griseofulvin (50 mg/kg orally once daily) or itraconazole (5 to 10 mg/kg orally once daily) are effective but expensive. In instances in which treating an infected herd is necessary or desirable, itraconazole at 5 to 10 mg/kg orally once daily for 15 days aborts the infection. Concurrent topical therapy with lime sulfur is necessary.

Elimination of dermatophytosis from single or multiple horse barns requires elimination of the organism from 685
infected hosts and decontamination of the environment. Box 11-3 summarizes one approach to treatment. 686
Commercial antifungal vaccines for equine dermatophytosis are not available in the United States. Autogenous vaccines are not recommended at this time because of lack of information on their efficacy and potential for serious adverse effects, including sterile abscesses, hypersensitivity reactions, generalized anaphylaxis, and death.

11.9.2.3 Bacterial Infections

Bacterial folliculitis in horses is caused most commonly by *Staphyloccoccus* spp. *Streptococcal* spp. infections also may occur. Infections most frequently occur in the summer when heat, moisture, increased insect populations, and increased use of the horse act in concert as predisposing factors.

The pathogenesis of bacterial folliculitis in the horse is similar to that in other animals. The causative agents normally are found on the host; when the natural protective barrier of the skin is compromised, bacteria invade and multiply within hair follicles. Infection results in inflammation, destruction of the hair follicle, and shedding of the hair. As the infection spreads, lesions enlarge. If hair follicles rupture, furunculosis and deep pyoderma may result.

Clinically, bacterial folliculitis may be indistinguishable from dermatophytosis. Bacterial lesions are much more likely to be painful and are only rarely pruritic. Infection is most common in the saddle and tack area but may be limited to the pastern area. Deep pyoderma of the tail base also occurs.

Definitive diagnosis is made best by cytologic examination, bacterial culture, and skin biopsy. Differentiating bacterial folliculitis from dermatophytosis is important. Skin biopsy reveals varying degrees of folliculitis, intraepidermal pustules, and perivascular inflammation. Neutrophils are the primary inflammatory cell. Keratinocytes often show intracellular edema; parakeratosis and orthokeratosis are common in the crust. Special stains often reveal bacteria within the crusts and hair follicles.

Treatment depends on severity of the clinical signs. Mild infections may be self-limiting but benefit from topical therapy. Severe cases require daily topical antibacterial shampoos with benzoyl peroxide or chlorhexidine and oral antibiotics. Ideally, one should base systemic antibiotic therapy on results of culture and sensitivity testing, but procaine penicillin at 22,000 IU/kg intramuscularly every 12 hours until resolution of clinical signs is usually effective.[51] A minimum of 10 to 14 days of therapy is recommended.

11.9.3 NUTRITIONAL CAUSES OF EXFOLIATION

Nutritional deficiencies are rare in horses, and much of this information has been extrapolated from work in other species or experimentally induced deficiencies.

11.9.3.1

BOX 11-3 TREATMENT OF EQUINE DERMATOPHYTOSIS (RINGWORM, FUNGUS) IN MULTIPLE HORSES

In the treatment of ringworm or superficial fungal infections of the skin, one must accomplish three things:

1. Kill the fungus on the animal to stop progression of the disease.
2. Kill the fungus on the animal to prevent spread to other animals, human beings, and the environment.
3. Decontaminate the environment.

The following suggestions have been helpful when treating outbreaks in the multiple-horse barn. One should keep in mind that fungal infections may take 6 to 8 weeks to heal completely.

1. *Isolate* all affected horses from normal horses.
2. Treat the barn by cleaning out all bedding and spraying stalls and exposed surfaces with 1:10 dilution of household bleach.
3. Treat all affected horses daily with lime sulfur sponge-on (4 oz/gal) for 7 days and then once weekly. Treating the *entire body* of each horse, not just the affected areas, is vital. One should allow sponge-on preparations to dry on the horse and should not bathe horses between treatments.
4. Use individual brushes and tack for each horse. Disinfect brushes and tack frequently. Do not transfer equipment between horses.
5. Blankets used with household bleach should be disinfected twice weekly or more often. *Not* using blankets or sheets is best to avoid enhancing the spread of the lesions.
6. Ideally, the persons who handle affected horses should not handle normal horses. If this is not possible, one should handle normal horses first and then affected horses. Washing *well* between animals with a chlorhexidine scrub is important.
7. Ringworm is contagious to other animals and human beings; handlers should be careful to wash well and report the development of any lesions. If possible, handlers should wear gloves when handling affected horses.
8. Ideally, horses affected with ringworm should be rested from training or riding until lesions are gone. Continued riding or training may contribute to skin microtrauma and spread of infection.

Modified from client education handouts used at the College of Veterinary Medicine, University of Florida, and the School of Veterinary Medicine, University of Wisconsin.

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11.9.3.2

Protein

Protein deficiency may occur in horses with fever, dysphagia, burns, proteinuria, liver disease, gastrointestinal diseases, or any disease in which metabolic demand increases and production of protein decreases. The skin uses 25% to 30% of the daily protein intake for hair production and maintenance of the epidermis,[53] a

requirement that may increase fivefold in disease states. In experimental deficiencies created in ruminants and laboratory animals, protein-deficient animals develop dry, dull, brittle, thin hair. Prolonged shedding also occurs.[54] Presumably, horses with protein-deficient diets would manifest similar clinical signs.

11.9.3.3 Zinc

Naturally occurring zinc deficiencies have not been reported in horses. Classically, this deficiency appears as crusting skin disease. Experimentally induced zinc deficiencies in foals resulted in alopecia, dense scaling on the lower limb that progressed to the trunk, inappetence, decreased growth rate, and decreased tissue and serum zinc concentrations.[54] Definitive diagnosis is by observing histologic lesions compatible with zinc deficiency (diffuse epidermal and follicular parakeratosis) and response to oral supplementation with zinc sulfate at 10 mg/kg once daily. A significant response usually occurs within 2 to 3 weeks, and therapy may or may not be lifelong.

11.9.3.4 Iodine

Suspected iodism caused by excessive supplementation therapeutically or in feeds has been reported in horses.[55] Noncutaneous signs include cough, lacrimation, nasal discharge, and joint pain. Severe dry seborrhea with or without dorsal alopecia is also present. No treatment is necessary and horses recover spontaneously on removal of the source of excess iodine.

11.9.4 PARASITIC CAUSES OF EXFOLIATION

Insect hypersensitivities (discussed earlier) are the most common parasitic cause of exfoliation in horses. The major differentiating feature is the presence of pruritus, lack of circumferentially spreading lesion, and the absence of pain. Skin biopsy findings of superficial or deep eosinophilic perivascular dermatitis with or without collagen degeneration suggest insect hypersensitivity.

11.9.5 IMMUNOLOGIC CAUSES OF EXFOLIATION: PEMPHIGUS FOLIACEUS

Pemphigus foliaceus is a rare autoimmune skin disease that results in severe crusting and matting of the hair coat. An autoantibody against the glycocalyx of keratinocytes results in the release and activation of keratinocyte proteolytic enzymes into the intercellular space. The glycocalyx is hydrolyzed and intercellular cohesion is lost, leading to acantholysis.[56]

Pemphigus foliaceus has no age or sex predilection. Appaloosa horses appear to be predisposed to developing pemphigus foliaceus.[57] Lesions usually begin on the face and limbs and may take weeks or months before becoming generalized. In some horses the disease affects only the coronary band. One rarely observes the primary lesion, a subcorneal pustule, because of the hair coat of the horse. The most common clinical presentation is severe matting and crusting of the coat with oozing of serum from the skin (Figure 11-15). Most horses are depressed and show signs of systemic illness. Swelling and pain of the distal extremities, in addition to lameness are common. Pruritus and pain on the trunk, ventral edema, and regional or generalized lymphadenopathy vary.

Definitive diagnosis is by routine histologic examination of a skin biopsy specimen. Submitting a skin biopsy with intact crusts attached to the underlying skin or hairs is critical. Histologically, pemphigus foliaceus is

characterized by intragranular to subcorneal acantholysis. One often observes layered rafts of acantholytic cells within crusts interspersed among neutophils.[57] Direct immunofluorescence is unreliable, but if positive reveals diffuse intercellular fluorescence. One may make a tentative diagnosis of pemphigus foliaceus based on impression smears of the content of intact pustules or the underside of exudative crusts (Figure 11-16). Rafts of deeply basophilic acantholytic cells and nondegenerate neutrophils are highly suggestive of this disease.

Pemphigus foliaceus in horses less than 1 year of age is usually self-limiting. Spontaneous remission is common in foals, and they often do not require long-term therapy. Because treatment is difficult and often unrewarding in adults, many owners elect euthanasia. Remission and control of the disease is induced with prednisone or prednisolone at 1 mg/kg orally every 12 hours until no new lesions develop, which usually occurs within 7 to 10 days, at which point alternate-day therapy continues at the same dose. If the horse does not respond to prednisone, one should attempt daily therapy with dexamethasone because of potential problems with absorption of prednisone given orally in some horses. Maintaining clinical normalcy with prednisone therapy alone is difficult, and one may have to add adjuvant immunosuppressive agents to the treatment program. Even in cases in which prednisone therapy is effective, the potential for laminitis and immunosuppression warrant the use of other agents. Chrysotherapy (gold salts) has been used in horses but is expensive.[58,59] One administers two doses of aurothioglucose (Solganal, Schering Corp., Kenilworth, New Jersey) 1 week apart intramuscularly, 20 and 40 mg, respectively. If one observes no adverse effects (stomatitis, urticaria, sloughing of skin, blood dyscrasia), one begins weekly therapy at 1 mg/kg intramuscularly until observing a response (6 to 12 weeks). One tailors maintenance therapy to the individual animal, which may involve biweekly or monthly therapy. Complete remission of clinical signs may not be possible to achieve even with combination therapy. The most common adverse effects of aurothioglucose therapy in human beings, dogs, and cats are immune-mediated thrombocytopenia and proteinuria. Presumably, these could occur in the horse. Weekly performance of a complete blood count and urinalysis is recommended for the first month and then monthly if one observes no abnormalities.

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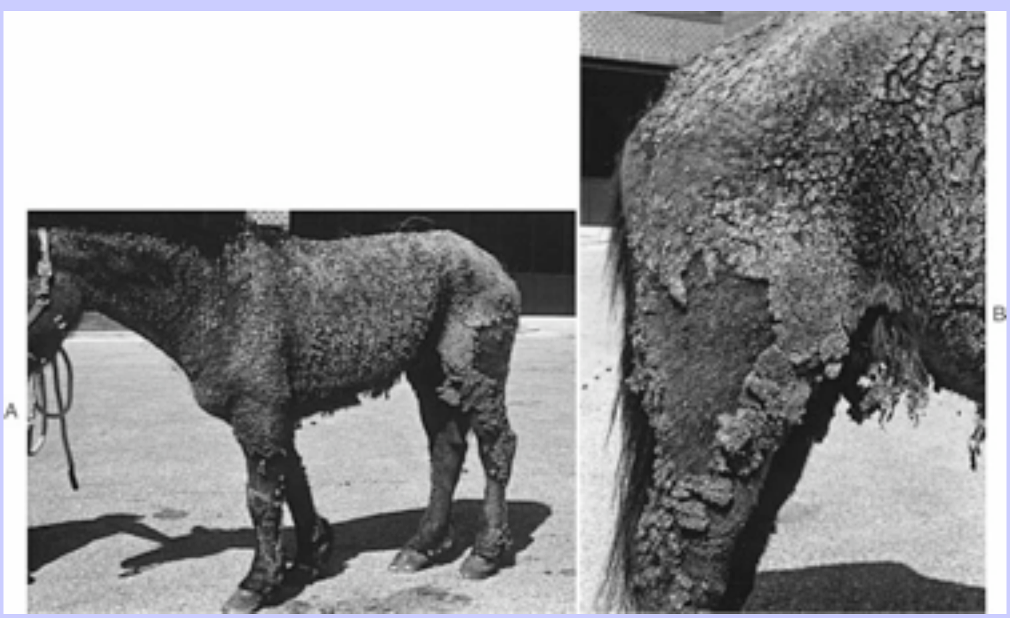
Figure 11-15 **A,** Pemphigus foliaceus in a horse. **B,** Skin with significant crusting.



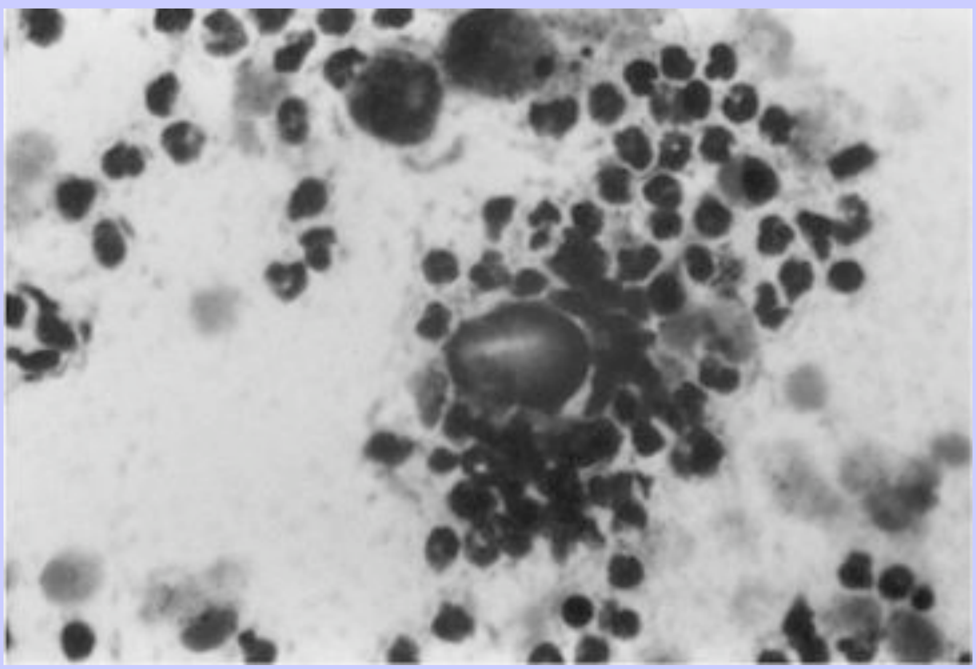
11.9.6 IRRITANT CONTACT DERMATITIS

Irritant contact dermatitis is fairly common in horses. This reaction differs from allergic contact dermatitis because the offending substance invariably causes a reaction if left in contact with the skin; prior sensitization is not needed.

The mechanism of irritant contact reaction depends on the causative chemical, but moisture and tissue maceration are important predisposing factors. Common irritating substances include feces, urine, wound secretions, caustic substances, crude oil, diesel oil, turpentine, blisters, leg sweats, improperly used insecticides, fly wipes, concentrated disinfectants, irritating plants, and soiled bedding.[60]

No age, sex, or breed predilection exists for irritant contact dermatitis.[60–62] Direct contact with the offending substance is required for irritant reactions to occur. Acute lesions are often erythematous, vesiculated, erosive or ulcerated, and painful. As the condition continues, necrosis, crusting, scaling, and hair loss occur. Leukoderma 688
and leukotrichia are common and may be permanent. Prutitus and pain vary. 689

Figure 11-16 Cytologic appearance of a touch preparation from a lesion of pemphigus. Rounded cells (acanthocytes) are notable.



Diagnosis is from the history, clinical signs, and inspection of the environment of the horse. Provocative testing is useful in identifying and confirming the causative agent as long as the risk is minimal to the horse. Histologic examination of skin reveals necrosis, spongiotic vesicles, and ulceration.

Treatment requires identification and removal of the offending substance. One should wash affected areas daily with copious amounts of water and apply topical 0.5% chlorhexidine soaks. Astringent topical solutions, for

example, aluminum acetate (Domeboro, available as an over-the-counter preparation), may be beneficial but often cause pain or delay wound healing. Topical antimicrobial creams free of corticosteroids may promote healing. Healing usually is rapid once the irritant is removed.

11.9.7 ALLERGIC CONTACT DERMATITIS

Allergic contact dermatitis has been discussed. An important note is that allergic contact dermatitis may cause scaling and crusting in addition to pruritus. Lesions usually are distributed in a pattern that reflects the offending substance.

11.9.8 PHOTODERMATITIS

Photodermatitis is caused directly by sunburn or indirectly by photoallergy or photosensitization.

11.9.8.1 Sunburn

Sunburn is a phototoxic reaction caused by excessive exposure to ultraviolet B light. Horses with white or light skin or hair are at risk. Affected areas become erythematous and scaly. If sunburn is severe, necrosis and exudation may occur. Diagnosis is based on history and clinical signs. Topical glucocorticoid creams alleviate pain, but excessive use has the potential to cause minor side effects (depigmentation, failure, or slowing of hair regrowth) or possibly serious side effects (laminitis) because these drugs are absorbed systemically. Stabling horses during periods of intense sunlight and application of water-repellent sunscreens may be helpful.

11.9.8.2 Photoallergy

Photoallergy is believed to be caused by contact with a chemical or plant (species unknown) and ultraviolet light. The condition is observed most commonly on white muzzles and extremities of horses housed on pasture containing clover, particularly alsike clover. Erythema, vesicles, and crusts develop. Diagnosis is by clinical findings. If one cannot identify the offending substance and remove it from the environment, then horses should be stabled during daylight, if possible, to minimize exposure to the offending substance.

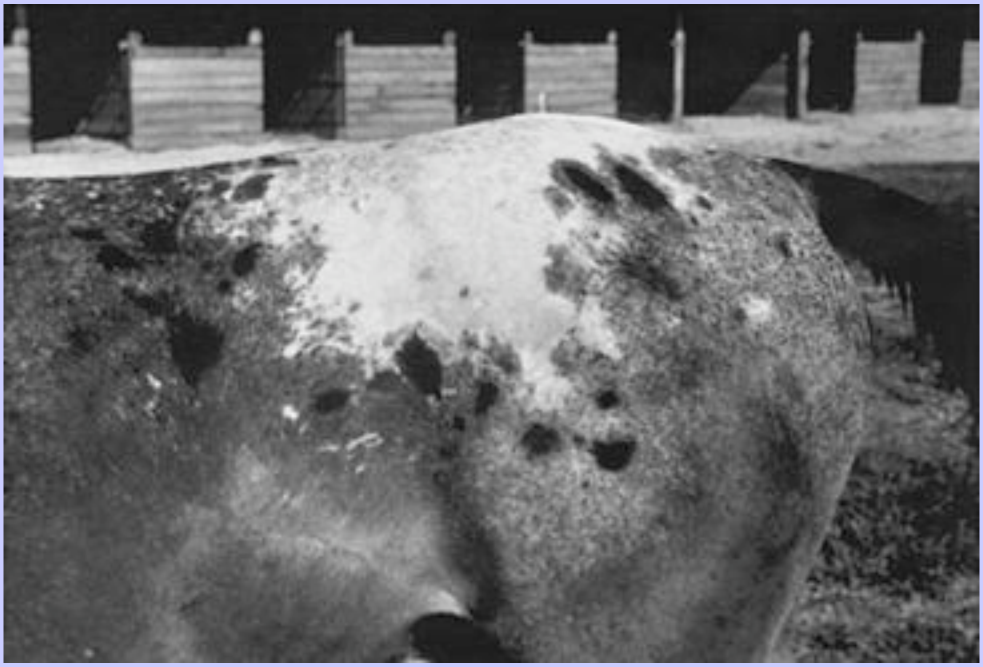
11.9.8.3 Photosensitization

Photosensitization may develop as a result of hepatogenous photosensitization; aberrant pigmentation formation; or ingestion, injection, or topical application of a primary photodynamic agent.[60,63] Examples of plants known to cause primary photosensitization in horses include St. John's wort, buckwheat, perennial rye grass, and burr trefoil. Chemicals that may cause primary photosensitization include phenothiazine, thiazide, acriflavine, rose bengal, methylene blue, sulfonamides, and tetracyclines. Plants, fungi, infection, neoplasms, and certain chemicals have been associated with hepatogenous photosensitization in horses. Plants that may cause equine hepatogenous photosensitization are burning bush or fireweed, ngaio tree, rape or kale, heliotrope, ragworts, tarweed or fiddleneck, and *Crotalaria* or rattleweed. Fungi that can induce hepatogenous photosensitization in horses include blue-green algae and *Phomopsis leptostromiformis* (on lupins). A detailed discussion on the pathogenesis of liver disease and its association with cutaneous photosensitization is included elsewhere in this text.

Regardless of the source of the photodynamic agent, the pathophysiology of skin disease is similar. Photodynamic agents are deposited in the skin and absorb energy when exposed to sunlight. These molecules are elevated to a high energy state and, in the presence of oxygen, produce free radicals that damage cell membranes. Lysosomes and other organelles release hydrolytic enzymes and other mediators of inflammation.

Lesions are most common in white or lightly pigmented areas but have been observed to extend into dark-colored areas.[51,60] Eyelids, lips, face, perineum, and coronary bands commonly are affected. Usually an acute onset of erythema, edema, pruritus, and pain occurs. Vesicles and bullae may develop (Figure 11-17). Necrosis, ulceration, and crusting often occur.

Figure 11-17 Horse with photosensitization.



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Diagnosis is based on clinical signs, history, and laboratory tests. One should perform liver function tests regardless of whether the horse is showing clinical signs of hepatic disease.

Treatment requires identification of the underlying cause. Prognosis varies in horses with liver disease. Therapy requires removal of the offending substance, avoidance of sunlight, and topical or systemic glucocorticoids.[51]

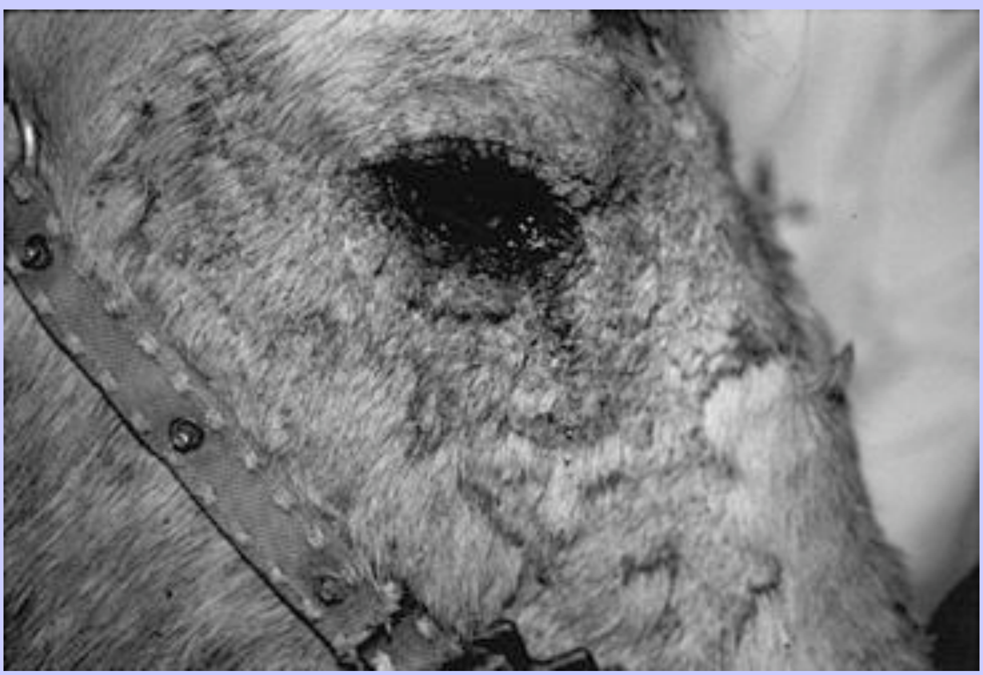
11.9.9 SEBORRHEA AND LOCALIZED DISORDERS OF KERATINIZATION AS CAUSES OF SCALING AND CRUSTING

11.9.9.1 Seborrhea

Seborrhea is a descriptive term for excessive scaling and crusting. The condition may or may not result from excessive sebum production. Most seborrheic skin conditions are secondary, resulting from dermatophytosis, dermatophilosis, or parasitic or bacterial skin diseases. The natural response of the skin to insult is proliferation, as a mechanism by which to remove an offending organism or as a result of inflammation. Primary seborrhea is a disorder of keratinization in which epidermal cell turnover time and basal cell proliferation increase. Genetic factors may control these mechanisms. Secondary seborrhea, the most common form of seborrhea, can occur in any inflammatory, infectious, or parasitic disease.

Idiopathic or primary seborrhea in horses may be generalized or localized to the tail and mane. No age, sex, or breed predilection exists. Generalized seborrhea appears clinically as diffuse matting of the coat. The texture of the coat may be oily and thick; adherent crusts may be removed easily. Oily or dry scales are present at the base of the hairs. Lesions usually are generalized and involve the face and legs (Figure 11-18). The animal is odoriferous and is not usually pruritic. Seborrhea of the mane and tail is common and presents as crusts or scales attached to the bases of hairs. Pain and inflammation are generally absent.

Figure 11-18 Horse with primary seborrhea.



Primary seborrhea is a diagnosis of exclusion. Severe generalized seborrhea must be differentiated from pemphigus foliaceus. One should perform skin scrapings, fungal cultures, dermatophilosis preparations, and skin biopsies on all suspect cases. Skin biopsies may help in determining whether primary or secondary

seborrhea is present.[64] In early cases of generalized equine seborrhea involving little secondary inflammation, skin biopsy findings strongly suggest a primary keratinization disorder. The noncornified epidermis is not hyperplastic (thickened), but a significant orthokeratotic hyperkeratosis is attached to an epidermis of normal thickness. The superficial dermis shows only a noncornified epidermis, suggesting a keratinization defect. In cases with secondary inflammation or in which the seborrheic condition is more long-standing, one may not interpret the biopsy findings as easily. The cornified epidermis is acanthotic (thickened) with significant orthokeratosis or parakeratotic hyperkeratosis. Superficial perivascular inflammation often is present in the superficial dermis.

Treatment of seborrhea involves eliminating the predisposing cause. Primary seborrhea is usually incurable, and management is symptomatic with topical therapy. The owner usually can manage seborrheic condition satisfactorily with antiseborrheic shampoos. The owner should wash the horse with a cleansing shampoo before using a medicated shampoo. This removes excess dirt and scale, improves efficacy of the medicated shampoo, and decreases the amount of medicated shampoo needed, minimizing potential for a contact reaction. The owner may use several brands of shampoo before finding a suitable shampoo for an individual patient. In general, dry seborrhea responds best to sulfur-based shampoos and oily seborrhea to tar-based shampoos. Benzoyl peroxide shampoos also work well in cases of oily seborrhea. Antiseborrheic shampoos formulated for small animals work well for horses but may be expensive. The owner should avoid human shampoos because these products are often more expensive than veterinary products and lather excessively, making rinsing difficult. Owners should allow the antiseborrheic shampoo to contact the coat for 10 to 15 minute before rinsing. Thoroughly rinsing the horse is important, especially in the axillary and inguinal region, because shampoo residues irritate the skin. Initially, owners may need to shampoo the horse daily for 1 to 2 weeks. After the coat of the horse is normal, the owner may decrease shampooing to twice a week but must realize that therapy is lifelong. If shampoo therapy dries the coat and worsens dry seborrhea, the owner may use a moisturizing spray or lotion.

11.9.9.2 Cannon Keratosis

Cannon keratosis is an idiopathic skin disease characterized by the presence of plaques and patches of 690
hyperkeratosis on the cranial aspect of the rear cannon bones.[65,66] This disorder of keratinization is 691
uncommon and does not appear to have any recognized age, breed, or sex predilection. Clinically, well-circumscribed plaques of tightly adherent crusts and scales with or without alopecia develop over the cranial surface of the rear cannon bones. The lesions are not pruritic.

Diagnosis is usually by clinical examination, and confirmation is by skin biopsy. Differentiating cannon keratosis from dermatophilosis and dermatophytosis is important. Treatment consists of antiseborrheic shampoos. Topical glucocorticoids may be useful. Topical vitamin A cream (tretinoin, Rein-A cream 0.1%, Ortho Pharmaceutical Corp., Raritan, New Jersey) may be beneficial in removing crusts and plaques.

11.9.9.3 Linear Keratosis

Linear keratosis is an uncommon idiopathic disorder of keratinization.[65,66] The disease appears to be heritable, especially in Quarter Horses. Lesions develop between 1 and 5 years of age. One or more painless, nonpruritic unilateral, vertically oriented bands of alopecia and hyperkeratosis develop on the neck or lateral thorax. Lesions vary in size, ranging from 0.25 to 3.5 cm in width and 5 to 70 cm in length. Early lesions may begin as coalescing groups of papules. Lesions are not painful or pruritic.

Diagnosis usually is based on clinical signs alone. Skin biopsy reveals regular, irregular, or papillated epidermal hyperplasia with significant compact orthokeratotic hyperkeratosis.[64] Superficial perivascular dermatitis may or may not be present. If the linear keratosis does not interfere with the function of the horse, no treatment is necessary; surgical excision of small lesions may be curative. Topical vitamin A cream or salicylic cream may be useful in nonsurgical cases. These agents are keratolytic and may decrease the height, width, or thickness or the keratosis, allowing the horse to be used. Owners should not breed affected animals.

11.9.10 IDIOPATHIC CAUSES OF SCALING AND CRUSTING

11.9.10.1 Equine Exfoliative Eosinophilic Dermatitis and Stomatitis

Equine exfoliative eosinophilic dermatitis and stomatitis is an idiopathic disease of horses characterized by ulcerative stomatitis, severe wasting, significant exfoliation, and eosinophil infiltration of the skin.[36,67,68] The disease also is referred to as multisystemic eosinophilic epitheliotrophic disease. No age, sex, or breed predilection exists, but lesions tend to occur more commonly in the winter.

Early lesions begin as scaling, crusting, exudation, and fissuring at the coronary band. Oral ulcers are usually present at this stage. Over several weeks a generalized exfoliative dermatosis develops. Hairs are epilated easily, and alopecia with multifocal areas of ulceration and exudation develops. The horse may be pruritic. Affected horses without ulcerative stomatitis have a good appetite and may even be ravenous, but exhibit rapid, severe weight loss. Some horses have concurrent protein-losing enteropathy or malabsorption syndrome.

Definitive diagnosis is based on the history, physical examination, and skin biopsy. Ruling out pemphigus foliaceus and bullous pemphigoid is important. The chronic wasting nature of the disease and multisystemic signs are key to making a diagnosis. Laboratory features include hypoalbuminemia, hypoproteinemia, impaired small intestinal carbohydrate absorption, and increased activities of γ-glutamyltransferase, serum alkaline phosphatase, and bile duct isoenzymes. A superficial and deep eosinophilic and lymphoplasmacytic dermatitis with irregular epidermal hyperplasia is present.[67,69] Exocytosis of eosinophils and lymphocytes and necrosis of keratinocytes occurs.[36,67,69] Perivascular collagen degeneration, lymphoid nodules, and a lichenoid inflammatory infiltrate may be present. Eosinophilic infiltrates of the pancreas, salivary glands, oral cavity, and gastrointestinal tract are common. Peripheral eosinophilia is usually absent.

Two horses have responded to dexamethasone at 0.2 mg/kg intramuscularly for the first 5 days, followed by prednisolone at 0.5 mg/kg orally every 12 hours for 7 days, and then 1.0 mg/kg every 24 hours for a week, followed by alternate-day therapy.[36,67,69] However, most horses respond poorly to systemic glucocorticoids, and the prognosis is grave. A single horse with this disease showed a partial response to hydroxyurea and dexamethasone therapy.[70]

11.9.10.2 Generalized Granulomatous Dermatitis (Equine Sarcoidosis)

Generalized granulomatous disease is a rare idiopathic skin disease characterized by exfoliation, severe wasting, and granulomatous inflammation of multiple organ systems.[71,72] This disease also has been called equine sarcoidosis because it resembles sarcoidosis of human beings. One should avoid using this term because of possible confusion with sarcoid tumors. The cause of the disease is unknown. The disease may

represent an abnormal host reaction to an infectious agent or allergen[71] or a nonmalignant neoplastic proliferation of cells.

No breed, age, or sex predilection exists for this condition. The skin disease begins as scaling, crusting, and alopecia on the face, limbs, and trunk (Figure 11-19). Clinical signs rapidly become generalized. Occasionally, the disease is focal or multifocal in distribution.[71] Peripheral lymphadenopathy may develop concurrently with weight loss, muscle wasting, anorexia, exercise intolerance, and fever. 691

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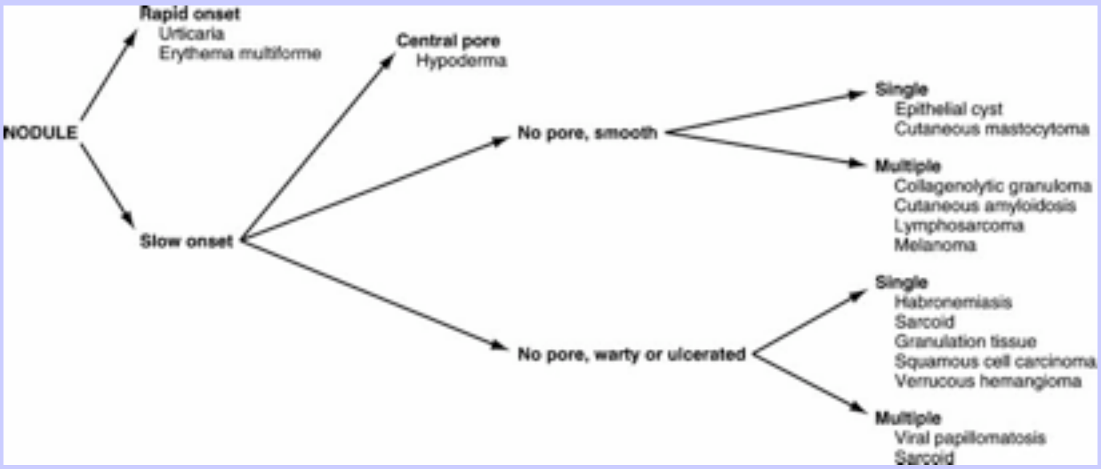
Figure 11-19 Horse with generalized granulomatous dermatitis. (Courtesy A.A. Stannard, University of California at Davis.)

Definitive diagnosis is by skin biopsy.[71] Perifollicular and middermal noncaseating granulomatous dermatitis occurs frequently with multinucleated giant cells. Neutrophils, lymphocytes, and plasma cells are present in small numbers. Granulomata are present in the superficial portion of the dermis. Granulomatous infiltrates also occur in other organs, including the lymph nodes, lung, gastrointestinal organs, liver, and spleen. A complete blood count reveals leukocytosis, increased fibrinogen, and hyperglobulinemia. Anemia caused by chronic infection may be present.

The prognosis is grave, and no treatment exists. Horses usually are euthanized within several months. Large doses of prednisolone (2 mg/kg orally every 24 hours) may be beneficial in the early stage of the disease. Occasionally, spontaneous remission may occur.

Figure 11-20 Flowchart for equine nodular dermatitis.



11.10 Diseases Characterized By Papules And Nodules

Many diseases are associated with single or multiple nodules in the skin of horses. Making a definitive diagnosis based on history and clinical signs alone is almost impossible. The most useful and cost-effective diagnostic test is a skin biopsy. Figure 11-20 illustrates a diagnostic algorithm for equine nodular skin disease.

11.10.1 INFECTIOUS CAUSES OF NODULES

11.10.1.1 Bacterial Granulomata (Botryomycosis)

Botryo- is a prefix that means grapelike. Affected horses have clusters of nodular, grapelike lesions. Many species of bacteria have been associated with bacterial granulomata in horses; however, the most commonly isolated organism for equine bacterial granulomata is *Staphylococcus* spp.

Bacterial granulomata begin as traumatic injuries to the skin during which an infectious organism is inoculated into the dermis. A granulomatous reaction develops because the organism is capable of eliciting a response from the host, but the host is only able to contain the infection and not eradicate it. Bacterial granulomata develop in areas of previous trauma and are common on the limbs and scrotum. Poorly circumscribed, firm, draining lesions may or may not be painful. The center of the lesion may be ulcerated. In some cases, variable numbers of white to yellow pieces of particulate matter resembling grains of sand (tissue grains) may be present in the exudate.[73]

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Diagnosis confirmation is by skin biopsy, and identification of the causative organism is by bacterial or fungal culture. Histologic examination of a skin biopsy reveals nodular to diffuse pyogranulomatous inflammation with or without tissue grains. One may observe bacteria.

Surgical excision or radical debulking followed by long-term antibiotic therapy based on culture and sensitivity has been the most successful treatment for bacterial granulomata.[52,73] Organisms may be difficult to isolate from biopsy tissue samples, and submission of large wedges of tissue obtained at the time of surgery

is recommended. If possible, one should obtain tissue to submit for culture from the deepest portion of the lesion to increase the likelihood of isolating an infectious agent and should submit tissue for aerobic, anaerobic, and deep fungal cultures.

11.10.1.2 Corynebacterial Infections

Corynebacterium spp. are gram-positive pleomorphic rods that are associated with two clinical syndromes in horses: abscesses and ulcerative lymphangitis.[47,52,74]

11.10.1.2.1 Abscesses

Corynebacterial abscesses occur frequently in the horse in the western United States. Lesions tend to be seasonal, occurring most commonly during the summer and fall concurrent with peak fly season, especially in dry dusty conditions. The organism is believed to be transmitted by flies, particularly horn flies. For unknown reasons, fly bites are susceptible to infection.

Lesions develop slowly and may be single or multiple. The pectoral and ventral abdominal areas are affected most commonly. These abscesses drain a creamy to caseous material that may be whitish to greenish. Pitting edema, ventral midline dermatitis, depression, fever, and lameness may be present. The course of the disease may be chronic, and recurrent internal abscesses are frequent.

Diagnosis is based on the season and the appearance of the abscess and exudate. Because identifying the organism on cytologic examination of exudate may be difficult, a culture is always recommended.

Treatment is protracted and is complicated by the limited response of the organism to systemic antibiotics. The veterinarian should allow the abscess to mature and then surgically incise and drain it.[52] Systemic antibiotic use before abscess rupture may be followed by recurrence of the abscess on discontinuation of the drug. If drainage cannot be accomplished, large doses of procaine penicillin (50,000 IU/kg intramuscularly every 12 hours) for up to 6 months may be curative.[47,52,73,74] Toxoids and bacterins have not been useful.

11.10.1.2.2 Ulcerative Lymphangitis

Ulcerative lymphangitis is an infection of the cutaneous lymphatics caused most commonly by *Corynebacterium pseudotuberculosis*. Infection is believed to begin by wound contamination. The disease occurs infrequently today.

No age, sex, or breed predilection exists. Lesions usually develop on the hindlimbs, especially at the fetlocks. Hard to fluctuant chains of nodules that abscess, ulcerate, and drain occur most commonly. Old lesions heal within 1 to 2 weeks, but new lesions develop.[48] The affected limb may be swollen and painful, and the horse may be depressed. Regional lymphatics appear corded. In chronic cases, permanent thickening of the tissue surrounding the regional lymphatics is common.

Diagnosis is based on the appearance of the lesion and identification of the organism. Direct smears of the exudate stained with a fast Giemsa or Gram stain and a culture and sensitivity usually confirm the diagnosis.

One must initiate treatment rapidly to prevent permanent debilitation and disfigurement. In early cases, hydrotherapy, exercise, surgical drainage, and large doses of procaine penicillin (20,000 to 80,000 IU/kg intramuscularly every 12 hours) for at least 30 days after clinical normalcy may be curative.[73] Nonsteroidal antiinflammatory drugs may be beneficial. If the area becomes fibrotic and joint motion is restricted, prognosis is poor.

11.10.1.3 Mycetomata

Mycetomata (Table 11-2) are chronic subcutaneous infections characterized by swelling, draining tracts, and tissue granules. Filamentous bacteria or eumycetic fungi (fungi that inhabit the soil or vegetation) may cause mycetomata. The most common fungal organisms that cause mycetomata in horses are *Curvularia geniculata* and *Pseudallescheria boydii*. The most common bacteria isolated from equine mycetomata are *Actinobacillus* spp., *Nocardia* spp., and *Actinomyces* spp. Regardless of causative agent, the organism usually gains entrance to the body through traumatic inoculation. As with other granulomatous reactions, the body is able to recognize the intruder and isolate it but not to eliminate it.

Single or multiple nodules of varying sizes can occur almost anywhere on the body of the horse. The nodules often are pigmented and ulcerated and may discharge tissue grains (small sandlike particles). The tissue grains are believed to be masses of hyphae from the organism. Gross examination of the cut surface often reveals dark-brown pigment or granules scattered in yellow to pink tissue.

Distinguishing clinically among bacterial or fungal mycetomata or any number of other causes of lumps in the skin is impossible. Definitive diagnosis requires an excisional or wedge biopsy of a lesion. Histologic examination shows diffuse to nodular pyogranulomatous to granulomatous inflammation surrounding septate branching hyphae. Tissue grains are often visible microscopically. The hyphae contained in tissue grains or tissue may be pigmented or nonpigmented. Fungi are cultured best from tissue grains and, occasionally, 693

694 exudate. If possible, surgical excision is curative.[52] Antibiotic therapy is usually ineffective, especially for fungal mycetomata.

TABLE 11-2 Fungal Diseases of Horses

DISEASE	COMMON CAUSATIVE AGENTS	CLINICAL SIGNS
SUPERFICIAL INFECTIONS		
Dermatophytosis	*Microsporum* and *Trichophyton* spp.	Circular patches or hair loss, crusting and scaling, urticaria, papules
Piedra	*Piedraia* spp. or *Trichosporon beigelii*	Black or white filamentous nodules on hair shaft
INTERMEDIATE INFECTIONS		
Sporotrichosis	*Sporothrix schenckii*	Papules and nodules occur along lymphatics; nodules may ulcerate, become thickened, and drain a thick brown- to red-colored exudate.
Phaeohyphomycosis (chronic, subcutaneous fungal infection caused by pigmented opportunistic fungi)	*Hormodendrum, Drechslera, Phialophora, Curvularia, Cladosporium*	Single or multiple nodules; lesions may be grossly or microscopically pigmented, nonpruritic, nonpainful, and cool to the touch.
Mycetoma (chronic subcutaneous infection)	Filamentous bacterial organisms and opportunistic fungi	Single or multiple nodular lesions are present; ulcerated, draining tracts are common; mycetomata discharge tissue grains in contrast to phaeohyphomycosis, which does not.

11.10.1.4

Phaeohyphomycosis

Phaeohyphomycosis (see Table 11-2), sometimes referred to as chromomycosis, is a chronic subcutaneous and occasionally systemic infection caused by pigmented opportunistic fungi, such as *Hormodendrum, Drechslera, Phialophora, Curvularia,* and *Cladosporium* species. Many of the organisms that cause fungal mycetomata can cause phaeohyphomycosis. The distinguishing difference is the lack of tissue grains. Pigmented fungi (dematiaceous fungi) causing this disease are soil and vegetative saprophytes that gain entrance into the body via a wound or abrasion. The body is not able to eliminate the organism, which proliferates in tissues. Immunosuppression may be a predisposing factor.

Lesions appear anywhere on the body and may be single or multiple. Some lesions are pigmented deeply on gross examination or pigmentation is only a microscopic feature. Nodules vary in size and are cool, nonpainful, and nonpruritic. Regional granulomatous dermatitis may be present.

Definitive diagnosis is by histologic examination of an excised nodule. Skin biopsy reveals suppurative to granulomatous dermatitis, with pigmented septate hyphae. Observation of pigmented septate hyphae in the skin indicates opportunistic invasion of the tissue. Fungal culture of tissue samples is necessary to identify the organism. An important note is that growth of these organisms is slow, often requiring weeks, and a reference laboratory is able to culture the organism best.

Excision is the only effective treatment but may be impractical when numerous lesions are present. These organisms are not susceptible to commonly used systemic or topical antifungal agents. However, the author has treated an affected dog successfully with itraconazole. The recommended dose is 3 mg/kg orally every 12 hours for 1 month beyond when the skin appears normal (usually 4 to 12 months). Ketoconazole therapy is not recommended in the horse because of poor bioavailability after oral administration. Even when the solution is acidified with hydrochloric acid, bioavailability is approximately 23%.

11.10.1.5 Sporotrichosis

Sporotrichosis (see Table 11-2) is a fungal diseases caused by *Sporothrix schenckii,* a dimorphic aerobic fungus. The organism is a soil and plant saprophyte that lives in decaying vegetation and gains entrance via traumatic inoculation, especially from puncture wounds.

In horses the cutaneous lymphatic form is most common.[75,76] Early lesions begin as papules that may exude a seropurulent material. Nodules are most common on the thigh or proximal foreleg and chest. If the immune system does not eliminate the organism, hard subcutaneous nodules develop along the lymphatics draining the area. The lymphatics become corded and drain thick, brown to red exudate. Regional lymph nodes are not involved. Occasionally, only a solitary nodule develops. Rarely, the disease becomes systemic.

Definitive diagnosis is by demonstration of the organism via cytologic examination of exudate, culture of tissue or exudate, or skin biopsy. The organism appears as a cigar-shaped yeast in macrophages and 694
neutrophils on Giemsa-stained smears. Histologic examination of tissue reveals nodular to diffuse, suppurative 695
to granulomatous dermatitis. Intraepidermal microabscesses may be visible. The yeast rarely is seen in tissue sections. The number of organisms present in the tissue may vary, and repeat cultures are often necessary.

Sodium iodide (20% solution) has been successful for treating sporotrichosis in horses.[76,77] One administers a loading dose of 20 to 40 mg/kg intravenously for 2 to 5 days, followed by once daily oral therapy (20 to 40 mg/kg) until all clinical lesions are gone. One should administer therapy for at least 3 weeks after lesions disappear. One may give sodium iodide orally via syringe or mixed in sweet feed. One may use topical hot packs of 20% sodium iodide on open wounds. Iodism (see the previous discussion) may develop and may require temporary discontinuation of therapy. Iodines cause abortion and should not be used in pregnant mares.[77]

11.10.1.6 Pythiosis (Phycomycosis, Florida Leeches, Gulf Coast Fungus, and Swamp Cancer)

Pythiosis is caused by an aquatic fungus, *Pythium insidiosum,* common in the Gulf Coast region of the United States, South America, and Australia. This organism is a plant parasite that normally lives on aquatic vegetation or organic debris. Damaged animal tissue is chemotactic for the organism, and the fungus probably gains entrance into animal tissue via wounds in prolonged contact with contaminated water.[78]

The disease commonly affects ventral body areas, including legs, abdomen, and chest (Figure 11-21), and also may involve nasal tissue. Wire cuts, puncture wounds, and ventral midline dermatitis caused by horn flies or *Culicoides* gnats are prime locations. Early signs of fungal invasion include single or multiple minute foci of necrosis that progress rapidly into circular, ulcerative, granulomatous masses with serosanguinous discharges. These masses are intensely pruritic and are often hemorrhagic from self-trauma. Thick, sticky material exudes

from the wound. This discharge often contains “kunkers,” hard, gritty, white to yellow masses that develop in tissue tracts and are considered a hallmark of this disease. These granules branch macroscopically, distinguishing them from granules observed in other skin diseases. Kunkers are composed of fungal hyphae, host exudate, and protein. Lameness, regional lymph node involvement, anemia, and hypoproteinemia are common noncutaneous findings. Anemia and hypoproteinemia develop following blood loss and serum exudation from the mass. Occasionally, the disease may become systemic. The disease is not a known zoonosis, but one should wear protective latex gloves when examining patients.

Pythiosis is difficult to differentiate from habronemiasis, exuberant granulation tissue, bacterial granulomata, and invasive squamous cell carcinoma. Definitive diagnosis requires biopsy, culture, and cytologic examination of exudate and kunkers. One should submit tissue specimens and kunkers for histologic examination. Common findings include pyogranulomatous inflammation with large numbers of eosinophils. If hyphae are visible, they are 2.6 to 6.4 μm in diameter, thick-walled, and irregular.[79] Pythiosis can be grown on fungal culture medium and requires incubation in a vegetable extract agar, and a laboratory familiar with appropriate isolation techniques is able best to culture it. One should collect kunkers, wash them in sterile saline, and embed them deeply in the media. Occasionally, one can diagnose the disease by cytologic inspection of the kunkers. One macerates and minces small kunkers with a scalpel, places the material on hydroxide, and stains it with India ink. Hyphae appear as black, broad, thick-walled branching structures.

Figure 11-21 Horse with bilateral pythiosis on the legs.

Prognosis is poor because treatment is difficult. With early diagnosis, surgical excision may be possible. However, relapses are common, and repeated excision may be required. One should monitor surgical sites closely for evidence of recurrence manifesting as focal areas of edema in the granulation tissue with dark hemorrhagic patches 1 to 5 mm in diameter and serosanguinous discharge.

Systemic antifungal drugs are not effective in treating this disease.[78,80] Systemic amphotericin B (Fungizone 695
IV, Bristol-Myers, Squibb, New Brunswick, New Jersey), combined with topical amphotericin B, may be 696
curative in rare, isolated cases. One administers amphotericin B at a dose of 0.3 mg/kg in 5% dextrose intravenously daily until reaching a total dose of 350 mg. One then administers this dose on alternate days until the horse is cured. In addition, one treats lesions topically with gauze dressing soaked in an amphotericin B and DMSO solution (50 mg amphotericin B, 10 ml sterile water, and 10 ml DMSO). One also may inject amphotericin B into the lesions. Amphotericin B is nephrotoxic, and one should monitor serum creatinine and serum urea nitrogen concentrations and hydration daily. Immunotherapy with phenolized, ultrasonicated proportion from fungal culture is reportedly curative if administered early in the course of the disease. In a small number of cases, vaccine administration resulted in a decrease in the size of the lesion. This therapy may be of benefit if used preoperatively to enhance complete removal of the infected tissue. Unfortunately, the vaccine is not commercially available. Because of the cost of medical or surgical therapy, the risks of treatment, and the high probability that excision is incomplete, owners often elect euthanasia.

11.10.2 PARASITIC CAUSES OF NODULES

A variety of parasites may infest equine skin and cause cutaneous nodules. Some of the more common causes of parasitic nodules in the horse are warbles, cutaneous habronemiasis, and ticks. The latter two have been discussed previously and are not covered in this section.

Warbles occasionally occur in horses and are caused by a larval stage of *Hypoderma bovis* and *Hypoderma lineatum*. In horses, larvae do not usually complete their life cycle.[23] Horses that develop infestation usually are pastured or housed with cattle. Adult flies lay eggs on the hairs, and larvae migrate toward the skin surface and eventually penetrate the skin. Once in the body of the host, the larvae migrate to reach the subcutaneous tissues of the neck and trunk. A swelling develops at the site of the larvae, which eventually becomes perforated as a breathing pore is formed. Nodules are visible most commonly over the withers, and almost all develop a breathing pore. The nodules are often painful. Spontaneous rupture may cause anaphylaxis.[23] Occasionally, aberrant migration causes neurologic signs.

The presence of dorsal swelling or a nodule with a breathing pore is diagnostic. The major differential consideration is collagenolytic granuloma; however, these nodules lack a breathing pore. One treats hypodermiasis by gently enlarging the breathing pore surgically and removing the grub, removing the entire nodule surgically, or allowing the grub to fall out spontaneously. In areas of high occurrence, one may use pour-on insecticides as a preventative. One should treat horses at the same time of year as one treats affected cattle in the area.

Figure 11-22 Urticaria in a horse following administration of penicillin.

11.10.3 URTICARIA

Urticaria is a nodular skin disease of horses characterized by wheals, edema, and often pruritus (Figure 11-22) and most commonly is caused by a type I hypersensitivity reaction. However, urticaria also may result from nonimmunologic factors such as pressure, sunlight, heat, exercise, stress, and drugs. Prerace stress, insect or arthropod bites, bacterial infections, topical parasiticides, systemic drugs (penicillin, phenylbutazone, aspirin, guaifenesin, phenothiazine, quinidine, streptomycin, oxytetracyline), feedstuffs, soaps, leather conditioners, vaccines, snake bites, inhalants, and plants can cause urticaria in horses. In the author's experience, urticaria is a common manifestation of insect hypersensitivity and atopy. Mosquito swarmings commonly result in urticaria.

No age, sex, or breed predilection exists for urticaria. Lesions may develop rapidly or slowly and may be localized or generalized. Lesions are raised, cool to the touch, pit when depressed, and may or may not exude serum or blood. Pruritus varies. In rare instances, lesions may coalesce to form bizarre patterns.

Although urticaria is usually recognizable, its cause can be difficult to identify. The cause of an acute urticaria episode (less than 6 weeks in duration) is much more likely to be identified than the cause of a chronic episode (greater than 6 weeks in duration). A detailed history is critical. Important questions to answer are these:

1. Is this a corticosteroid-responsive urticaria? This suggests an allergic cause such as atopy, insect hypersensitivity, or food allergy.
2. Does the development of the lesion correlate with the administration of systemic drugs or the application of topical medications? This would suggest a drug reaction.

3. Does the application of fly repellent aggravate or alleviate the urticaria? Worsening of the urticaria suggests an allergic contact reaction, whereas alleviation suggests an insect hypersensitivity. 696 697

4. Do the lesions resolve with stabling the horse for 24 to 48 hours or moving to a new environment? If so, this would suggest an environmental cause of the urticaria (molds, inhaled dust from bedding, hay, feedstuffs, feathers from roosting birds).

5. Do lesions improve if the horse is moved off pasture? A positive answer would suggest plant antigen as a cause of the urticaria.

6. Do the lesions improve if the feed is changed? Improvement in clinical signs suggests an ingested allergen or inhaled allergen from feedstuffs.

In cases in which lesions are bizarre in appearance, chronic, or exudative, one should perform a skin biopsy to rule out other causes of nodular disease. Typical histologic findings for urticaria include vasodilation, edema in the dermis, and mild to moderate eosinophilic perivascular inflammation. Complete blood cell counts and serum chemistry profiles are rarely useful. Before beginning expensive or involved diagnostic testing, one should be sure to rule out the possibility of allergic reactions to shampoos, tack cleaners, soaps, etc. All horses with chronic urticaria should undergo a food trial, be moved to a new environment for 5 to 7 days, and undergo a program of insect control before being referred for intradermal skin testing. Intradermal skin testing is the best method for identifying the cause of allergic inhalant dermatitis. Horses should not receive antihistamines or tranquilizers for 1 week or corticosteroids for 4 weeks before intradermal skin testing.

Treatment aims at eliminating the underlying cause, if possible. Hydroxyzine at 200 to 400 mg orally every 12 hours is effective in eliminating urticarial swellings in most horses with acute or chronic urticaria.[34] Transient sedation of several days may occur with this drug. If the horse is nonresponsive to hydroxyzine, prednisone at 1 mg/kg orally, intravenously, or intramuscularly may be beneficial on a short-term (less than 1 week) basis.

11.10.4 IDIOPATHIC INFLAMMATORY CAUSES OF NODULES

11.10.4.1 Eosinophilic Collagenolytic Granuloma (Nodular Collagenolytic Granuloma and Nodular Necrobiosis)

Eosinophilic granulomata are a frequent cause of nodules in the skin of horses. The exact cause of these lesions is unknown, but they may result from a hypersensitivity reaction to insect bites.[51] Lesions, however, have been reported to develop spontaneously or as a result of trauma, suggesting multiple causes.[81] Regional variability in the occurrence of lesions has been noted.

These lesions have no sex, breed, or age predilection but occur most commonly in warmer months. They may be single or multiple and vary greatly in size (0.5 to 10 cm in diameter). The nodules may occur anywhere on the body but are most common on the neck, withers, and dorsal trunk (Figure 11-23). Individual lesions are rounded, well circumscribed, firm, haired, nonpainful, and nonpruritic. Owners of horses with seasonal problems have reported that early lesions are often soft and fluctuant and develop into firm masses over weeks to months. If lesions are under the saddle, the horse may exhibit pain.

Definitive diagnosis is by skin biopsy. Histologic examination of tissue reveals multifocal areas of collagen degeneration surrounded by granulomatous eosinophilic inflammation. Chronic lesions may have significant dystrophic mineralization.[64]

Solitary lesions that do not cause the horse discomfort require no treatment. Problem lesions respond to excision or sublesional injections of triamcinolone acetonide (3 to 5 mg per lesion) or methylprednisolone acetate (5 to 10 mg per lesion).[51] One should inject no more than 20 mg per horse of triamcinolone acetonide because of the danger of laminitis. Horses with multiple lesions may be treated with prednisolone orally at 1 697
mg/kg once daily for 2 to 3 weeks. After lesions have resolved, one should taper the dose of prednisolone and 698
discontinue it over a 5- to 10-day period. Horses with seasonal recurrences in which insect hypersensitivity is involved and documented via intradermal skin testing might benefit from hyposensitization to insects.

Figure 11-23 Horse with multiple eosinophilic granulomata.

11.10.4.2 Equine Axillary Nodular Necrosis

Equine axillary nodular necrosis is a rare idiopathic skin disease of horses. The disease is similar to equine eosinophilic granuloma with collagen degeneration, except that the lesions are localized to the axillary region. [27]

The condition has no known age, sex, or breed predilection. Clinically, single or multiple nodules develop unilaterally in the axillae. The nodules are painless, nonpruritic, haired, well-circumscribed firm masses that vary in size from 0.5 to 4.0 cm or greater in diameter. Multiple lesions tend to be arranged in a row.

Skin biopsy is diagnostic. Biopsy findings include pyogranulomatous, eosinophilic dermatitis with foci of coagulation necrosis. Collagen degeneration is not a common finding.

Treatment is the same as for equine eosinophilic granuloma. Corticosteroids often do not work well, and surgical excision may be necessary. Lesions tend to recur.

11.10.4.3 Equine Unilateral Papular Dermatosis

Equine unilateral papular dermatosis is a rare idiopathic skin disorder of horses.[82] This disease has been seen in several breeds of horses but may be more common in Quarter Horses. Lesions develop in the warm months and are characterized by the unilateral development of multiple (30 to 300) papules and nodules on the trunk (Figure 11-24). Lesions are firm, well circumscribed, nonpainful, and nonpruritic.

Diagnosis is by skin biopsy. Histologic examination of tissue reveals eosinophilic folliculitis and furunculosis. Spontaneous remission may occur; otherwise, prednisolone at 1 mg/kg orally once daily for 2 to 3 weeks is recommended until nodules resolve. Relapses may occur.

11.10.5 NEOPLASTIC CAUSES OF NODULES

Reviewing all of the neoplastic skin conditions of horses is beyond the scope of this chapter. Only the skin tumors of horses that commonly masquerade as nodules are discussed: sarcoids, melanomas, mast cell tumors, and cutaneous lymphoma. One can diagnose some cutaneous neoplasms via exfoliative cytologic examination, but biopsy is the diagnostic test of choice.

11.10.5.1 Sarcoids

Sarcoids are the most common skin neoplasms in horses, accounting for up to one third of all reported tumors in horses. Sarcoids are locally invasive fibroblastic neoplasms.[83–89]

Figure 11-24 Horse with unilateral papular dermatitis. (Courtesy V. Fadock, University of Florida.)

The cause of equine sarcoids is controversial; currently the papovavirus is believed to be involved in development of lesions.[82,84,85] Polymerase chain reaction nucleotide sequences strongly suggest that equine sarcoids are caused by bovine papillomavirus types 1 and 2.[86] Evidence for this includes the clinical observations that lesions often develop in areas of previous trauma, lesions may spread to other areas on the same horse or to other horses, epizootics of equine sarcoid have been described, and autotransmission is possible under experimental conditions. Equine sarcoids were produced in donkeys inoculated experimentally with bovine papillomavirus. A predisposition or susceptibility also appears to play a part in the pathogenesis. Equine sarcoids occur with increased incidence in certain families, and a genetic link with specific major histocompatibility genes (W13 MHC class II antigen) has been demonstrated. Exposure to the virus and genetic susceptibility are probably required for a horse to develop sarcoids.

No sex or breed predilection exists, but greater than 70% of sarcoids develop in horses less than 4 years of age. They may arise spontaneously or at a site of previous trauma and may occur anywhere on the body. Sarcoids have a predilection for the head, ears, and limbs. One third of affected horses have multiple lesions.

Equine sarcoids may be classified as (1) nodular sarcoids, (2) fibroblastic sarcoids (Figure 11-25), (3) verrucous sarcoids, (4) occult or flat sarcoids (Figure 11-26), (5) malevolent sarcoids, and (6) mixed sarcoids. Each type of sarcoid differs clinically in its morphologic appearance and lesion location.[89] Nodular sarcoids are firm, raised, circular nodules that are 5 to 20 mm in diameter and tend to occur on the sheath/groin areas and the eyelids. Fibroblastic sarcoids resemble proud flesh (proliferative, fleshy, and ulcerated lesion) and usually are located around the groin, lower limbs, coronet, and eyelid. Verrucous sarcoids are warty and tend to occur on the face, body, and sheath/groin areas. Occult sarcoids normally appear as flat, circular, hyperkeratotic areas. Occult sarcoids also may appear as one or more small nodules 2 to 5 mm in diameter. The typical lesion location for occult sarcoids is the neck, mouth, eyes, and the medial aspects of the forearm and thigh. Malevolent sarcoids are locally invasive tumors with multiple nodules and a fibroblastic character. Some malevolent sarcoids infiltrate lymphatics and produce a cordlike appearance. Although local lymph nodes are enlarged, no evidence of saroid tumor has been detected within the lymph nodes themselves. Mixed sarcoids consist of sarcoid lesions that appear as a confluence of several different types of sarcoids. Mixed sarcoids contain verrucous/occult type tissue along with fibroblastic/nodular-appearing tissue. This type of sarcoid is more common in long-standing sarcoid lesions or lesions that have experienced minor trauma.

Figure 11-25 Fibroblastic (proliferative) sarcoid.

Figure 11-26 Flat sarcoid.

A skin biopsy is the only way to diagnose an equine sarcoid definitively. Flat or small verrucous forms may become more aggressive after a biopsy. For this reason, some veterinarians are reluctant to take a biopsy sample of these lesions.

The histologic pattern observed with equine sarcoids is a fibroblastic proliferation of cells that form whorls or interlacing bundles.[64] The usual overall orientation of this proliferation is perpendicular to the basement membrane, but exceptions exist. The epidermis, when present, is hyperplastic with characteristic elongated rete ridges, but normal or even atrophic epidermis with significant hyperkeratosis can occur (flat sarcoids).

Several different treatments are described for managing equine sarcoids.[77,87–89] Previous experience suggests that the best treatment for occult and verrucous sarcoids is benign neglect. These types of sarcoids become more aggressive after biopsies have been performed. For fibroblastic sarcoids, the treatment of choice is cryotherapy along with surgical debulking of the tumor. The inclusion of cryotherapy in the treatment regimen is associated with a lower rate of recurrence than with surgery alone (30% to 40% recurrence rate for cryosurgery versus 50% to 64% recurrence rate with traditional surgery). Adverse effects associated with cryosurgery include swelling, hyperemia, hemorrhage, necrosis, and local edema after treatment. Average healing time after cryosurgery is 2.4 months with a range of 1 to 3.5 months. Cryotherapy usually destroys hair follicles in the treated area, and hair regrowth is white. If cortical bone is accidentally freezes, the strength of the bone may decrease by 70%. For lesions around the eyes, recommended treatment is surgical debulking of the tumor followed by injections with bacille Calmette-Guérin (BCG) vaccine or mycobacterial cell wall products. BCG is thought to work by stimulating the immune response of the host to sarcoid cells. 699 700
Mycobacterial antigens are thought to stimulate host lymphocytes and also may stimulate an increase in natural killer cells. One injects the tumor with BCG every 2 to 3 weeks for four treatments. One should

pretreat horses with flunixin meglumine and prednisolone 30 minutes before using BCG vaccine to help reduce the risk of anaphylaxis.

Local radiation therapy using a variety of implants also has been recommended for treating equine sarcoids. This form of treatment is limited to smaller lesions because radiation does not effectively penetrate deeper skin lesions. Relapse rates with this form of therapy vary from 0% to 50%. Some disadvantages of radiation therapy are that the horse must be kept in a radiation-approved area and cost may be significant if the tumor is large.

Cisplatin (Platinol, Bristol Myers Squibb, Princeton, New Jersey) in oil recently has been described as a treatment for horses with sarcoids. This treatment may be up to 100% effective, but requires multiple intralesional injections. Emulsions are made of equal volumes of an aqueous solution of 1 mg cisplatin per milliliter of sesame or almond oil. A dose of 1 mg cisplatin per cubic centimeter of tumor is suggested. With denser sarcoid tumors, injecting cisplatin solution into the tumor may be difficult. Precautions recommended when using cisplatin include wearing gloves and protective clothing. One should place all material in a biohazard container after use and should use a Luer-Lok syringe and extension set to help minimize potential human exposure. Local skin reactions may occur when cisplatin contacts the skin and mucous membranes and require thoroughly washing the area with soap and water. One should not use cisplatin in patients with renal impairment or in breeding animals. Cisplatin is associated with teratogenic and embryotoxic reactions in mice and azoospermia and impaired spermatogenesis in human beings.

Another recently described therapy for equine sarcoids is a topical treatment known as Xxterra (Larson Laboratories, Fort Collins, Colorado).[82] This product contains a caustic substance and an extract of the bloodroot plant. Native Americans have used the bloodroot plant to treat a variety of conditions including warts. This product is thought to work by changing the antigenicity of the sarcoids so that the body recognizes these cells as foreign and produces antibodies against them. One applies Xxterra (while wearing gloves) to the sarcoid lesion and places a bandage over it for 4 days. If the sarcoid has not sloughed by 4 days, one repeats the treatment. If one cannot bandage the area with the sarcoid, then one should apply the product to the sarcoid daily for 4 days or until sloughing occurs. Interestingly, when this product was applied to normal skin nothing happened, but when the product was applied to a sarcoid, the skin became inflamed and the sarcoid sloughed. An exception to this is pigmented skin. If freckles are present on the skin, Xxterra may react with them. Horses with mixed coat colors or Paint Horses have not had problems with this tissue reaction. Application of Xxterra to the ears may result in crinkling of the ears. If this would be an unsatisfactory side effect for the owner, one should use a different treatment. In addition, the veterinarian should warn owners that the treated area may ooze a purulent material and become malodorous. This often occurs before the tissue sloughs.

Other therapies for equine sarcoids that have been used with success are limited in their use because of expense and availability. Examples of such treatments include radiofrequency-induced hyperthermia, carbon dioxide laser surgery, and immunotherapy using a tumor vaccine. With time, these therapeutic options may become more readily available and accepted.

Regardless of the form of therapy chosen, the client needs to be aware that sarcoid tumors tend to recur. Verrucous or flat tumors are best left untreated.

11.10.5.2 Melanomas

Melanomas may arise from dermal melanocytes or melanoblasts and may be benign or malignant. These tumors occur most commonly in older horses of Arabian and Percheron breeding. The relationship between

development of melanomas and gray coat color is recognized widely.[90] Melanomas appear to occur exclusively in horses that are gray or become dapple-gray with age. Up to 80% of gray horses more than 15 years of age are estimated to have melanomas.[91]

Lesions may be solitary or multiple and occur most commonly on the perineum or ventral surface of the tail. Tumors are usually firm and nodular and may be hairless and ulcerated. They are almost always black. Vitiligo may precede the development of the lesions.[90] Three growth patterns have been described: (1) slow growth without metastasis, (2) slow growth with sudden metastasis, and (3) rapid growth and malignancy from the onset.

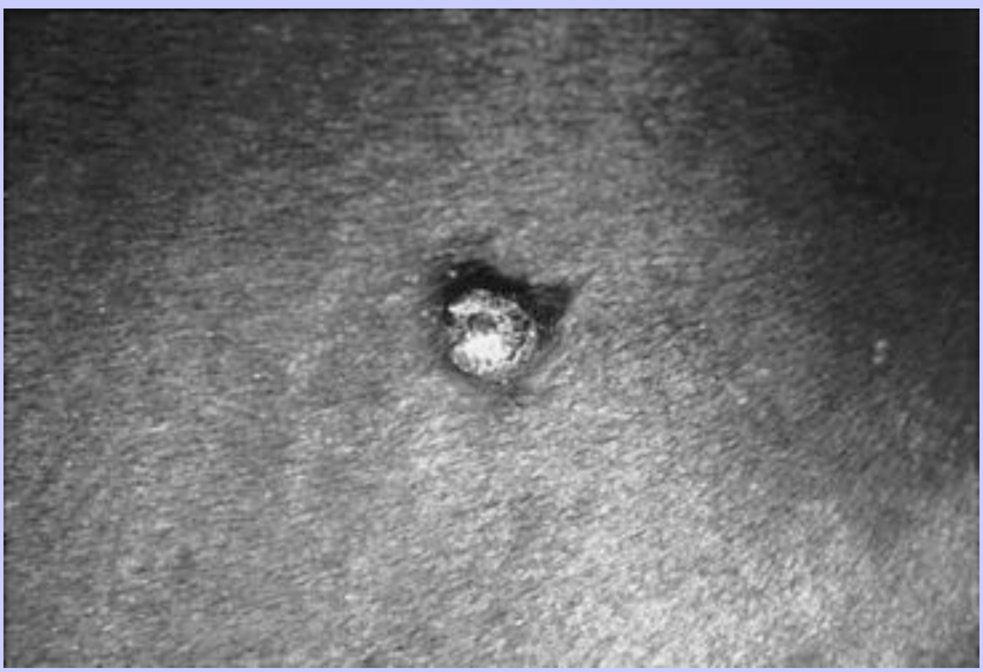
Diagnosis usually is based on clinical signs. Biopsy usually is not needed to confirm the diagnosis unless the lesion is bizarre in appearance.

No treatment is needed unless the melanoma interferes with function. Cimetidine (Tagamet) at 2.5 mg/kg every 8 hours has been reported to cause partial to complete regression of some melanomas. The number and size of the melanomas decrease by 50% to 90% in horses treated for 3 months. After tumors have regressed, daily maintenance therapy at 1.6 mg/kg orally once daily is recommended.[92]

11.10.5.3 Mastocytoma (Mast Cell Tumor)

Mast cell tumors are uncommon in horses. Equine mast cell tumors are 5 times more common in males than 700
females. Arabian Horses appear to be predisposed to developing mast cell tumors. The breed that is least 701
predisposed to developing mast cell tumors is the Thoroughbred.[27,51] No age predilection exists for equine mast cell tumors.

Figure 11-27 Hyperplastic mast cell tumor in a horse.



Two distinct types of mast cell tumors in horses are a hyperplastic type and a neoplastic type. Most equine mast cell tumors are hyperplastic. Clinically, two forms of equine mast cell tumors exist.[51] The most common form is a single cutaneous nodule, often located on the head (Figure 11-27). Nodules range in size from 2 to 20 cm in diameter. The surface of the nodules may be normal, hairless, or ulcerated. Alternatively, mast cell tumors may present as a diffuse swelling on a lower extremity, usually below the carpus or hock. The swelling is firm, and the overlying skin is normal in appearance. Radiographs of the limb commonly show multifocal areas of soft tissue mineralization.[64]

Diagnosis is by biopsy. Diffuse to nodular proliferations of mast cells occur in the dermis. Tumor cells may be well differentiated or more atypical. Tissue eosinophilia, collagen degeneration, and dystrophic mineralization occur commonly in horses.

One may treat solitary tumors by excision, with sublesional triamcinolone acetonide (5 to 10 mg per lesion), or with cryosurgery. Spontaneous remission may occur following incomplete excision or in young horses. Metastasis has not been reported.[27]

11.10.5.4 Cutaneous Lymphoma

Cutaneous lymphoma is rare in horses.[81,93] No age, sex, or breed predilection exists for this tumor. Most affected animals have another concurrent systemic disease, and weight loss, anorexia, anemia, and lymphadenopathy are commonly present. Lesions develop slowly or rapidly. They may occur anywhere on the body, but the trunk and neck are affected most commonly. Individual lesions may be firm or fluctuant, resembling urticaria. The overlying skin is usually normal. Involvement of internal organs may occur.

Definitive diagnosis is by histologic examination of a skin biopsy. One should submit several representative tissue samples for examination. Histologically, lymphosarcoma is characterized by diffuse dermal and subcutaneous infiltration of malignant lymphocytes.[94]

If the owners want to pursue treatment, several options are available. Prednisolone at 1 mg/kg orally once daily for 7 days and then every other day has been used successfully. In addition, PEG-L-asparginase at 10,000 IU/m^2 once weekly may be beneficial, although expensive. Recently, low-dose cyclophosphamide and immunization with vaccinia virus-infected autologous tumor cells was successful in inducing a 19-month remission in a 13-year old horse; however, this therapy is experimental.[95]

11.10.5.5 Pseudolymphoma

Pseudolymphomata are common in horses compared with lymphomata.[51] Differentiating the two diseases is important because the prognosis for pseudolymphoma is excellent.

Pseudolymphomata are nodular to papular lesions that develop in the skin as a result of chronic antigenic stimulation. The most common antigenic stimuli are believed to be insect bites and drug reactions. Lesions occur most commonly in late summer and fall and are usually solitary but may be multiple.[51,93] Pseudolymphomata usually occur on the head and trunk. Individual lesions are firm, raised, and haired.

Definitive diagnosis is by a skin biopsy. Histologic examination of tissue shows dense dermal and subcutaneous infiltration of lymphocytes, histiocytes, plasma cells, and eosinophils. Lymphoid nodules and

eosinophils are present and are an important aid in the differentiation of pseudolymphoma from lymphoma.[64,93]

Pseudolymphomata often spontaneously regress but may be excised surgically or injected sublesionally with triamcinolone acetonide at 3 to 5 mg per lesion, not to exceed a total dose of 15 to 20 mg.[93]

11.10.6 CONGENTIAL CAUSES OF NODULES

11.10.6.1 Dermoid Cysts

A dermoid cyst is a tumor of developmental origin consisting of a wall of fibrous tissue lined with stratified epithelium containing hair follicles, sweat glands, sebaceous glands, nerves, or any combination of these. Dermoid cysts may be congenital or hereditary, have been reported in horses 6 months to 9 years of age, and are common in Thoroughbreds.[96] They occur frequently on the dorsal midline between the rump and withers. The cysts appear as soft, fluctuant swellings with normal overlying skin. Diagnosis is by histologic examination after excision. 701

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11.10.6.2 Atheroma (False Nostril Cyst)

An atheroma is a cyst in the false nostril. These lesions are believed to develop from hair follicle retention cysts or from displaced germ material.[92] Atheromata are present at birth, usually are unilateral, and enlarge with time. They usually are not noticeable until they enlarge to greater than 2 cm. The tumor is firm on palpation but is rarely painful.

Diagnosis is by clinical signs. Treatment is not necessary unless the tumor compromises breathing or the owner is disturbed by the lesion. Surgical excision is the treatment of choice, but one must take care to remove the entire cyst, lest recurrence or chronic drainage occur.

11.11 Disorders Of Pigmentation

Skin and hair color depend on melanin production in the skin. Melanocytes produce pigment at the dermal-epidermal junction and in the outer root sheath of the hair follicle. The ratio is one melanocyte for every 10 to 20 keratinocytes. Melanin pigments have a wide range of colors including black-brown eumelanins, yellow-red pheomelanins, and a range of intermediate pigments. Special tyrosinase-rich organelles called melanosomes, which are found in the cytoplasm of melanocytes, produce melanin pigment. Tyrosine is converted to dopa, which is oxidized to dopaquinone. The copper-containing enzyme tyrosinase catalyzes both reactions. Subsequent intermediate products polymerize, eventually forming melanin. Melanocytes secrete or inject melanosomes into adjacent keratinocytes. Production of melanin is controlled by genetics, hormones, local keratinocytes, and Langerhan's cells. Ultraviolet light, inflammation, by-products of the arachidonic acid cycle, androgens, estrogen, glucocorticoids, and thyroid hormone also may influence pigmentation.[93]

The number, size, type, and distribution of melano-somes determines skin and hair color. Melanosomes are responsible for coat color, photoprotection, free radical scavenging, and heat conservation.

11.11.1 CAUSES OF HYPERPIGMENTATION

Hyperpigmentation is almost always an acquired change. The skin and hair may become hyperpigmented. The usual cause of localized or patchy hyperpigmentation is chronic inflammation or irritation. Macular patches of noninflammatory hyperpigmentation, called lentigo, can occur in horses. Lentigo occurs most frequently at mucocutaneous junctions. The most important differential diagnosis for lentigo is cutaneous melanoma. A skin biopsy of the affected area is the most useful diagnostic test. Generalized hyperpigmentation of the coat or skin has not been reported; however, if observed, the condition may suggest an underlying hormonal disorder.

11.11.2 CAUSES OF HYPOPIGMENTATION

Hypopigmentation is a decrease in normal melanin pigmentation. Depigmentation specifically refers to a loss of preexisting melanin. *Leukoderma* and *leukotrichia* are clinical terms used to describe the loss of color in skin and hair, respectively. Amelanosis is a total lack of melanin.

11.11.2.1 Albinism and Lethal White Foal Disease

Albinism is rare in horses and is transmitted by an autosomal dominant gene. Affected animals have white skin and hair, hypopigmented irides, and photophobia. Lethal white foal disease has two forms. One form is caused by an autosomal dominant gene characterized by early embryonic death in the homozygous state. The second form results from breeding of two overo Paint Horses and is an autosomal recessive disorder. Affected foals are characterized by a white hair coat and congenital ileocolonic aganglionosis.

11.11.2.2 Leukoderma

Leukoderma develops in areas of previous trauma or inflammation and may be temporary or permanent. Affected areas appear normal but depigmented. Leukoderma occurs commonly in horses with onchocerciasis, lupus erythematosus, pressure sores, ventral midline dermatitis, viral skin diseases, freezing, burns, or sun damage (Figure 11-28). Leukoderma can result from the contact of skin with chemicals that inhibit or interfere with melanogenesis, such as phenol and rubber products containing monobenzyl ether or hydroquinone.

Diagnosis usually is by clinical signs, but elliptic biopsy of the junction of the pigmented and nonpigmented area confirms the diagnosis. Leukoderma causes no discomfort to the horse, but may be a source of great agitation to the owner. No treatment is known.

Figure 11-28 Idiopathic leukoderma in a horse.

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Figure 11-29 Horse with Arabian fading syndrome. Depigmented areas on the face are notable.

11.11.2.3 Vitiligo of Arabian Horses (Arabian Fading Syndrome and Pinky Syndrome)

The term *vitiligo* refers to an idiopathic acquired depigmentation. Leukoderma develops in areas with no known history of previous trauma. Vitiligo can occur in any age, sex, or breed of horse but is most common in the Arabian (Figure 11-29).[51] Vitiligo appears to be more common in pregnant or postpartum mares, suggesting a hormonal or stress influence. In addition, the condition may be hereditary.[96]

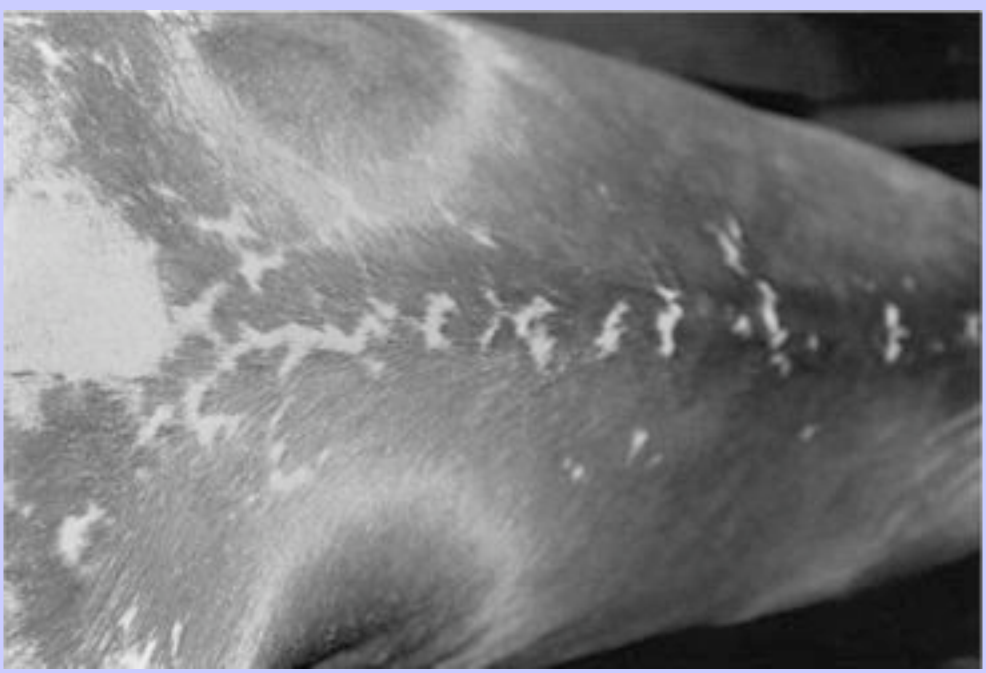
With vitiligo, annular areas of depigmentation develop symmetrically on the muzzle, face, lips and periocular areas. Depigmentation also can occur at the mucocutaneous junctions of the genital and anal area. Areas of depigmentation vary from 1 mm to 2 to 3 cm in size, and spots may become confluent at the mucocutaneous junctions. Depigmentation may wax and wane, and rarely the skin repigments completely within 1 to 2 years.

History, physical examination, and skin biopsy are diagnostic. Skin biopsy shows complete absence of epidermal melanin and lack of an inflammatory infiltrate. Success of treatment with topical glucocorticoids and vitamin-mineral supplement (i.e., cooper) is unpredictable. One should consider the condition untreatable, and owners should not breed affected animals because the condition is heritable and is considered a flaw. More practically, these areas are prone to sunburn and may predispose the horse to squamous cell carcinoma.

11.11.2.4 Leukotrichia

Leukotrichia is an acquired loss of pigment in hairs that occurs most commonly in areas of previous trauma or inflammation. Leukotrichia also may occur at the site of previous injections of epinephrine with or without lidocaine.

Figure 11-30 Reticulated leukotrichia in a Quarter Horse.



Reticulated leukotrichia is an inherited disorder of Quarter Horses, Thoroughbreds, and Standardbreds[51,96,97] (Figure 11-30). The condition has no sex predilection. Affected horses begin to show clinical signs as yearlings. Linear crusts in a netlike to crosshatched design develop over the dorsum from the withers to the tailhead region. Crusts are shed and after the temporary alopecia resolves, new hair growth is white. The underlying skin is normal, and leukotrichia is permanent. Leukotrichia causes no discomfort to the horse or interference with training or use of the animal. No therapy is available, and owners should not breed affected animals because leukotrichia is considered a breed flaw.

Spotted leukotrichia is a similar condition observed most commonly in Arabian Horses.[51,96,97] Permanent spots of leukotrichia develop over the hindquarters and trunk. Occasionally, lesions resolve spontaneously. No treatment is known, and owners should not breed affected animals because the condition is a breed flaw.

Hyperesthetic leukotrichia is a rare disease reported only in California.[98] Single or multiple crusts that are intensely painful characterize this disorder. Lesions develop on the dorsal midline from the withers to the tailhead. Within a few weeks, white hairs develop in the areas of crusting. The intense pain and crusting may last weeks to months. Eventually, lesions subside, but leukotrichia is permanent. Skin biopsy shows significant subepidermal and intraepidermal edema. No treatment is known, and large doses of glucocorticoids have not been of value.

11.11.2.5 Discoid Lupus Erythematosus

Discoid lupus erythematosus is a rare skin disease in horses that is believed to be a variant of systemic lupus 703
erythematosus. Affected horses develop areas of patchy erythema, crusting alopecia, and scaling on the face, 704
ears, and neck.[36] Leukoderma and leukotrichia may be present.

The diagnostic test of choice is a skin biopsy. The skin biopsy reveals an interface dermatitis (hydropic or lichenoid or both). Focal hydropic degeneration of basal epidermal cells, pigmentary incontinence (pigment granules in the dermis or in dermal macrophages), and focal thickening of the basement membrane are important histologic features. Direct immunofluorescence testing may show a linear deposition of immunoglobulins at the basement membrane zone. This test is not reliable and is not recommended as a substitute for a routine histopathologic study.

One treats discoid lupus erythematosus on an individual basis. Ideally, the owner should stable the horse during the hours of strong sunlight because sunlight aggravates the disease.[36] Topical sunscreens and topical glucocorticoids, for example, 0.1% betamethasone 17-valerate may be beneficial. Severe or refractory cases of discoid lupus erythematosus may require immunosuppressive doses of oral prednisolone (see the section on pemphigus foliaceus).

11.12 Disorders Of Collagen Formation

Hyperelastosis cutis is a defect in collagen that occurs primarily in cutting horse strains of Quarter Horses and horses of Quarter Horse lineage (Paints and Appaloosas).[99–102] A similar condition has been described in an Arabian cross-breed. Male and female horses are affected equally. Hyperelastosis cutis is thought to be an autosomal recessive trait. Foals are born with hyperelastosis cutis but the condition may take months to years before becoming clinically apparent to the owner. Clinical signs include loose skin that is hyperextensible, hyperfragile, and easily torn and that exhibits impaired healing (Figures 11-31 and 11-32). Lesions may be solitary

or multiple, and the most common lesion location is the dorsal body surface. Affected horses rarely if ever exhibit hypermobility of the joints. Occassionally, owners complain that the horse develops hematomas and subcutaneous abscesses.

Figure 11-31 Horse with severe hyperelastosis cutis.

Figure 11-32 Horse with mild hyperelastosis cutis.

Diagnosis is based on clinical signs, physical examination, and skin biopies. The most common histologic findings are an absent or greatly diminished deep dermal collagen layer. Other changes that may occur are thinning of the dermis and thinning, fragmented, and disordered collagen fibers. Zonal dermal seperation is a distinctive histopathologic lesion associated with hyperelastosis cutis in the Quarter Horse.[102]

No treatment for hyperelastosis cutis is available. Owners should attempt to minimize trauma to affected horses and promptly treat secondary infections. Most affected horses are euthanized because their fragile skin impairs their usefulness. Owners should remove sires and dams of affected horses from the breeding stock.

11.13 Hair Abnormalities

Horses have simple hair follicles, which are accompanied by a sebaceous and sweat gland and an arrector pili muscle. Photoperiods and ambient temperature predominantly govern the hair cycle. Nutrition, hormone, genetics, and general well-being also may influence hair growth. Hair replacement in the horse occurs in a random pattern (mosaic) with the exception of the coarse permanent hair of the mane, tail, and fetlock.

11.13.1 CHEMICAL AND PLANT CAUSES OF COAT ABNORMALITIES

11.13.1.1 Selenium Toxicosis

Selenium is a trace mineral found in high concentrations in the Great Plains and Rocky Mountain areas of the
United States. Toxicosis occurs when high levels of selenium in the soil are concentrated in selenium- 704
concentrating plants. Interestingly, many of these plants grow selectively in selenium-rich soil and can contain 705
up to several thousand parts per million of selenium. Selenium toxicosis also may occur when water contains 0.1 to 0.2 ppm selenium or feed contains greater than 5 ppm.[60] The exact mechanism of action is unknown, but selenium is believed to interfere with oxidative enzyme systems that possess sulfur-containing amino acids by displacing the sulfur groups. Selenium is substituted for sulfur in sulfur-containing amino acids. Keratinization of the hoof and hair especially are affected by the defective amino acids.

Selenium toxicosis occurs in any age, breed, or sex of animal but is most common in horses on pasture.[103] Early lesions begin as lameness and soreness of the coronary band and feet. The hindfeet usually are affected first. The coronary band and hoof develop cracks and separation and eventually the hoof may slough. The hair coat becomes rough, and generalized epilation of the long hairs of the mane and tail occurs. Generalized alopecia may be visible.

Definitive diagnosis is by history, clinical signs, and tissue analysis for selenium levels. Selenium in excess of 1 to 4 ppm from blood, 11 to 45 ppm for hair, and 8 to 20 ppm for hoof indicates chronic toxicity. Identifying and removing the exogenous source of selenium is critical. Treatment of individual animals is frustrating because of the prolonged recovery period. Chronically affected animals have a 50% chance of recovery. A high-protein diet rich in sulfa-containing amino acids and 2 to 3 mg orally per day of DL-methionine may be beneficial. Naphthalene orally at 4 to 5 g per adult horse also may be of benefit. The horse should receive one cycle of treatment: 5 days of naphthalene, stop for 5 days, and 5 days of naphthalene treatment. Inorganic arsenic at 5 ppm in drinking water or salt with 35 to 40 ppm arsenic may protect the sulfur-containing structures of the body.[103]

11.13.1.2

Arsenic Poisoning

Arsenic commonly is found in some parasiticidal dips, weed and orchard sprays, insect baits, and arsenic-containing medications. Arsenic is a general tissue poison, and 1 to 5 mg/kg may be toxic to horses.[104] Signs of arsenic toxicosis include gastrointestinal disturbances, weight loss, and a dry, dull, easily epilated hair coat. Some horse may develop a long shaggy coat with severe seborrhea.

Diagnosis is based on history of ingestion of arsenic or an arsenic-containing compound and clinical signs. Definitive diagnosis may be difficult because the test of choice is the measurement of arsenic levels in kidney or liver (greater than 10 ppm).[60,104]

Treatment of arsenic toxicity is complicated by the peracute nature of the disease. One should wash off topically absorbable arsenic and should empty the digestive tract of the horse with the aid of laxatives. Orally administered sodium thiosulfate at 50 to 75 g every 6 to 8 hours binds unabsorbed arsenic. Intraveously administered sodium thiosulfate at 25 to 30 g of a 20% solution in distilled water may counterabsorb arsenic. Dimercaprol (BAL) is the classic antidote for arsenic toxicity and is effective if administered within hours of ingestion. Initial treatment is 5 mg/kg intramuscularly and then 3 mg/kg every 6 hours for the first day, followed by a dosage of 1 mg/kg intramuscularly every 6 hours for the next 48 hours. Intramuscular injections are painful.[105] One must identify the source of arsenic and eliminate it.

11.13.1.3

Mercury Poisoning

Mercury poisoning occurs when the horse ingests feed contaminated with fungicide or absorbs or ingests mercury from counterirritants.[60] Affected horses may have gastrointestinal disorders, depression, anorexia, weight loss, and emaciation. Generalized alopecia with subsequent loss of mane and tail hairs is common. Diagnosis is complicated because the sample of choice is kidney tissue, where mercury is concentrated. Orally administered potassium iodide at 4 g per day for 14 days may be beneficial in treating affected horses.[92]

11.13.1.4

Leucenosis Poisoning

Leucenosis is a toxicosis resulting from the ingestion of plants of the genus *Leucaena*.[60,105] In the United States, these plants are found in Hawaii. This plant contains a toxic amino acid (mimosine) that is a potent depilatory agent.

Horses with this toxicosis show a gradual loss of the mane, tail, and fetlock hairs. Severely intoxicated animals may undergo complete hair loss 7 to 14 days after exposure. Laminitis and hoof dystrophies have been reported.[60,105]

Diagnosis is based on history and clinical signs. One treats affected horses symptomatically after preventing further exposure to the plants. Ferrous sulfate 1% added to the feed may benefit affected horses and limit further hoof damage.[94]

11.13.2 HAIR FOLLICLE ABNORMALITIES

11.13.2.1 Mane and Tail Dystrophy

Mane and tail dystrophy may be congenital or acquired. Whether the condition is hereditary is unknown. Affected horses (juvenile or adult) have short, brittle, dull hairs on the mane and tail. No treatment is known, but the condition does not affect the health or function of the horse.

11.13.2.2 Black and White Follicle Dystrophy

Affected horses (juvenile and adult) have abnormal hairs growing in patches of white- or black-haired areas. These hairs are short, brittle, and dull. Affected areas may be hypotrichotic. No treatment is available. 705

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11.13.2.3 Curly Coat

Curly coat is a hereditary condition of Percherons, Missouri Fox Trotters, and Bashkin Horse breeds that is inherited as an autosomal recessive trait. Clinically affected horses have unusually curly coats.

11.13.3 ENDOCRINE CAUSES OF HAIR ABNORMALITIES

11.13.3.1 Hyperadrenocorticism

Equine hyperadrenocorticism most commonly results from an adenoma or dysfunction of the pars intermedia of the hypophysis.[89,106] The abnormal pars intermedia secretes adrenocorticotropic hormone, α- and β-melanocyte–stimulating hormone, corticotropin-like intermediate lobe peptides, and β-endorphins.

Hyperadrenocorticism is most common in older horses (12 to 15 years old or older)[106–108] but is reported occasionally in horses 7 to 10 years of age. The first clinical sign noted by an owner is often a rapid regrowth of long hair after the shedding season or failure to shed the coat (Figure 11-33). The coat becomes shaggy, and body hairs can reach lengths of 10 to 12 cm. Interestingly, mane, tail, and fetlock hairs are unaffected. Secondary seborrhea may develop. Affected horses may be predisposed to dermatophytosis, dermatophilosis, or secondary bacterial pyoderma. Nondermatologic signs include polydipsia, polyuria, muscle wasting, lethargy, weight loss, pendulous abdomen, laminitis, and flaccid muscles. Neurologic disorders and blindness may develop.

Diagnosis is based on clinical signs and laboratory test results.[108–110] Complete blood counts often reveal neutrophilia, lymphopenia, and eosinopenia. Urinalysis may reveal a low specific gravity and occasionally glucosuria. A serum chemistry panel may reveal any combination of the following: hyperglycemia, hypercholesterolemia, and lipemia.[108] Definitive diagnosis of hyperadrenocorticism in horses caused by pituitary pars intermedia dysfunction is discussed elsewhere in this text.

Figure 11-33 Horse with pituitary-dependent hyperadrenocorticism. The long hair coat did not shed in the spring.

Treatment options for horses with hyperadrenocorticism include bromocriptine mesylate, cyproheptadine hydrochloride, and pergolide.[111] Unlike small animals, horses are not responsive to mitotane treatment. These treatment options are discussed elsewhere in this text.

11.13.3.2 Hypothyroidism

Hypothyroidism in the horse is rare, and much of the information on this endocrinopathy is anecdotal.[112–114] Horses with naturally occurring hypothyroidism reportedly have a wide range of clinical signs including loss of mane and tail hairs, muscle weakness, hyperpigmentaion, seborrhea, lethargy, poor performance, infertility, agalactia, myxedema, and dry, dull, brittle hair. Diagnosis and treatment of equine hypothyroidism is discussed elsewhere in this text.

11.13.4 MISCELLANEOUS DISORDERS AFFECTING THE HAIR COAT

11.13.4.1 Anagen Defluxion and Telogen Defluxion

Anagen defluxion refers to a condition in which a disease or drug interferes with anagen hair growth, resulting in abnormal hairs or hair shaft abnormalities or both. Hair loss occurs within days of the insult or drug. The condition occurs most commonly in horses with high fevers, systemic illnesses, or malnutrition[115] (Figure 11-34). Typically, the mane and tail are not affected.

Telogen defluxion refers to a condition in which a stressful situation causes the sudden cessation of anagen hair growth and the sudden synchrony of many hair follicles in telogen hair cycle stage.[115] Two to 3 months later a sudden shedding and a wave of new hair growth occur.

These conditions cannot be distinguished clinically. Diagnosis is based on examining the hair shaft of easily epilated hairs. Anagen defluxion is characterized by dysplastic hairs, the hair shaft may be weak or narrow, and the root end contains a root sheath. Telogen defluxion is characterized by uniform hairs with no shaft abnormalities and a nonpigmented root end lacking a root sheath. Both conditions resolve spontaneously when the animal recovers from the predisposing condition.

11.13.4.2 Trichorrhexis Nodosa

Trichorrhexis nodosa is an acquired hair shaft disorder of horses caused by physical or chemical trauma.[97] Overzealous grooming, shampoos, pesticides, alcohol, or solvents are the most common causes. The lesions are visible without magnification and appear as small white to gray nodules on the hair shaft. The hair shaft fractures and breaks easily at these sites. Microscopically, affected hairs have the appearance of two brooms shoved together. Therapy consists of identifying and eliminating the trauma. 706

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Figure 11-34 Foal with telogen effluvium after illness.

11.13.4.3 Piedra

Piedra is a rare superficial fungal disease of horses that causes nodules on the hair shaft. Black or white filamentous nodules consisting of tightly packed hyphae most commonly are visible on the mane and tail. Affected hairs break at the site of infection. Definitive diagnosis is by fungal culture and microscopic identification of the agent, *Piedraia* sp. or *Trichosporon beigelii*. Treatment of affected horses includes clipping the hair and applying topical fungicides.

11.13.4.4 Abnormal Shedding

Photoperiod and temperature control shedding. Most horses have a spring and fall shed. Abnormal shedding in horses may result in large areas of alopecia.[97] Large areas of hair loss may develop on the face, shoulders, or even over the entire body. The pathogenesis is unknown. Skin biopsy is recommended to eliminate infectious or parasitic causes of hair loss. Skin biopsy is normal and shows normal hair follicles in various stages of development. Horses spontaneously recover in up to 3 months.[98]

11.13.4.5 Alopecia Areata

Alopecia areata is a rare idiopathic skin disease of horses characterized by focal areas of alopecia.[96] Lesions may be single or multiple, and the underlying skin is otherwise normal. If and when hair regrows, it may be a different color. Diagnosis is by biopsy. Classically, in early lesions, skin biopsy shows an accumulation of lymphoid cells around the proximal end of the anagen hair follicles.[97] This change is diagnostic but may require numerous biopsies before being observed. Prognosis is uncertain. In human beings, alopecia areata may resolve spontaneously in 3 to 5 years. Whether spontaneous resolution occurs in horses is unknown.

11.14 Mucocutaneous Vesicular Diseases

A vesicle is an elevated, fluctuant, fluid-filled lesion of less than 1 cm in size. A vesicular eruption suggests a viral, autoimmune, or irritant cause. In horses, vesicles are transient because the epidermis is thin and these lesions rupture easily. Erosions or ulcerations are often the first clue that a vesicle-producing disease is present. Additionally, vesicular diseases may masquerade as pustular eruptions. Vesicles in horses rapidly fill with inflammatory cells, making them indistinguishable from frank pustules. In rare instances, horses may develop bullae (>1 cm in diameter) or large vesicles. Occasionally, bullae fill with blood and are red-purple.

11.14.1 VIRAL CAUSES OF VESICULAR ERUPTIONS

11.14.1.1 Equine Herpes Coital Exanthema

Coital exanthema is a rare contagious venereal disease of horses cause by equine herpesvirus type 3, which occurs worldwide. The disease is transmitted via coitus, insects, fomites, and inhalation. Early lesions begin as papules that rapidly progress to vesicles or bullae or pustules on the vulva or perineum of mares and the penis and prepuce of stallions.[116] Vesicles and bullae also may be present in the mouth or nostril or on the lips. Affected areas commonly become eroded and ulcerative. Lesions are more often pruritic rather than painful.

Depigmentation often occurs in areas where lesions have developed and healed. Stress may precipitate recurrences.

Diagnosis is based on the appearance of lesions on the vulva, perineum, penis, or prepuce; skin biopsy; and virus isolation. Histologic findings include hyperplastic superficial and deep perivascular dermatitis with ballooning degeneration and eosinophilic intranuclear inclusion bodies.

Treatment is symptomatic, but corticosteroids are contraindicated. Owners should isolate affected horses and remove broodmares and stallions from the breeding program for a minimum of 4 weeks. The disease has no known effect on fertility.

11.14.1.2 Vesicular Stomatitis (Sore Nose and Sore Mouth)

Vesicular stomatitis is caused by a rhabdovirus with two major serotypes: New Jersey and Indiana.[117] The 707
disease affects horses, cattle, and swine and is enzootic in North, Central, and South America. In North 708
America, the disease is reportable.

The disease has a seasonal occurrence in summer and fall and is believed to be transmitted to horses by insect bites. Within a group of horses, however, the disease may be transmitted via direct contact. The incubation period is short (24 to 72 hours), and lesions may last for only 3 to 4 days. Infected horses rapidly develop vesicles up to 2 cm in diameter in the mouth and on the lips. These lesions rupture, leaving large painful erosions and ulcers. Affected animals salivate profusely, and fever and anorexia are common. Rarely, lesions develop on the hooves, prepuce, and teats.

Diagnosis confirmation is by serologic examination. Histologic findings are nonspecific and include a hyperplastic epidermis, inter- and intracellular edema of the epidermis, reticular degeneration, spongiotic microvesicles, and focal necrosis. Superficial and deep perivascular dermatitis is present.

Owners should feed affected horses a soft gruel until oral lesions heal, usually within a few days. Permanent depigmentation may occur in areas of former ulceration. Infection results in immunity for up to 6 months.

11.14.1.3 Horsepox

Horsepox is uncommon and has been reported only in Europe. Horsepox is a benign disease, caused by an unclassified poxvirus.

The virus gains access to the body by the respiratory tract or the skin.[118] Systemic spread occurs by the lymphatics. Poxvirus inoculated into the skin may multiply locally, directly enter the blood, and create a primary viremia. The virus causes degenerative changes in the epithelium as a result of virus replication and results in development of vesicular lesions. Poxviruses also cause epithelial hyperplasia by stimulating host-cell DNA synthesis before the onset of cytoplasmic virus-related DNA replication. Ischemia and necrosis occur in the dermis and subcutaneous areas as a result of vascular damage.

Clinical lesions of equine poxvirus infections develop as follows. An erythematous maculopapular eruption develops, followed by the development of vesicles. This stage is transient and may not be observed. Vesicles develop into umbilicated pustules with a depressed center and a raised erythematous border. When the pustule ruptures, a crust develops and the lesion heals, often with scarring.

Horsepox may affect horses of any age. The disease is transmitted by direct contact with an infected host or with contaminated grooming tools or tack. Lesions of horsepox are typically vesicles and umbilicated pustules with crusts. Three clinical presentations (oral, leg, and vulvar) have been described.[64] Oral horsepox (buccal horse pox, contagious pustular stomatitis) is characterized by the development of lesions on the inner lips and buccal mucosa. In severe forms, pox lesions may develop in the pharynx, larynx, nostrils, or all of these sites. Leg pox usually develops on the pasterns and fetlocks and often is confused with “scratches” or “greasy heel.” Pain and lameness are common. Vulvar pox (genital horsepox) is uncommon, occurring predominantly in severely affected animals. One may observe pyrexia and anorexia early in the course of the three forms of horsepox.

Diagnosis is based on history and clinical signs, and confirmation is by histologic examination. Intracytoplasmic inclusion bodies demonstrated by routine histopathologic examination of tissue or electron microscopy are diagnostic. Other findings include ballooning degeneration of the epidermis (stratum spinosum), reticular degeneration, acantholysis (loss of cohesion of cell in the granular area), intraepidermal microvesicles, a superficial and deep perivascular dermatitis, and intraepidermal microabscesses and pustules.

Treatment is symptomatic. Most horses recover in 2 to 4 weeks. Occasionally, the disease is fatal in severely affected young horses. Recovery produces lifelong immunity. This disease affects human beings and cattle.

11.14.2 AUTOIMMUNE DISEASES CAUSING VESICULAR LESIONS

Bullous pemphigoid is a rare autoimmune skin disease of horses characterized by the development of vesicles and ulceration of the oral mucosa and occasionally the skin. Bullous pemphigoid is caused by production of an autoantibody (pemphigoid antibody) against a component in the basement membrane of the skin and mucous membranes.[56] The pemphigoid antibody binds to the basement membrane zone and complement fixation occurs, resulting in production of inflammatory mediators chemotactic for neutrophils and eosinophils. These cells release proteolytic enzymes that disrupt the dermal-epidermal cohesion, separation occurs, and a vesicle forms.

In horses, vesicles and bullae develop at the mucocutaneous junction in the axillary or inguinal regions or in the oral cavity.[96] Skin lesions occur in the axilla and groin. Vesicles and bullae rupture easily and often are crusted and secondarily infected with bacteria. Pain, pruritus, anorexia, fever, and salivation are common.

Definitive diagnosis is by eliminating other causes of vesicular lesions from the differential list and by skin biopsy. Biopsy of a vesicle or intact bullae is essential; biopsy of crusted or ulcerative lesions will be nondiagnostic. Bullous pemphigoid is characterized by subepidermal vacuolar alterations and subepidermal clefts and vesicles.[36] Neutrophilic and eosinophilic infiltration of the superficial epidermis is common. If one 708
performs direct immunofluorescence testing and the results are positive, one observes a linear deposition of 709
immunoglobulin at the basement membrane zone. Direct immunofluorescence testing is not recommended.

Few reports exists of treatment of bullous pemphigoid in horses.[36,57,96] Treatments have included an initial large dose of prednisolone (1 mg/kg orally every 12 hours) or dexamethasone (0.2 mg/kg orally every 24 hours) to induce remission. After the arrest of new lesion development and healing, one institutes maintenance therapy with alternate-day administration of glucocorticoids at the smallest effective dose. Therapy must be individualized and most likely will be lifelong. The use of adjuvant therapy (e.g., gold salts or azathioprine) has not been reported in horses with bullous pemphigoid but may be useful.

11.14.3 IRRITANT AND TOXIC CAUSES OF MUCOCUTANEOUS VESICLES

Irritant reactions, toxic chemicals, or allergic reaction to drugs may result in oral mucocutaneous vesicles. One should suspect these causes when lesions develop acutely in the absence of signs of systemic illness, in single animals within a herd, or in horses receiving medications or counterirritants. Mercurial compounds (commonly used in counterirritants) also can cause generalized hair loss, lameness, and emaciation in chronically intoxicated animals. Creosol and cantharidin beetles are common causes of oral ulcers. Foreign bodies such as grass awns may cause mucocutaneous ulceration and vesicles.

11.14.4 VASCULITIS

Oral ulcers may develop in horses with vasculitis (see the section Vasculitis and Purpura Hemorrhagica).

11.15 Necrotizing Skin Disorders

Necrotizing skin diseases develop from a wide range of causes. The unifying characteristic is that each of these diseases may cause local or widespread full-thickness death of the skin, sloughing of tissue, ulceration, and exudation. Definitive diagnosis of the underlying cause may be difficult in some cases.

11.15.1 DECUBITAL ULCERS (PRESSURE SORES, SETFASTS, SADDLE GALLS, AND SADDLE SORES)

Decubital ulcers or sores are caused by prolonged application of pressure to an area. Thin and emaciated animals are at greatest risk for development of decubital ulcers.

Pressure sores may develop from poorly fitting tack on the back, neck, or girth of the horse; from recumbency during surgery, even while on suitable bedding; and from casts, leg wraps, or elastic bandages that have become wet or are unevenly wrapped, too tight, or ill-fitting. The amount of pressure required to create a decubital ulcer is not great. Tack and saddles may cause lesions over bony prominences or other areas subjected to extended pressure. Capillary circulation is stopped or severely limited, resulting in tissue anoxia and retention of metabolic wastes with tissue damage and death. Most lesions begin as small areas of tissue necrosis and progress to areas of ulceration. Secondary infections are common.[60]

Clinically, early lesions begin with hair loss, swelling, and erythema. A red-purple area of tissue discoloration may be visible. Within a few days, oozing, necrosis, and ulceration become visible. In severe cases, the skin loses its elasticity, subcutaneous tissues slough, and the skin may harden. Strands of living tissue may be attached to necrotic tissue. Lesions are often malodorous. Healed lesions tend to scar, and leukotrichia or leukoderma are common sequela.[92]

Diagnosis is based on history and clinical examination. Areas of skin that have lost sensation or have a parchmentlike feel should be suspect.

Treatment of these lesions is difficult. Because of significant capillary and venous congestion, systemic antibiotics are often not effective.[60,92] Wounds are managed best topically. One should clean the wounds daily with copious amounts of water to remove tissue debris and stimulate circulation and should use topical antibiotic

creams or ointments (i.e., iodine and chlorhexidine) to prevent secondary infection and to prevent the wound from becoming desiccated. Reepithelialization and wound healing greatly improve with the use of surgical dressings, but bandaging some lesions may be difficult. Surgical debridement and skin grafting may be indicated in some cases.

11.15.2 GANGRENE

Gangrene is a clinical term used to describe wet or dry tissue necrosis. Gangrene may result from external pressure, severe edema, burns, frostbite, snake bites, vasculitis, ergotism, fescue toxicosis, or bacterial or viral infections.[60] The characteristic lesion results from occlusion of the venous or arterial blood supply.

Dry gangrene occurs when the arterial blood supply to an area is occluded but the venous or lymphatic drainage is intact. Wet or moist gangrene is caused by occlusion or impairment of lymphatic and venous drainage plus putrefaction caused by a bacterial infection. Lesions of dry gangrene are dry, leathery, discolored, sunken, and cold to the touch. The skin may take a long time to slough. Lesions of wet gangrene are swollen, discolored, and malodorous.

Diagnosis is most often made by clinical examination, and confirmation is by biopsy. One must identify and treat the primary cause of gangrene. 709

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11.15.3 THERMAL INJURIES

11.15.3.1 Burns

Excessive heat (burns) can occur from barn fires, brush fires, accidental spillage of hot solutions, lightning, electrocution, rope burns, counterirritants, or radiation. Barn fires cause most burns in horses.

Large thermal injuries in horses are difficult to manage.[119–122] The large surface area of the burn dramatically increases the potential for the loss of fluids, electrolytes, and caloric losses. Burns on 50% or more of the body are usually fatal, but this depends on the depth of the burn.[119,122] Massive wound contamination is almost impossible to prevent because of the impossibility of maintaining a sterile environment.[118,121] Horses require long-term restraint to prevent continued trauma; wounds are often pruritic and self-mutilation is common.[120] Burned horses frequently are disfigured, preventing them from returning to full function.

Burns are classified by the depth of the injury.[119–122] First-degree burns involve only the most superficial layers of the epidermis. These burns are characterized by erythema, edema, desquamation of superficial layers of the skin, and pain. The germinal layer of the epidermis is spared, and these burns heal without complication.[121] Second-degree burns involve the entire epidermis and may be superficial or deep. Superficial second-degree burns involve the stratum corneum, stratum granulosum, and a few cells of the basal layer. Deep second-degree burns involve all layers of the epidermis. Clinically, these burns are characterized by erythema, edema at the epidermal-dermal junction, necrosis of the epidermis, accumulation of white blood cells at the basal layer of the burn, eschar (slough produced by a thermal burn) formation, and minimal pain. The only germinal cells spared are those within the ducts of sweat glands and hair follicles. Second-degree burns heal well with good wound care.[121] Third-degree burns are characterized by loss of epidermal and dermal components. Fluid loss occurs, along with significant cellular response at the margins and deeper tissue, eschar formation, lack of pain, shock, wound infection, and possible bacteremia and septicemia.

Healing is by contraction and epithelization and occurs only from the wound margins. Infection and problems with wound healing frequently complicate these burns.[121] Fourth-degree burns involve all the skin and underlying muscle, bone, and ligaments.

Burns cause local and systemic effects.[120–123] Local tissue damage results from massive protein coagulation and cellular death. In the immediate area of the burn, arteries and venules constrict and capillary beds dilate. Capillary wall permeability increases in response to vasoactive amines released as a result of tissue damage and inflammation.[122] These vascular responses result in fluid, protein, and inflammatory cells accumulating in the wound. Vascular sludging, thrombosis, and dermal ischemia occur, resulting in further tissue damage. The tissue ischemia continues for 24 to 48 hours after the injury and is believed to be caused by the local release of thromboxane A_2.[121,122] Lipid layers in the skin are destroyed, and a fourfold increase in the loss of fluid occurs. Fluid losses result in increased heat loss from evaporation and an increased metabolic rate. The full extent and depth of the burn may not be evident for several days. Neutrophil function and chemotaxis greatly decrease, predisposing the wound to local infection, bacteremia, and septicemia. The following microorganisms frequently colonize burns: *Pseudomonas aeruginosa, Staphylococcus, Escherichia coli, Klebsiella* spp., nonhemolytic *Streptococcus* spp., *Proteus* spp., *Clostridium* spp., and *Candida* spp.[118–121] Systemic effects are life-threatening and include hypovolemia, fluid and electrolyte losses, protein loss, pulmonary edema, anemia, increased basal metabolic rate, increased caloric needs, and depressed cell-mediated and humoral immune responses. Hypovolemia exacerbates a decrease in cardiac output following a circulating myocardial depressant factor.[123]

One should treat first-degree burns and superficial second-degree burns immediately with ice or cold water to prevent further tissue necrosis.[118,121] One should apply aloe vera cream or a water-soluble antibacterial cream to the wound to prevent infection. In addition, one should administer nonsteroidal analgesics to alleviate pain and to help reduce dermal ischemia. Aspirin at 10 to 20 mg/kg orally once or twice daily decreases thromboxane production, may halt further dermal ischemia, and may be the initial drug of choice.[121]

One manages deep second-degree burns initially as described previously; however, these burns tend to form blisters that should be left intact as long as possible because the vesicular fluid is a good medium for reepithelialization.[119–121] One should trim ruptured blisters, clean the area with copious amounts of water, and cover the wound with an antibacterial dressing or xenograft or allow an eschar to form.[119–122] The bandage should allow drainage and should be changed at least daily.

One may manage full-thickness burns (third and fourth degree) by occlusive dressing (closed technique), eschar production (exposure technique), continuous wet dressings (semiopen technique), or excision and grafting techniques.[122] The most practical therapy for large burns in horses is the semiopen method, leaving the eschar intact with continuous application of moist bandages and antibacterial agents. The moist dressings help prevent heat and moisture loss from the eschar, provide protection of the eschar, and help prevent bacterial invasion in the wound.

Routine use of systemic antibiotics is not recommended in burn patients. Short-term systemic antibiotics may 710
be useful in the initial 3 to 5 days after the burn to minimize bacterial colonization of burns and systemic 711
sepsis.[122] In the absence of sepsis, systemic antibiotics are contraindicated. Extensive use of antibiotics may cause altered microbial flora in the gut and in mucous membranes, which may predispose the patient to infections from antibiotic-resistant gram-negative bacteria or from fungi.[124] Topical medications should be water-based, should be applied and removed easily, should not interfere with wound healing, and should be

excreted or metabolized readily. Silver sulfadiazine and aloe vera are effective. Silver sulfadiazine is effective against gram-negative bacteria, causes no discomfort to the horse, penetrates eschar, and has a 24-hour duration of action Aloe vera is reported to relieve pain, decrease inflammation, stimulate cell growth, and kill bacteria and fungi. Aloe vera is most useful in the early treatment of wounds. Several excellent references for further information on long-term medical and surgical management of horses with large thermal burns are available.[119,121,122]

11.15.3.2 Frostbite

Frostbite occurs when tissue is exposed to extreme cold. Sick, debilitated, and neonatal animals are at increased risk. Cold temperatures inhibit cell metabolism and cause tissue dehydration, cell disruption by ice crystals, ischemia, and vascular damage. The most commonly affected areas are the glans penis, ear tips, coronary bands, and heels.[60] The initial lesion of frostbite is paleness of the skin. Erythema, scaling, and hair loss follow. Pigment loss may occur. In severe cases, necrosis and dry gangrene occur.

Mild cases of frostbite do not require treatment. More severe cases require rapid thawing in warm water (41° to 44° C).[60] After rewarming, one should apply nonsteroidal antibiotic ointments to the area. In severe cases with necrosis and sloughing, topical wet soaks and symptomatic therapy with antibiotics may be needed to prevent sepsis. One should not attempt surgical debridement until an obvious demarcation is present between viable and nonviable tissue. Previously frostbitten areas may be more susceptible to cold injury.

11.15.4 CHEMICAL TOXICOSIS

In addition to causing disorders of the hair coat, selenosis may result in necrosis and sloughing of the hoof (see the section Chemical and Plant Causes of Coat Abnormalities).

11.15.5 STACHYBOTRYOTOXICOSIS

Stachybotryotoxicosis is a mycotoxicosis caused by the toxins of the fungus *Stachybotrys atra*.[60] This fungus grows on hay and straw and produces toxins referred to as macrocytic trichothecenes. These toxins cause bone marrow suppression, profound neutropenia, thrombocytopenia, and necrotic-ulcerative lesions of the skin and mucous membranes.

Commonly, lesions begin at the mucocutaneous junction as focal areas of necrosis and ulceration. Petechiae, ulcers, large areas of necrosis, catarrhal rhinitis, suppurative rhinopharyngitis, and laryngitis follow. Skin lesions occur as early as 24 hours after ingestion of the toxin. Systemic signs include lethargy, anorexia, weight loss, hyperactivity, proprioceptive deficits, colic, muscular stiffness, and second-degree atrioventricular block. Affected animal develops hemorrhagic diathesis, hemorrhagic enteritis, and septicemia and die.

Diagnosis is based on history, clinical signs, and finding the toxin in the feed. If one recognizes toxicosis early in the course of the disease, withdrawal of affected feed has resulted in resolution of signs. Animals with extensive lesions and chronic exposure have a poor prognosis.

11.15.6 BITES AND STINGS OF VENOMOUS INSECTS, SPIDERS, AND REPTILES

Many species of snakes, spiders, and insects have venomous bites and stings. Except for snake bites, these animals lack sufficient quantity of venom to cause more than transient pain or local inflammation. Venoms contain a variety of substances, including enzymes, peptides, polypeptides, amines, and glycosides, that act locally by causing tissue necrosis and vascular thrombosis and hemorrhage or systemically by causing widespread hemolysis and neurotoxicity.

11.15.6.1 Snake Bite

Of the poisonous snakes in the United States, bites from rattlesnakes, water moccasins, and copperheads occur most commonly in horses.[125] The venom from snakes is hemotoxic and proteolytic and produces extreme local swelling with significant tissue and red blood cell destruction.

Most bites occur in the spring and summer. Horses most commonly are bitten on the nose, head, neck, and legs. Snake bites may or may not involve envenomation and may be dry or wet. Bites in which no venom is injected swell minimally and are only slightly painful (dry bite). When envenomation occurs (wet bite), rapid swelling, pain, and local hemorrhage develop, usually within 60 minutes of the bite. Fang marks are often difficult to find because of tissue swelling. Edema, erythema, and tissue necrosis develop over days, and the skin may slough (Figure 11-35). Bites occurring on the face or head are serious because of the risk of respiratory and nasal edema. If respiratory distress is severe, the horse may need a tracheotomy.

One treats snake bites symptomatically. One should clean the wound and provide hydrotherapy with cold water to minimize swelling. If swelling is already present, warm hydrotherapy may stimulate circulation and 711
removal of tissue edema. Whether this increases the absorption of venom in the early stages of the bite is 712
unknown, however. Broad-spectrum antibiotics are indicated to prevent secondary infection. The use of glucocorticoids is controversial but may be beneficial in decreasing inflammation and pain. Nonsteroidal antiinflammatory agents also may be beneficial. Surgical debridement and wound closure may be necessary if severe necrosis and sloughing occur. Although specific antivenoms are available, they are of limited use. Maximum benefit is attained only when they are administered within hours of the bite. Additionally, the volume of antivenom necessary to treat snake bites in horses may be cost-prohibitive.

Figure 11-35 Face of a horse after being bitten by an Eastern diamondback rattlesnake.

11.15.6.2 Fire Ant Bites

Fire ants, *Solenopsis* spp., are common in the southern United States. Single or small numbers of stings are acutely painful and rapidly develop into a pustule or crust. Horses are bitten most commonly on the legs, nose, and ventrum, but massive exposure can occur if the horse rolls on an anthill and is bitten by hundreds or thousands of ants. Complications of massive exposure include infection, anaphylactic shock, bronchospasms, and sloughing of the epidermis. Single or multiple small numbers of solitary stings usually require little or no treatment. Applying an antibiotic ointment to minimize the chance of myiasis is prudent, but otherwise wounds heal without complication. If massive exposure has occurred, one may need to use systemic antibiotics and nonsteroidal antiinflammatory drugs to decrease pain and swelling.

11.15.6.3 Spider Bites

Black, brown, and red widow spiders *(Latrodectus* species) and the brown recluse *(Loxosceles reclusa)* spider are common in America. In Australia, the black house spider *(Ixeuticus)* causes painful bites.[125] Spider bites are characterized by hot, edematous, painful swellings in the area of the bite. The brown recluse spider has a dermal necrotoxin that may cause severe dermal necrosis, but this has not been reported in horses.

Treatment is symptomatic. Cold ice packs applied to the area and systemic glucocorticoids or antihistamines, or both, may be helpful. Spraying the environment of the horse with a parasiticide is important. One should remove discarded furniture, newspapers, old cloths, and trash from the premises because these are favorite living areas for venomous spiders.

11.15.7 VASCULITIS AND PURPURA HEMORRHAGICA

Vasculitis is an inflammatory reaction occurring in the wall of the blood vessel (Figure 11-36). Vasculitides are classified by inflammatory cell type. Neutrophilic vasculitides are subdivided further into leukocytoclastic (neutrophil nuclei undergo karyorrhexis) or nonleukocytoclastic.

The immunologic mechanisms involved are typically type I and type II hypersensitivity reactions.[59] Equine viral arteritis, equine influenza, *Corynebacterium pseudotuberculosis,* and *Streptococcus* spp. (especially *S. zooepidemicus* subsp. *equi*) infections may result in vasculitis.[34] Vasculitis also may occur following 712
Rhodococcus equi pneumonia, bronchopneumonia, cholangiohepatitis, and some antibiotic treatments. Often the 713
underlying cause is not identified.

Figure 11-36 Horse with vasculitis resulting from strangles.

Idiopathic vasculitis has no known breed, sex, or age predilection. The most common locations for lesions are the distal limbs, ears, lips, and periocular areas.[36] Oral ulcers and bullae may be present. Skin lesions consist of purpura, edema, and erythema. Systemic signs may include pyrexia, depression, anorexia, weight loss, and lameness.

Purpura hemorrhagica is an acute noncontagious disease of horses characterized by extensive edema and hemorrhage of the subcutaneous tissue. Hemorrhage in the mucosae and viscera are common. Most cases occur following strangles or equine influenza.[36] Clinical signs usually develop within 2 to 4 weeks of the respiratory infection. Urticaria followed by pitting edema of the distal limbs, head, and ventral abdomen is common. Severe edema of the head may compromise breathing.[16] Tissue exudation and sloughing may occur. Pain and pruritus are rare. Affected horses usually are depressed, reluctant to move, and often anorectic.

Diagnosis of vasculitis is by skin biopsy. Obtaining skin biopsy specimens from lesions 8 to 24 hours of age is important because these lesions tend to have the most diagnostic changes. Lesions more than 24 hours old may be nondiagnostic because of intense secondary cellular infiltrates or necrosis. Skin biospy reveals neutrophilic, eosinophilic, lymphocytic, or mixed cellular infiltrates in the vessel wall. Fibrinoid degeneration and hemorrhage are common.[64] Direct immunofluorescence testing is occasionally useful as an adjunct diagnostic aid.

Prognosis for horses with vasculitis is unpredictable.[126] Therapy of vasculitis is discussed elsewhere in this text.

11.15.8 PANNICULITIS AND FAT NECROSIS

Panniculitis refers to inflammation of the subcutaneous fat. This condition is rare in horses and results from widespread death of lipocytes. Fat cells are vulnerable to trauma, ischemia, and neighboring inflammation. When lipocytes are damaged, lipid is released and undergoes hydrolysis into glycerol and fatty acids. Fatty acids are potent inflammatory agents that elicit further inflammatory reactions.[127]

Panniculitis may be precipitated by a wide range of causes including trauma, infections, autoimmune disease, pancreatic disease, glucocorticoid therapy, vasculitis, vitamin E deficiency, and idiopathic causes. In the horse, few cases have been reported, and the causes of those were obscure. Vitamin E deficiency was suspected in a few horses.[82]

Clinically, horses with panniculitis show deep-seated nodules and plaques.[127] Lesions may be single or multiple and vary in size. Nodules may be hard and well-defined or soft and ill-defined. Initially, lesions are not fixed to the overlying skin, but as the disease progresses, nodules become cystic and rupture onto the skin surface. The ulcerating nodules drain a yellow to brown to bloody oily material. Pain varies. Healed lesions may leave depressed scars. Affected animals may be febrile, depressed, lethargic, and anorectic.

Definitive diagnosis is by skin biopsy. One should obtain samples for histopathologic evaluation by deep excisional biopsy using a scalpel blade because skin biopsy punches do not obtain sufficiently deep samples to be diagnostic. One should request special stains (i.e., positive acid–Schiff, Gomori's methenamine silver, Brown and Brenn) for causative agent. Skin biopsy reveals lobular to diffuse pyogranulomatous inflammation in the panniculitis.

One should identify and treat underlying causes appropriately. Idiopathic panniculitis may respond well to prednisolone at 1 to 2 mg/kg orally once daily for 7 to 14 days or to a single treatment of dexamethasone at 20 to

30 mg intramuscularly.[82,127] Clinical improvement usually occurs within 7 to 14 days. Relapses occur, and the horse may require lifelong therapy.

11.16 Dermatosis Of The Lower Limb

Skin diseases of the lower limbs of horses are common dermatologic problems. These diseases may be extensions of generalized dermatoses or unique clinical syndromes.

11.16.1 INFECTIOUS DISEASES OF THE LOWER LIMB

11.16.1.1 Bacterial Pastern Folliculitis

Bacterial folliculitis of the pastern is caused by *Staphylococcus aureus, Staphylococcus hyicus,* or β-hemolytic streptococcus.[92,125] This disease is considered a primary pyoderma, but the mechanism by which organisms initiate disease is unknown.

Lesions are limited to the posterior aspect of the pastern and fetlock region and may affect single or multiple limbs. The initial lesion consists of papules and pustules that eventually coalesce and produce large areas of ulceration and suppuration. The disease is not associated with systemic signs.

Diagnosis is by clinical signs, skin scrapings, cytologic examination of the exudate, Gram stain, and bacterial culture and sensitivity. Cytologic examination of pustule contents usually reveals large numbers of neutrophils engulfing bacteria.

Sedation may be necessary for initial treatment because lesions are painful.[125] One should clip hair from affected areas and wash the area daily in an antimicrobial scrub or shampoo (e.g., chlorhexidine shampoo or 713
scrub) and should apply an appropriate antibiotic ointment without corticosteroids twice daily. Systemic 714
antibiotic therapy is not usually necessary.

11.16.1.2 Ulcerative Lymphangitis

Ulcerative lymphangitis is a bacterial infection of the cutaneous lymphatics of horses. Lesions are most common on the hindlegs, especially distal to the hock, and consist of hard to fluctuant nodules that abscess, ulcerate, and drain pus. Individual nodules may heal, but new lesions develop. Cording of the regional lymphatics, edema, and fibrosis are common. Regional lymph nodes usually are not involved.

Definitive diagnosis is by direct smear, Gram stain, and culture of a nodule. Skin biopsy reveals superficial and deep perivascular dermatitis that may be suppurative or pyogranulomatous. Special stains (Brown and Brenn, and Gram) may reveal the organism.

If one treats the disease early, therapy may be effective and may prevent permanent disfigurement and debilitation. The drug of choice pending culture and sensitivity is procaine penicillin 20,000 to 80,000 IU/kg intramuscularly every 12 hours for 30 days or longer. Adjunct hydrotherapy may be beneficial. After fibrosis develops, prognosis is poor.[125] One should clean the affected area daily with copious amounts of water and wash it with an antimicrobial scrub (e.g., chlorhexidine).

11.16.1.3 Viral Papillomatosis

Equine viral papillomatosis (warts) is caused by a DNA papovavirus.[118] Viral papillomatosis is common in horses and occurs most frequently in horses less than 3 years of age. No sex or breed predilection exists. Transmission appears to be by direct contact. Groups of young horses often are affected more frequently than horses of the same age housed individually. Lesions are most common on the muzzle, genitalia, and distal legs and are usually multiple and resemble papillomatosis of other species.

Definitive diagnosis is by skin biopsy. Epithelial proliferation without connective tissue proliferation is typical.

Benign neglect is the best treatment for these lesions; spontaneous remission almost always occurs. One should consider surgical removal only when the lesion interferes with function. Anecdotal reports that surgical removal or damage to part of the lesion induces remission of the lesion are unproven.[93] In fact, controlled studies suggest that such intervention might increase the duration of the lesions.[93,125] Efficacy of autogenous vaccines is unproven.

11.16.1.4 Sporotrichosis

The dimorphic fungus *Sporothrix schenckii* causes sporotrichosis, which was discussed in detail under Infectious Causes of Nodules.

A cutaneous lymphatic form of sporotrichosis may localize to the distal extremities of horses. The lymphatics become corded, and large nodules ulcerate and drain a thick brown-red discharge. Regional lymph nodes are not involved. Edema is rare. Diagnosis and treatment have been discussed previously.

11.16.2 PARASITIC DISEASES OF THE LOWER LIMB

The common parasitic diseases of horse have been discussed previously (see Parasitic Skin Diseases). Infestations of lice, *Chorioptes* spp., and chiggers are the most common parasitic dermatoses that cause lower limb pruritus.

11.16.3 NEOPLASTIC AND NONNEOPLASTIC PROLIFERATION DISEASES OF THE LOWER LIMBS

11.16.3.1 Keloids

A keloid is a nonneoplastic fibroblastic response that occurs on the pastern of horses. Clinically, the keloid resembles a cluster of grapes. Definitive diagnosis is by biopsy. These lesions do not respond to surgical excision, and in fact surgery may exacerbate them.[89,125] The best treatment for keloids is by intralesional injection of triamcinolone acetonide (not to exceed 20 mg per horse). Unfortunately, most lesions are so massive that response to therapy is poor.

11.16.3.2
Hemangiomata

Hemangiomata are benign tumors of the endothelial cells of blood vessels. Lesions occur most commonly in horses less than 1 year of age, and some horses are born with them.[93] Hemangiomata tend to be solitary tumors occurring on the distal limbs. The clinical appearance varies and may be circumscribed, nodular, firm to fluctuant, blue to black in color, dermal or subcutaneous, hyperkeratotic, or verrucous. Ulceration and bleeding are common.

Diagnosis confirmation is by skin biopsy, which reveals proliferation of blood-filled vascular spaces lined by single layers of well-differentiated endothelial cells.[37,93] Equine hemangiomata are characterized by a multinodular capillary hemangioma with hyperplasia and hyperkeratosis of the overlying epidermis.

Surgical excision is the treatment of choice. Equine verrucous hemangioma is difficult to excise, and cryosurgery may be beneficial. Recurrence is common.

11.16.4
AUTOIMMUNE DISEASES OF THE LOWER LIMBS

11.16.4.1
Pemphigus Foliaceus

Pemphigus foliaceus was discussed under Immunologic Causes of Exfoliation. In some horses the condition affects only the coronary band.[51] The coronary band of all four limbs is crumbly, degenerating, and exudative. Pain, lameness, and edema of the lower limb may occur. 714

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11.16.4.2
Systemic Lupus Erythematosus

Systemic lupus erythematosus is a multisystemic autoimmune disease that is rare in horses. Profound lymphedema of the lower limbs may be the only clinical sign. One treats the disease with immunosuppressive doses of prednisone (2 mg/kg orally every 12 hours). The prognosis is grave.

11.16.4.3
Vasculitis

Vasculitis was discussed earlier under Vasculitis and Purpura Hemorrhagica. In some horses, cutaneous vasculitis is restricted to the white skin of the pasterns and face of horses in the summer.[51] Characteric lesions are erythema, swelling, pain, and exudation, and lesions may resemble photodermatitis, which suggests a photoinduced cause. Edema of the pastern and face is significant.

11.16.4.4
Pastern Dermatitis (Greasy Heel and Scratches)

Skin disease of the lower limbs is a common and frustrating problem in horses. *Pastern dermatitis, scratches, greasy heel,* and *mud fever* are colloquial terms for a moist exudative dermatitis of the caudal heel and pastern area. One must remember that these terms are nonspecific and describe a variety of inflammatory skin conditions of the lower limbs of horses.

The pathogenesis of this syndrome is not understood completely. The initial lesion may be a primary infection or may follow a predisposing factor such as pemphigus foliaceus or mites. Horses with long fetlock hair or

horses housed in muddy paddocks, unsanitary conditions, or rough stubbly pasture are at risk for pastern dermatitis. The problem is common in horses worked on tracks consisting of grit particles, which can cause microtrauma to the skin.

Clinical signs depend greatly on whether the condition is acute or chronic and whether the owner has treated the lesions. Many horse owners do not recognize the acute development of skin diseases, minimizing the opportunity to identify the underlying cause. To complicate matters further, many owners treat these lesions before seeking veterinary care. Many common over-the-counter medications can induce irritant or allergic reactions, making distinguishing between these reactions and the original skin disease almost impossible.

Regardless of the cause, clinical signs are similar (Figure 11-37).[125] Acute lesions usually begin at the heel. Pain, swelling, moist exudation, and hair loss are common. As the disease process continues, lesions spread proximally and anteriorly. Matting of the hair occurs, and the horse may be noticeably lame. Crusting is common. If the underlying disease process involves a vasculitis, ulceration may be present. If the disease is left untreated, a foul odor develops. Because of the constant flexion in the area, fissures often develop. In Draft Horses, vegetative granulomatous growths commonly result. Lesions may occur on one or multiple limbs or just on extremities with white markings.

Figure 11-37 Pastern dermatitis.

Definitive diagnosis requires a complete medical history. Liver function tests are essential in any horse in which the lesions are limited to the unpigmented areas of the skin. One should perform *Dermatophilus* preparations, fungal cultures, and skin scrapings in all cases. In horses with long hairs on the fetlocks, one should comb the hair thoroughly with a fine-toothed metal comb, which is often the only successful method for finding lice. In difficult cases, one should submit tissue samples for bacterial or fungal culture.

Correct therapy requires identifying the underlying cause. The reader can find specific therapy for most of the diseases elsewhere in this chapter or in the references. In all cases, one should clip the long hairs of the fetlock area and thoroughly wash the affected area with an antimicrobial scrub (e.g., 2% chlorhexidine). One should avoid iodine preparations because they can be irritating. One should remove all crusts and exudation, daily if necessary, and should avoid exposing the horse to moistures and irritants. Horses with idiopathic greasy heel often respond well to cleaning of the area, improved hygiene of the stall, and systemic corticosteroid therapy

(assuming bacteria are not the cause). Large doses of prednisolone (e.g., 1 mg/kg orally once daily) may be necessary to induce remission of clinical signs. One should not decrease the dose of glucocorticoids too rapidly or relapse may occur. If oral prednisolone does not induce remission, dexamethasone at 0.02 to 0.04 mg/kg orally once daily for 3 to 5 days may be effective.

11.17 Systemic Diseases With Cutaneous Manifestations

Reviewing all the skin diseases that may have systemic clinical signs is beyond the scope of this chapter. The 715
author has made an effort to include the most common systemic findings in this chapter. Table 11-3 provides a 718
brief summary.

TABLE 11-3 Skin Diseases With Systemic Manifestations

DISEASE	CUTANEOUS SIGNS	SYSTEMIC SIGNS
ENVIRONMENTAL		
Gangrene	Wet: moist swelling, discoloration, malodorous, tissue decomposition	Depends on underlying cause; fever
	Dry: dry, discolored, leathery skin	
Burns	Superficial: erythema, edema, pain, vesicles	Shock, respiratory compromise
	Deep: necrosis, ulceration, anesthesia, scarring	
Selenosis	Painful coronary band, sloughing and necrosis of hoof, rough hair coat, progressive loss of long hairs of mane and tail	Lameness, weight loss
Arsenic poisoning	Severe seborrhea, ulcer, nonhealing wounds, hypertrichosis	Gastroenteritis, emaciation, variable appetite
Mercury poisoning	Progressive alopecia	Gastroenteritis, lameness, emaciation
Iodism	Severe dry seborrhea with or without hair loss	Cough, variable appetite, joint pain, seromucoid nasal discharge, lacrimation
Hepatogenous photosensitization	Erythema, edema, pruritus, pain, in white or light-skinned area; vesicles and bullae may progress to oozing, necrosis, and sloughing	Acute: hepatic encephalopathy, icterus, depression, decreased appetite Chronic: weight loss, depression neurologic signs
Ergotism	Swelling of coronary band, necrosis of feet, sloughing of ears, tail, feet	Lameness of hindlegs, fever, weight loss, poor appetite
Leucenosis	Hoof dystrophies, shedding of hair	Laminitis, lameness
Hairy vetch toxicosis	Cutaneous plaques and papules that ooze yellow pus; pruritus; hair loss	Conjunctivitis, anorexia, pyrexia, weight loss
BACTERIAL DISEASES		

Strangles	Limb edema; edema of lips, eyelids; petechial hemorrhages of mucous membranes and sclera	History of acute contagious upper respiratory infection; pyrexia, mucopurulent nasal discharge; abscesses in the mandibular or retropharyngeal lymph nodes
Corynebacterium pseudotuberculosis abscesses	Single or multiple deep abscesses that develop slowly or rapidly; 50% occur in the pectoral or ventral abdominal area; ventral midline edema	Pitting edema, depression, fever, lameness, internal abscess, prolonged fever, abortion
Dermatophilosis	Exudative crusted lesions on dorsal trunk, face, pastern, or coronets	Depression, fever, lethargy, poor appetite, weight loss, lymphadenopathy; lesions on legs may cause edema, pain, and lameness.
Actinobacillosis	Thick-walled abscess of soft tissue	In newborn foals, disease is a highly fatal septicemic disease, and skin lesions are rare.
Clostridial infections	Malignant edema: swelling at site of infection, pitting edema, local erythema; skin may become hot to touch, painful, or slough; crepitus	High fever, anorexia, muscle tremors, acute death possible within 24–48 hours
	Blackleg: hot, painful, swelling that progresses to a cold painful swelling with edema and subcutaneous emphysema	
Glanders (farcy) *Pseudomonas mallei* (not in United States)	Subcutaneous nodules begin most commonly on medial aspect of hock; lesions rapidly ulcerate and drain a honey-colored material; lymphadenopathy and cording of lymphatics is common	Respiratory infection that rapidly leads to death
FUNGAL DISEASE		
Histoplasma farciminosa (epizootic lymphangitis)	Unilateral nodules on face, head, neck and occasionally trunk; nodules initially are firm but rupture and exude light-green, blood-tinged exudate; large ulcer may form and lesions may spread bilaterally	Lacrimation, conjunctivitis, and respiratory signs may occur
PARASITES		
Lice	Pruritus, scaling, alopecia	Anemia in severe infestations with sucking lice

Black flies	Painful papules, and wheals that may become vesicular, hemorrhagic, and necrotic; lesion may be localized to ears or intermandibular areas	Toxin in bite can cause increased capillary permeability; depression, weakness, staggers, tachypnea, tachycardia, weak pulse, shock, and possible death
IMMUNE-MEDIATED DISEASES		
Atopy	Chronic pruritic urticaria, excoriations, alopecia, lichenification	Respiratory difficulty, especially on expiration
Pemphigus foliaceus	Crusts, scales, oozing, annular eruption, matting of the coat; lesions may be limited to coronary band	Depression, weight loss, poor appetite, pyrexia
Bullous pemphigoid	Vesicles and bullae in mouth, groin, and axilla; crusts, ulcers, and epidermal crusts	Anorexia, depression, fever
Systemic lupus erythematosus	Lymphedema, panniculitis, alopecia, leukoderma; scaling of face, neck, and trunk	Polyarthritis, thrombocytopenia, proteinuria, fever, depression, weight loss
Transfusion reactions and graft-versus-host disease (may occur in horse after unmatched blood transfusion)	Exfoliative to ulcerative dermatitis, ulcerative stomatitis	Diarrhea, increased heart and respiratory rates, lacrimation, muscle fasciculation
Erythema multiforme	Symmetric maculopapular lesions, urticaria that results in annular arciform or polycyclic shapes; wheals that do not disappear	Occurs following pregnancy, drugs, neoplasia, connective tissue disease, and infections or may be idiopathic
Vasculitis	Purpura, edema, erythema, necrosis, crusts; purpura hemorrhagica causes edema and hemorrhagic swelling in tissue, mucosa, and viscera	May occur following strangles or influenza; depression, fever, reluctance to move, colic, diarrhea
Equine exfoliative eosinophilic dermatitis and stomatitis	Scaling and crusting that progress to generalized exfoliation, alopecia, ulceration, and exudation; variable pruritus	Severe progressive weight loss; no diarrhea, ravenous appetite
Equine cutaneous amyloidosis	Papules, nodules, and plaque over the head and neck that develop rapidly	Diffuse nodules in upper respiratory tract may cause severe dyspnea
ENDOCRINE DISEASES		

Hypothyroidism	Dull rough hair coat, delayed shedding of coat, edema of face and limb	Anecdotal reports of laminitis, infertility, anhidrosis, anemia, myopathy; weight gain and decreased food intake; skeletal limb disorders have been reported in foals
Hyperadrenocorticism	Long shaggy hair that fails to shed; mane and tail are unaffected	Polydipsia, polyuria, muscle wasting, weight loss, lethargy, swayback appearance, pendulous abdomen, blindness, chronic infections, neurologic disorders or signs
SWEAT GLAND DISORDERS		
Anhidrosis	Acute episode: none	Acute: labored breathing, fever, flared nostrils, lack of sweating, collapse, death
	Chronic: dry hair coat, excessive scaling, partial alopecia, pruritus	Chronic: polydipsia, polyuria, poor appetite, loss of body condition
MISCELLANEOUS DISEASES		
Panniculitis	Firm to fluctuant nodules most commonly found on trunk in subcutaneous tissue that ruptures and drains an oily yellow-brown to bloody discharge	Anorexia, depression, lethargy, pyrexia
NEOPLASTIC DISEASES		
Hemangioma and hemangiosarcoma	Two types: (1) well-circumscribed nodules that are blue-black in appearance; (2) dark hyperkeratotic and verrucous lesions that bleed easily	Anemia
Lymphosarcoma	Single or multiple dermal-to-subcutaneous nodules, especially on trunk	Internal organ involvement; usually fatal

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12 CHAPTER 12 DISORDERS OF THE HEMATOPOIETIC SYSTEM

Debra C. Sellon

721

The hematopoietic system includes the blood and blood-forming tissues of the body. Peripheral blood cells are essential for tissue oxygenation, immune surveillance and clearance of foreign antigens, coagulation, and inflammatory reactions. Because of the interactions of blood with other body tissues and organs, alterations in blood parameters often reflect dysfunctions elsewhere in the body, and evaluation of the blood and its constituents has become an essential component of most diagnostic efforts.

This chapter discusses the basics of hematopoiesis and hematopoietic metabolism as they relate to specific disease conditions in the horse. Included is discussion of the erythron, leukon, platelets, and hemostatic mechanisms of the horse.

12.1 Erythron

Erythropoietic tissue of the bone marrow and the circulating erythrocytes often are referred to as the erythron, to emphasize their organlike function. Erythrocytes are essential for delivery of oxygen to all tissues of the body. The complex maturation and development of erythrocytes and their pivotal role in survival of all body tissues make them uniquely reflective of many pathologic conditions. An understanding of the erythron and its responses to perturbations in homeostatic mechanisms throughout the body can provide the astute clinician with invaluable clues in unraveling difficult diagnostic problems.

12.1.1 PHYSIOLOGY

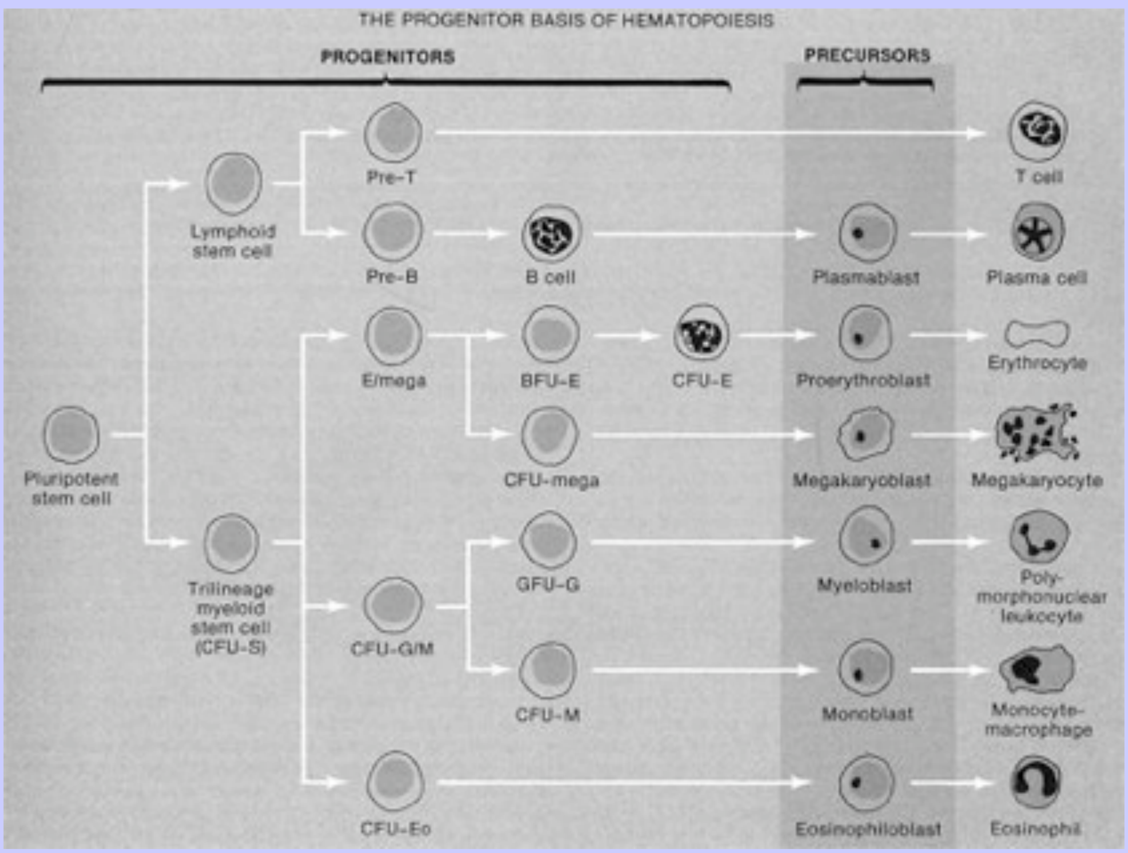
Erythropoiesis refers to the process of development and maturation of erythrocytes. Knowledge of the metabolic processes of these cells is critical to understanding pathologic processes that interfere with their primary function of tissue oxygenation.

In the fetus the cellular elements of blood are produced almost exclusively in the liver and spleen. As the body matures and differentiates in utero, hematopoiesis gradually shifts to the marrow cavities, so that at birth the bone marrow is the main organ of hematopoiesis. As an animal ages, bone marrow hematopoiesis decreases and fat infiltrates much of the previously active marrow. In the older adult animal, active hematopoiesis is limited to the marrow of the vertebrae, ribs, sternum, skull, and pelvis and the epiphyseal marrow of the humerus and femur.

Erythrocyte production begins from a pluripotent colony-forming unit stem cell capable of differentiating into erythroid-, myeloid-, megakaryocytoid-, or lymphoid-producing cell lines. The stem cell is capable of self-renewal to provide a continuing supply of pluripotent stem cells. The direction of differentiation is determined in part by the types and quantities of cytokine mediators to which the stem cell is exposed at the time it begins to divide. The exact combinations of mediators necessary to direct differentiation into each line are not understood entirely. In erythrocyte development the stem cell undergoes sequential mitotic divisions to produce the 721
committed erythrocyte progenitor burst-forming unit, followed by erythroid colony-forming units. These two 722
stages are capable of limited replicative self-renewal but are committed ultimately to differentiate into erythrocytes (Figure 12-1). Burst-promoting activity depends on the presence of various cytokines, including interleukin 3 (IL-3), IL-4, and granulocyte-macrophage colony-stimulating factor. The erythroid colony-forming

unit cell is the immediate precursor stage of the first morphologically recognizable erythrocyte precursor, the proerythroblast.

Figure 12-1 Schematic representation of the progenitor basis of hematopoiesis. Maturation is depicted from left to right with circulating blood cells as the final product on the far right of the drawing. Progressive amplification of progenitors and precursors as they mature and differentiate is not shown. *CFU-S,* Colony-forming unit–spleen; *CFU-G/M,* CFU–granulocyte macrophage; *CFU-Eo,* CFU–eosinophil; *BFU-E,* burst-forming unit, erythroid; *CFU-mega,* CFU–megakaryocyte; *CFU-G,* CFU–granulocyte; *CFU-M,* CFU–monocyte; *CFU-E,* CFU–erythrocyte. (From Nathan DG: Hematologic diseases. In Wyngaarden JB, Smith LH, editors: *Cecil textbook of medicine,* ed 18, Philadelphia, WB Saunders, 1988.)



Initial stages of erythrocyte development depend greatly on the presence of the glycoprotein hormone erythropoietin, which the kidney produces in response to renal hypoxia and is an absolute requirement for erythrocyte progenitor cell maturation and differentiation. The absence of erythropoietin, as in patients with

chronic renal disease, is associated with potentially severe nonregenerative anemia. Erythrocytosis, an increase in the circulating red blood cell (RBC) mass, is associated with excess secretion of erythropoietin in certain disease conditions.

Bone marrow proerythrocytes undergo four successive divisions followed by a period of maturation to produce 16 erythrocytes. Sequential divisions produce basophilic erythroblasts, polychromatophilic erythroblasts I and II, and orthochromic erythroblasts. The orthochromic erythroblast becomes a reticulocyte by ejecting its nucleus. 722
Reticulocyte production requires approximately 72 hours; 24 to 48 hours later the reticulocyte will have matured 723
into an erythrocyte. Iron is an essential component for hemoglobin synthesis and thus for erythropoiesis. The diet of the horse normally contains an abundance of readily available iron. Absorption is most efficient in the duodenum but may occur at almost any part of the gastrointestinal tract. The amount of iron absorbed from the gastrointestinal tract varies with the systemic iron status of the animal. Absorption increases in iron-depleted animals and decreases in animals with ample iron stores. Unabsorbed iron passes through the remainder of the tract and is lost in feces. Horses, especially young foals, supplemented with excessive dietary iron can develop hemochromatosis, hepatic cirrhosis, and liver failure.[1–4]

After absorption the blood transports iron complexed with the protein transferrin. In the liver, spleen, and bone marrow, iron is transferred to molecules of the storage protein ferritin. Approximately 30% of body iron is present in the storage proteins ferritin and hemosiderin (a complex aggregation of ferritin molecules). Only minute amounts of iron are complexed to the transport protein transferrin at any given time. Minor quantities of iron are also present in myoglobin and various electron transport molecules and enzymes. The hemoglobin of senescent erythrocytes removed from the peripheral circulation is degraded in cells of the mononuclear phagocyte system in the spleen, liver, and bone marrow. Iron ultimately is recycled to the bone marrow or liver to be used in synthesis of hemoglobin for new erythrocytes. Almost 70% of total body iron is present in hemoglobin.

Hemoglobin is synthesized in the mitochondria of the developing erythroblast and consists of four polypeptide chains (two α and two β in the adult) and a heme moiety. Heme is the functional portion of the hemoglobin molecule and contains an iron atom held in place by the four pyrrole rings of a porphyrin molecule. The iron in hemoglobin must be in the ferrous form (Fe^{2+}) to bind oxygen reversibly. Heme iron oxidized to the ferric form (Fe^{3+}) forms methemoglobin, and the molecule is no longer capable of transporting oxygen.

The normal life span of equine erythrocytes in circulation is approximately 150 days.[5,6] Mononuclear phagocytes of the spleen, liver, and bone marrow remove senescent RBCs from circulation. Transferrin transports released iron to RBC precursors in the marrow. Heme in phagocytic cells undergoes enzymatic degradation to biliverdin and then bilirubin. Phagocytic cells release unconjugated bilirubin into the circulation, where it binds to plasma albumin. Hepatocytes take up unconjugated bilirubin, which is conjugated and excreted via the bile into the gastrointestinal tract, where it is converted to urobilinogen and then stercobilin. Intravascular or extravascular hemolysis often results in increased serum unconjugated bilirubin concentrations.

During intravascular hemolysis, ruptured erythrocytes release heme, which combines with haptoglobin, an acute phase protein normally present in plasma, to be carried back to the liver, where heme is converted to bilirubin and excreted. If the level of haptoglobin exceeds the carrier capacity of the blood, the result is free hemoglobin in the plasma. Free plasma hemoglobin is filtered readily across the renal glomerulus and reabsorbed by renal tubular epithelial cells. Hemoglobin and other related pigment molecules, including myoglobin, are potentially nephrotoxic, and excessive intravascular hemolysis may result in acute renal failure.

The mature erythrocyte is an anucleate cell incapable of de novo protein synthesis to replace enzymes or other proteins that have been used during normal metabolism. Binding, transport, and delivery of oxygen to tissues does not require energy expenditure by the erythrocyte, but maintaining iron and various cellular proteins in a reduced state essential for proper function does require energy. Maintenance of appropriate electrolyte gradients across the RBC membrane also requires energy. Glucose is the main in vivo energy source for erythrocytes. RBCs differ from other cell types in the absence of the Krebs (tricarboxylic acid) cycle. Glucose must be metabolized via the anaerobic glycolytic (Embden-Meyerhof) pathway or via the hexose monophosphate shunt. The enzymes involved in these metabolic pathways are critical to RBC survival, and hereditary deficiencies described in other species are associated with severe anemia.

The body must maintain iron, hemoglobin, some critical RBC enzymes, and several membrane proteins in a reduced form for appropriate activity. High intracellular concentrations of glutathione (GSH), a sulfhydryl-containing tripeptide, are important for reduction of sulfhydryl groups of hemoglobin and other erythrocyte proteins. Reduction reactions involve the conversion of GSH to its oxidized form (GSSG), which then is returned to the reduced form (GSH) by the action of glutathione reductase. When the functional ferrous form of iron (Fe^{2+}) in hemoglobin is oxidized to the ferric form (Fe^{3+}), the resulting methemoglobin is incapable of carrying oxygen. Small amounts of methemoglobin (1% to 2% of total hemoglobin) are produced normally in erythrocytes. This methemoglobin is reduced to functional hemoglobin primarily via the action of the enzyme methemoglobin reductase.

12.1.2 EVALUATION OF THE ERYTHRON

Laboratory evaluation of the blood and bone marrow provides diagnostic clues that may aid the practitioner in diagnosing and treating a variety of disorders. Knowing the tests that are available and developing an 723
understanding of how and why they are performed aids one in interpreting the results. 724

TABLE 12-1 Reference Ranges for Equine Hematologic Parameters[*]

PARAMETER	UNITS	LIGHT HORSE[†]	DRAFT HORSE[‡]	MINIATURE HORSE[§]	DONKEY[||]
RBC count	$10^6/\mu l$	6.0–10.0	5.5–9.5	4.3–10.3	4.7–9.0
Hemoglobin	g/dl	12.0–17.0	8.0–14.0	9.0–16.0	9.5–16.5
PCV	%	32–50	24–44	24–42	28–47
MCV	fl	42–58	—	38–61	46–67
MCH	μg	15–20	—	14–23	16–23
MCHC	g/dl	32–38	—	33–40	32–36

RBC, Red blood cell; *PCV,* packed cell volume; *MCV,* mean corpuscular volume; *MCH,* mean corpuscular hemoglobin; *MCHC,* mean corpuscular hemoglobin concentration.

* Numbers are for comparison only. Each laboratory should establish its own normal values.

† North Carolina State University clinical pathology laboratory equine reference values.

‡ Data from Jain N: *Schalm's veterinary hematology,* ed 4, Philadelphia, 1986, Lea & Febiger.

§ Data from Harvey R, Hambright M, Rowe L: Clinical biochemical and hematologic values of the American miniature horse: reference values, *Am J Vet Res* 45:987, 1984.

|| Data from Zinkl J, Mae D, Merida P et al: Reference ranges and the influence of age and sex on hematologic and serum biochemical values in donkeys *(Equus asinus)*, *Am J Vet Res* 51:408, 1990.

12.1.2.1 Peripheral Blood Evaluation

Most clinical laboratories now use automated cell counters for evaluation of peripheral blood. These counters are capable of determining RBC counts, hemoglobin, packed cell volume (PCV), and various erythrocyte indices. Tables 12-1 and 12-2 list normal values for the horse.[7,8] These values are intended as guidelines only. Each laboratory should establish its own normal values for the horse.

RBC count, hemoglobin, and PCV are the most frequently used parameters for assessing the quantity of erythrocytes in circulation. In the normal horse, hemoglobin concentration is approximately one third of the PCV. This value increases in horses with intravascular hemolysis and hemoglobinemia. RBC count, hemoglobin concentration, and PCV decrease rapidly during the first weeks of life in the equine neonate. This decrease has been attributed to decreased erythrocyte production, a shorter RBC life span in the neonate, and hemodilution. During this period, mean RBC volume also decreases to the point that cells would be classified as microcytic if compared with normal adult values.[9]

TABLE 12-2 Influence of Breed on Normal Erythron Values (Mean Standard Deviation) in Adult Horses

BREED	RBC (×106/μl)[*]	Hgb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
Thoroughbred	9.35 ± 1.05	14.8 ± 1.3	41.7 ± 3.8	44.7 ± 3.4	15.9 ± 1.4	35.8 ± 1.4
Standardbred	8.37 ± 1.02	13.6 ± 1.6	38.3 ± 3.5	46.1 ± 4.0	16.3 ± 1.4	35.5 ± 1.6
Quarter Horse	8.26 ± 1.02	13.3 ± 1.6	38.0 ± 4.0	46.2 ± 3.9	16.1 ± 1.7	34.9 ± 1.6
Appaloosa	8.60 ± 1.11	13.3 ± 1.6	38.4 ± 4.7	44.8 ± 4.4	15.5 ± 1.3	34.5 ± 0.8
Arabian	8.41 ± 1.21	13.8 ± 2.1	39.3 ± 5.0	46.9 ± 1.9	16.4 ± 0.9	34.9 ± 1.0
Clydesdale	7.30 ± 0.87	12.4 ± 1.1	33.0 ± 3.0	44.6	—	38.1
Percheron	7.39 ± 1.08	11.7 ± 1.4	—	—	—	—
Mixed cold-blooded	7.76 ± 1.23	—	33.0 ± 7.0	42.3	—	—

From Moms DD: Review of anemia in horses. 1. Clinical signs, laboratory findings and diagnosis, *Equine Pract* 11:27–34, 1989; modified from Jain NC, editor: *Schalm's veterinary hematology*, ed 4, Philadelphia, 1986, Lea & Febiger.

* *RBC,* Red blood cell; *Hgb,* hemoglobin; *PCV,* packed cell volume; *MCV,* mean corpuscular volume; *MCH,* mean corpuscular hemoglobin; *MCHC,* mean corpuscular hemoglobin concentration.

Evaluation of RBC indices can be helpful in characterizing anemia. If these parameters are not included in automated blood analyses, they may be calculated from the PCV, RBC count (in millions), and hemoglobin (in g/dl) as presented in Box 12-1.

Some automated cell counters also report RBC distribution width. This value may increase in some horses with anemia. Peripheral blood evaluation is not complete without a thorough examination of a stained blood smear to assess erythrocyte morphology. The normal equine erythrocyte is a biconcave disc, but in contrast to other species, most equine RBCs lack distinct central pallor. Equine RBCs normally exhibit a strong tendency 724
toward rouleau formation, a coinlike stacking or grouping of erythrocytes. This tendency causes equine 725
erythrocytes to sediment rapidly after collection, and one should mix all samples thoroughly immediately before any evaluation. Excellent pictures of normal and abnormal equine erythrocyte morphology are available in a variety of texts, and the reader is referred to those to aid in recognition of the following cell types[11,12]:

Poikilocyte: Any abnormally shaped erythrocyte. Poikilocytosis usually is reserved for description of RBCs that exhibit a variety of morphologies. If one particular shape predominates, one should use a more specific description.

Anisocytosis: A variability in the size of erythrocytes, usually associated with an increased RBC distribution width. Polychromasia indicates a variability in the color of erythrocytes usually caused by a variable hemoglobin and RNA content.

Spherocyte: Spherical erythrocyte in some horses with immune-mediated hemolysis.

Echinocyte: Burr cell with short, regularly spaced spicules projecting from the RBC surface. Echinocytes may be associated with uremia.

Acanthocyte: Spur cell with irregularly shaped spicules extending from the RBC surface. Acanthocytes may be associated with liver disease or gastrointestinal malabsorption.

Elliptocyte: Ellipsoid or oval erythrocyte found in animals with iron deficiency or myelophthisic anemia.

Leptocyte: Thin, flat RBC frequently associated with hepatic disease or iron deficiency.

Codocyte: Target cell with a dense central area of hemoglobin surrounded by a pale zone. Codocytes may be associated with hypochromic anemias or hepatic disease.

Howell-Jolly bodies: Basophilic nuclear remnants seen in the cytoplasm of erythrocytes. Approximately 10 in 10,000 erythrocytes contain Howell-Jolly bodies in the normal horse.[12]

Heinz bodies: Oxidized precipitated hemoglobin indicating oxidative damage to the RBC, usually resulting in intravascular or extravascular hemolysis. One may see Heinz bodies best using new methylene blue stain but may observe them in smears using Wright's stain as round structures protruding from the edge of the RBC membrane.

12.1.2.1.1

BOX 12-1 EVALUATION OF RED BLOOD CELL INDICES: CALCULATION FROM THE PACKED CELL VOLUME, RBC COUNT (IN MILLIONS), AND HEMOGLOBIN (IN g/dl)

$$\text{Mean corpuscular volume} = \frac{\text{Packed cell volume} \times 10}{\text{RBC count}}$$

Expressed as femtoliters (fl)

Increased in some horses with regenerative anemia

Decreased with iron deficiency anemia

Increased in older horses[10]

$$\text{Mean corpuscular hemoglobin} = \frac{\text{Hemoglobin} \times 10}{\text{RBC count}}$$

Expressed as picograms (pg)

Increased with intravascular hemolysis

Decreased with iron deficiency anemia

$$\text{Mean corpuscular hemoglobin concentration} = \frac{\text{Hemoglobin} \times 100}{\text{Packed cell volume}}$$

Expressed as grams per deciliter (g/dl)

Increased with intravascular hemolysis

Decreased with iron deficiency anemia

Occasionally, specialized tests of erythrocyte function or stability are indicated. Osmotic fragility tests measure the resistance of erythrocytes to in vitro hemolysis when incubated in increasingly hypotonic NaCl solutions. Erythrocytes from horses with immune-mediated hemolytic anemia frequently are more fragile than RBCs from normal horses.[13,14] The direct antiglobulin or Coombs' test is also helpful for diagnosing immune-mediated hemolytic anemia. Coombs' test detects immunoglobulin or complement on the surface of circulating erythrocytes. The Coombs' reagent should contain antisera to immunoglobulin G (IgG), IgM, and the third component of complement (C3). One incubates washed erythrocytes from the patient with Coombs' reagent and observes for agglutination. One may incubate cells at 10° C to detect cold reactive antibodies or at 30° C to detect warm reactive antibodies.[12] Because agglutination is the end point of the direct Coombs' test,

autoagglutination of blood is considered diagnostic of autoimmune hemolysis. One must differentiate autoagglutination from rouleau formation by dilution of an RBC suspension with isotonic saline. Saline disperses rouleau formation but does not affect true autoagglutination. The indirect Coombs' test detects the presence of circulating anti-RBC antibody in the serum by incubating patient serum with normal equine erythrocytes and monitoring for agglutination.

12.1.2.2 Bone Marrow Evaluation

Examination of the bone marrow of a horse is indicated if one identifies or suspects a disorder of the hematopoietic system but cannot diagnose it from information gathered by history, physical examination, and routine laboratory tests.[15] Bone marrow aspirates or core biopsies may be useful in characterizing anemias, 725
evaluating iron stores, or explaining quantitative or qualitative abnormalities of blood cells. 726

One may collect equine bone marrow aspirates or biopsies from the sternum,[15–17] tuber coxae, [15,18] or proximal ribs.[15,19] One obtains sternal aspirates from the ventral midline between the front legs. This site is preferable in most horses because hematopoietic activity persists throughout life, bones are not covered by a large muscle mass, and the marrow cavity is covered by only a thin layer of bone.[15] One may attempt an aspirate of the tuber coxae in young horses by directing the needle toward the opposite coxofemoral joint. One collects costal marrow from the proximal portion of an easily palpable cranial rib, usually beneath the latissimus dorsi and serratus posticus muscles.[19] One may use any large-gauge (16-gauge or larger) bone marrow needle with stylet to collect marrow aspirates. The needle should be at least 2 in long. The veterinarian clips the appropriate area and surgically scrubs it, injects a small amount of local anesthetic into the subcutaneous and periosteal tissues, and makes a stab incision through the skin and subcutaneous fascia. Forcing the bone marrow needle through the bone and into the marrow cavity may require considerable pressure and rotational movement.

The veterinarian removes the stylet from the needle; uses a sterile syringe containing a small quantity of anticoagulant to aspirate red-colored marrow, discontinuing aspiration when blood is visible in the hub of the syringe; withdraws the needle; and smears marrow from the hub of the syringe and the needle on glass microscope slides as for blood smears. Alternatively, one injects the marrow sample into a Petri dish. On visualization, one transfers spicules to a glass slide for staining. One may obtain core marrow biopsies using a 10-gauge Jamshidi needle and fixing the sample for routine histopathologic analysis.[15,20]

One should examine marrow samples first at low magnification for evidence of cellularity, distribution of fat cells, and heterogeneity of progenitor cell populations. At higher magnification, one may evaluate individual progenitor series and estimate a bone marrow myeloid to erythroid (M:E) ratio by counting 500 to 800 cells.[11] Descriptions and pictures of bone marrow progenitor cells are reported elsewhere, and the reader is referred to these for identification of cell types present in bone marrow aspirates or biopsies.[11,12,18] Normal M:E in the horse has been reported to range between 0.5 and 3.76.[16,17,19] Ratios of less than 0.5 are considered indicative of erythrocyte regeneration or myeloid suppression.[21] Special histochemical stains for iron (Prussian blue stain) are available to evaluate peripheral iron stores in the horse.[22] In many cases of hemolytic anemia, bone marrow macrophages may be visible with phagocytosed RBCs.

Myelophthisis is a reduction in the cellular elements in the bone marrow, frequently a result of myelofibrosis, proliferation of fibrous tissue in the marrow. Myelodysplasia implies accumulation of abnormal cells in the

bone marrow. These cells are often not dysplastic in the truest sense of the word but rather result from myeloproliferative neoplasia.

12.1.2.3 Iron Status Evaluation

Evaluation of systemic iron status is crucial to characterizing many hematologic abnormalities, especially in the investigation of anemias. Several laboratory parameters can aid in the identification and classification of abnormalities of iron metabolism. The peripheral blood smear may provide initial clues. Microcytic, hypochromic erythrocytes often are seen in animals with disturbances of iron metabolism. One may confirm these observations by calculating the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) of peripheral blood erythrocytes. Measurement of serum ferritin provides a reliable index of stored hepatic and splenic iron in the horse. Serum ferritin concentration in 28 normal horses was 152 ± 54.6 ng/ml. A bone marrow aspirate examined after special iron stains is also an excellent means of assessing the pool of storage iron. Serum iron concentration reflects the total quantity of transport iron in the plasma (i.e., the quantity of iron bound to transferrin). Normal equine values of 120 ± 5.0 μg/dl[23] and 108 μg / dl[24] have been reported. The total iron-binding capacity (TIBC) reflects the amount of iron that plasma transferrin could bind if fully saturated. Normal TIBC in the horse has been reported as 388 ± 8.1 μg/dl.[23] One calculates the percentage of transferrin saturation from the serum iron concentration and the TIBC. Percentage transferrin saturation is normally approximately 30%. Serum iron concentration increases dramatically for 48 to 72 hours after administration of corticosteroids, but TIBC and serum ferritin concentrations do not.[25]

12.1.3 DISORDERS OF THE ERYTHRON

12.1.3.1 Erythrocytosis

Erythrocytosis or polycythemia is a real or apparent increase in the circulating RBC mass and may be classified as relative, caused by a decrease in plasma volume, or absolute, caused by a real increase in RBC numbers. Animals with persistent erythrocytosis have muddy red to blue mucous membranes, prolonged capillary refill time, weakness, lethargy, and exercise intolerance. Despite the large RBC mass in the circulation, oxygen delivery to the peripheral tissues often decreases because of increased blood viscosity and sludging in small vessels. Persistent erythrocytosis may lead to complications including hypertension, tissue hypoxia, thrombosis, and hemorrhage.[26] 726

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12.1.3.1.1 Relative Erythrocytosis

Relative erythrocytosis or polycythemia may occur because of hemoconcentration or splenic contraction. Hemoconcentration occurs when total plasma volume decreases without any change in total RBC numbers in circulation. Clinical dehydration is usually evident, with slow capillary refill time, dry mucous membranes, and prolonged skin tenting. Dehydration may occur with excessive water losses accompanying diuresis, diarrhea, or excessive sweating or with decreased water intake. Endotoxic shock results in dehydration and hemoconcentration by causing a shift of water from plasma to the interstitial space. An increase in total plasma protein usually accompanies relative erythrocytosis caused by hemoconcentration. An exception to this occurs with concurrent loss of protein and body water (e.g., in horses with severe protein-losing diarrhea or glomerulonephritis). In these horses, total plasma protein may be normal or

decreased in the presence of a high PCV. The PCV of hemoconcentrated horses usually returns to normal after intravenous fluid therapy, unless water losses continue.

The spleen of the resting horse may harbor up to one third of the total circulating erythrocyte volume. Exercise, endogenous epinephrine release caused by excitement or stress, and exogenous epinephrine administration results in splenic contraction that may increase the PCV by as much as 50%.[27–30] This relative erythrocytosis is not accompanied by a significant increase in total plasma protein. If the horse is allowed to relax in a nonstressful environment, PCV usually returns to normal within a few hours.

12.1.3.1.2 Absolute Erythrocytosis

Absolute erythrocytosis occurs when increased erythropoiesis causes an increase in PCV, RBC count, and hemoglobin concentration. Plasma volume and plasma protein concentration remain normal.

Primary absolute erythrocytosis (including polycythemia vera) is rare and is considered a myeloproliferative disorder of the bone marrow.[26] Serum erythropoietin concentrations decrease to normal and Pao_2 is normal. This condition may be accompanied by thrombocytosis or leukocytosis. Severe erythrocytosis has been described in two horses with normal serum erythropoietin levels.[26,31]

Secondary absolute erythrocytosis may be appropriate or inappropriate, depending on the presence or absence, respectively, of tissue hypoxia. Appropriate secondary absolute erythrocytosis is associated with increased circulating erythropoietin levels caused by chronic hypoxia from right-to-left shunting of blood in the heart or great vessels, chronic pulmonary disease, or adaptation to high altitude. The most common cardiac anomalies associated with erythrocytosis are complex defects such as tetralogy or pentalogy of Fallot, although a number of other defects, including ventricular septal defect, eventually may result in right-to-left shunting and secondary erythrocytosis.[32] Horses with this condition frequently have cyanotic mucous membranes and an audible heart murmur. One can document chronic hypoxia by measuring the Pao_2. Oxygen saturation concentrations of less than 92% usually are required for stimulation of erythropoiesis.[26,33] One may confirm the diagnosis with cardiac radiography, ultrasonography, angiocardiography, or cardiac catheterization with blood gas and pressure analyses.

Erythrocytosis is rare in horses with chronic lung disease. Auscultation of animals with chronic lung disease severe enough to result in hypoxia likely would reveal abnormal lung sounds such as wheezes and crackles. Thoracic radiographs aid in assessing the extent and severity of the problem. One should direct treatment of these animals toward correction or alleviation of the primary problem. If this is not possible, periodic phlebotomy and removal of several liters of blood helps decrease blood viscosity and may improve the quality of life of these horses.

Inappropriate secondary absolute erythrocytosis occurs following increased erythropoietin or other hormone release in the absence of tissue hypoxia. Although reported in a horse with metastatic carcinoma,[34] this condition is most common in horses with hepatic neoplasms or renal abnormalities. These animals have a normal or nearly normal Pao_2. In human beings, hydronephrosis, renal cysts, and embryonal nephromata have been associated most frequently with this paraneoplastic syndrome. In the horse, secondary absolute erythrocytosis has been described along with hepatocellular carcinoma and hepatoblastoma. All affected horses have been less than 3 years of age, and concurrent elevations in serum erythropoietin and/or α-fetoprotein concentrations have been described.[35–37] These horses had a persistently elevated PCV that did not decrease with intravenous fluid therapy, normal plasma protein concentrations, and mild to moderate

elevations in hepatic enzymes. A horse with erythrocytosis following metastatic carcinoma also has been described.

Most horses with primary or secondary erythrocytosis have a guarded to poor prognosis. If an underlying disease exists, one should treat it appropriately. Intermittent phlebotomy may provide supportive therapy.[26]

12.1.3.2

Anemia

Anemia is a decrease in the circulating RBC mass caused by an imbalance in the rate of loss or destruction of erythrocytes and the rate of their production in the bone marrow. Anemia is not considered a primary diagnosis but rather a hematologic abnormality resulting from an underlying disease process. *Anemia* is defined most frequently as a decrease in PCV or RBC numbers. Hemoglobin also is decreased, except in cases 727
of intravascular hemolysis. A systematic approach to characterization of the anemia can provide valuable clues 728
in the diagnosis of an underlying disease process. All anemias may be classified as regenerative or nonregenerative based on bone marrow response to the decrease in circulating RBC mass. Regenerative anemia results from loss of intact erythrocytes from circulation (hemorrhage) or accelerated destruction of RBCs (hemolysis) and is characterized by an increase in effective erythropoiesis in the bone marrow. Nonregenerative anemia occurs following systemic abnormalities or because of intrinsic bone marrow disease and results from a lack of appropriate marrow erythropoiesis in response to normal or accelerated RBC senescence or destruction.

Anemia also may be characterized on the basis of erythrocyte size (MCV) and hemoglobin content (MCHC). Normocytic normochromic anemia accompanies many chronic systemic disease processes, including renal and hepatic failure, endocrine abnormalities, neoplastic conditions, and chronic infections. Microcytic hypochromic anemia (low MCV and MCHC) is associated classically with iron deficiency and megaloblastic anemia. Macrocytic anemia (increased MCV) occasionally occurs in horses after a severe hemolytic or hemorrhagic crisis.

Clinical signs of severe anemia relate to decreased tissue oxygenation and the physiologic compensatory mechanisms intended to alleviate this hypoxia. Signs include pallor of the mucous membranes, tachycardia, polypnea, weakness, lethargy, and a systolic heart murmur caused by decreased viscosity and increased turbulence of the blood as it flows through the heart and great vessels. Horses with mild to moderate anemia may have no obvious clinical signs or may have only lethargy and slightly pale mucous membranes. Other clinical signs, including fever, icterus, and hemoglobinuria, may be present in anemic horses and reflect the primary pathophysiologic process involved.

12.1.3.2.1

Regenerative Anemia

When erythrocytes are lost from the circulation at an accelerated rate by hemorrhage or hemolysis, the bone marrow responds by increasing its rate of erythrocyte production and release into the peripheral circulation. Documentation of an active regenerative response is important in diagnosis and prognosis of many cases of anemia. An increase in the number of reticulocytes in the peripheral circulation and an increase in the MCV are considered accurate indicators of a regenerative response in the bone marrow of most species. Unfortunately, in the horse, reticulocytes are not released into the peripheral circulation, even during a strong regenerative bone marrow response. Increases in MCV are inconsistent but slightly more common after hemolysis than after acute blood loss.[38–40] The only change in equine peripheral blood after acute

hemorrhage or hemolysis may be a slight anisocytosis that is quantitatively assessed through changes in RBC distribution width.[40–42]

Because of the difficulty in documenting a regenerative bone marrow response with conventional methods, several erythrocyte parameters have been investigated as markers of RBC regeneration in the equid.[43–45] Glucose-6-phosphate dehydrogenase, creatine,[43] and adenosine-5-triphosphate[46] increase in equine erythrocytes during a regenerative response, but tests for these are not available in most veterinary laboratories. The most practical, reliable method of assessing the erythrocyte regenerative response in an anemic horse is bone marrow analysis. A bone marrow M:E of less than 0.5 is considered evidence of erythrocyte regeneration.[21] Reticulocyte counts in the bone marrow are also indicative of a regenerative response in the horse. Normal equine bone marrow contains approximately 3% reticulocytes, but this may increase to as high as 66% in response to severe blood loss.[20,21]

12.1.3.2.1.1

Hemorrhage.

Blood loss anemia may develop acutely or chronically. In many cases the source of the hemorrhage is obvious, but in others is not. Internal hemorrhage (into body cavities) permits the body to reuse blood components. Approximately two thirds of the erythrocytes lost into the abdomen or thorax are autotransfused back into the circulation within 24 to 72 hours. The other one third are lysed or phagocytized and the iron and protein are reused. External hemorrhage (including hemorrhage into the gastrointestinal tract) prevents reuse of these components. Accelerated bone marrow erythropoiesis is usually evident by 3 days after acute hemorrhage and is maximal by 7 days.[11] Box 12-2 lists differential diagnoses for blood loss in the horse.

Clinical signs of hemorrhage in the horse vary depending on the duration and severity of blood loss. Horses may lose up to one third of their blood volume acutely without dying. Acute loss of large quantities of blood results in severe hypovolemic shock with tachycardia, polypnea, pale mucous membranes, poor venous distention, weakness, and oliguria. Initially, RBC parameters in the remaining circulating blood appear normal because all blood components have been lost in equal volumes. Physiologic compensatory mechanisms induce redistribution of interstitial fluid into the vasculature and eventually result in decreased RBC numbers and total protein in the peripheral blood. This redistribution may take up to 24 hours after acute hemorrhage. The difficulty in estimating the severity of blood loss during the first 24 hours after hemorrhage is compounded by the many erythrocytes that can be stored in the equine spleen and released into the circulation as a consequence of endogenous catecholamine release. After vascular equilibration, hematologic parameters should reveal a decrease in PCV, total RBC 728
count, and hemoglobin with no change in associated RBC indices (MCV, MCHC, and MCH). Anemia 729
caused by hemorrhage usually is accompanied by panhypoproteinemia because of significant loss of plasma proteins. A neutrophilic leukocytosis commonly is apparent by 3 hours after hemorrhage; platelets may increase as well if they have not been consumed by excessive coagulation.[11]

12.1.3.2.1.1.1

BOX 12-2 DIFFERENTIAL DIAGNOSES FOR BLOOD LOSS IN THE HORSE

12.1.3.2.1.1.1.1

Epistaxis

Guttural pouch mycosis

Pulmonary abscess

Exercise-induced pulmonary hemorrhage

Ethmoid hematoma

Par anasal sinus abscess or infection

Traumatic nasogastric intubation

Upper respiratory tract neoplasm

Coagulopathy

Trauma

Pneumonia/pleuritis

12.1.3.2.1.1.1.2

Hemothorax

Thoracic trauma

- Fractured rib
- Lacerated heart or vessels

Ruptured pulmonary abscess

Ruptured great vessel

Neoplasia

Coagulopathy

12.1.3.2.1.1.1.3

Hematuria

Pyelonephritis

Cystitis/urolithiasis

Neoplasia

Trauma

Urethral ulceration

Coagulopathy

12.1.3.2.1.1.1.4

Hemoperitoneum

Trauma

- Splenic rupture
- Hepatic rupture

Mesenteric vessel rupture

Verminous arteritis

Uterine artery rupture

Abdominal abscess

Neoplasia

Coagulopathy

12.1.3.2.1.1.1.5

Gastrointestinal Conditions

Ulcerations

Nonsteroidal antiinflammatory drug toxicity

Parasites

- *Strongylus vulgaris*
- Small strongyles

Granulomatous intestinal disease

- Histoplasmosis
- Tuberculosis
- Granulomatous enteritis

Neoplasia

- Squamous cell carcinoma

Lymphosarcoma

Coagulopathy

12.1.3.2.1.1.1.6 External Conditions

Trauma

Surgical complication

Coagulopathy

External parasites

One may diagnose blood loss into the thoracic cavity by thoracic radiography, ultrasound, or thoracocentesis and may diagnose blood loss into the abdominal cavity by ultrasound or abdominocentesis. One may differentiate intraabdominal hemorrhage from inadvertent splenic aspiration or laceration of a subcutaneous blood vessel by the presence of erythrophacogytosis and absence of platelets and may differentiate recent hemorrhage into the abdominal cavity from diapedesis across compromised bowel or old hemorrhage by the presence of platelets in fresh blood. One may observe gastrointestinal blood loss as melena or hematochezia. More commonly, gastrointestinal hemorrhage is not severe enough to result in these overt signs, and feces appear normal. One may confirm a diagnosis by a simple and inexpensive fecal occult blood test.

Effective treatment of acute blood loss anemia must begin with finding and eliminating the source of hemorrhage. In many animals, this source is readily apparent, and direct pressure or surgical ligation of ruptured vessels is indicated. Internal hemorrhage is more difficult to control because immediate surgery to access the site of hemorrhage is usually not an option, as is most commonly the case in peripartum 729
hemorrhage of brood mares. Older mares[47–49] are at greatest risk for fatal hemorrhage because of rupture 730
of middle uterine, uteroovarian, or external iliac arteries.[49,50] A link between age and declining serum copper concentrations, vascular degeneration, and fatal hemorrhage has been proposed.[50] Therapy for mares with ruptured middle uterine artery is generally supportive with analgesics, sedatives to relieve anxiety and lower blood pressure, and oxytocin to enhance uterine involution. Blood transfusions, fluid therapy, hypertonic saline, and polymerized ultrapurified bovine hemoglobin have been administered to affected mares with variable success. However, volume expansion has the potential risk of exacerbating hemorrhage if one cannot achieve hemostasis.

In horses with acute severe hemorrhage the administration of isotonic crystalline intravenous fluids may aid tissue oxygenation by increasing peripheral blood volume and perfusion pressures. Administration of hypertonic saline solution (4 to 5 ml/kg of 7.5% NaCl) may be beneficial for rapid volume expansion after achieving adequate hemostasis.[51] Polymerized ultrapurified bovine hemoglobin is an alternative oxygen-carrying fluid that has been administered to horses in lieu of whole blood transfusion.[52,53] Although expensive, this product has the advantages of compatibility without crossmatching and long shelf life.

If 20% to 30% of the total blood volume is lost acutely, blood transfusion from a compatible donor horse is indicated. More detailed discussion of principles of blood and blood product therapy occur later in this

chapter. Hemorrhage into a large body cavity frequently is followed by autotransfusion of erythrocytes back into the peripheral circulation. Therefore one should not remove blood from these spaces unless necessary to stop continued bleeding, because the blood is interfering with vital organ function, or to lavage potentially septic sites.

Horses with subacute hemorrhage (over a few days) usually begin to show clinical signs of severe anemia when the PCV declines to 15% to 20%. The same clinical signs may not be noticeable in horses with chronic blood loss (over weeks to months) until the PCV reaches 12% or less. Because these laboratory parameters are so variable and potentially misleading, one should base treatment decisions on the clinical signs in the horse and not necessarily on the clinical laboratory parameters. A horse with subacute to chronic blood loss anemia severe enough to result in peripheral tissue anoxia because of inadequate RBC mass to supply needed oxygen exhibits tachycardia, polypnea, pale mucous membranes, weakness and lethargy, and possibly a mild to moderate systolic heart murmur caused by reduced viscosity and increased turbulence of the blood. If these signs are severe, blood transfusion is indicated. As with acute hemorrhage, one must identify the source of the blood loss. Other treatment is supportive and should be designed to eliminate the primary problem. If blood loss has occurred over weeks to months, body iron stores may be depleted, a particularly common problem with chronic gastrointestinal bleeding. Depletion of iron slows or stops effective bone marrow erythropoiesis so that these animals have laboratory parameters typical of iron deficiency anemia. One may identify low serum iron, low marrow iron stores, and increased TIBC, accompanying a hypochromic, microcytic anemia. One may supplement dietary iron orally with ferrous sulfate at a dose of 2 mg/kg body mass. One should not administer iron dextran parentally to horses because it has been associated with sudden death in this species. If one must administer iron intravenously, iron cacodylate solutions are available.

Because of the lack of peripheral signs of a bone marrow regenerative response in the horse, accurately assessing the regenerative efforts of bone marrow after hemorrhage may be difficult. One may evaluate serial bone marrow aspirates if this information is critical, but in most cases, monitoring increases in PCV over time is adequate. After experimental phlebotomy to mimic blood loss anemia in the horse, PCV increased about 0.672% per day, more slowly than in other species.[46] Experimentally, the bone marrow erythroid response peaked at 9 days after phlebotomy, coinciding with the lowest marrow M:E. Regeneration of the erythroid compartment was incomplete 31 days after hemorrhage.[54]

12.1.3.2.1.2 Hemolysis.

Intravascular or extravascular destruction of erythrocytes can occur in a variety of disorders. During intravascular hemolysis, hemoglobin released from destroyed erythrocytes combines with plasma haptoglobin, and tissue mononuclear phagocytes remove the haptoglobin-hemoglobin complex. Plasma haptoglobin levels decrease as intravascular hemolysis increases.[55,56] When plasma haptoglobin binding is exceeded, free hemoglobin accumulates in the plasma and is eliminated via the kidneys. Thus the hallmarks of intravascular hemolysis are hemoglobinemia and hemoglobinuria. Horses recovering from a severe hemolytic episode are more likely to have a demonstrable increase in MCV than are horses recovering from acute blood loss.[38,39,57]

Acute hemolytic anemia of adult horses has been associated with a variety of pathologic conditions (Box 12-3). Dimethyl sulfoxide administered intravenously at concentrations of 50% or greater results in severe intravascular hemolysis.[58] Hemolytic-uremic syndrome is characterized by acute renal failure, microangiopathic hemolytic anemia, and intravascular coagulation. Erythrocytes become damaged as

they pass between fibrin strands deposited in the lumen of small renal vessels. Thrombocytopenia may occur concurrently.[59,60] Hemolysis has been reported in horses ingesting leaves of the northern red oak (*Quercus rubra* L. var. *borealis*). Other associated clinical signs included abdominal pain, constipation, and increased coagulation times.[61] Administration of significantly hypotonic or hypertonic solutions to horses also may result in intravascular hemolysis.[51] All these conditions are uncommon in the horse. The most common causes of hemolysis are immune-mediated disease, oxidant-induced damage to erythrocytes, and infectious diseases.

12.1.3.2.1.2.1

BOX 12-3 DIFFERENTIAL DIAGNOSES FOR HEMOLYSIS IN THE HORSE

12.1.3.2.1.2.1.1

Infectious Diseases

Piroplasmosis

Equine infectious anemia

12.1.3.2.1.2.1.2

Immune-Mediated Disease

Autoimmune disease

Idiopathic

Secondary

- Bacterial infection
  - *Clostridium perfringens*
  - Streptococcal infections
- Viral infection
  - Equine infectious anemia
- Neoplasia
  - Lymphosarcoma
- Drug reaction
  - Penicillin

Neonatal isoerythrolysis

12.1.3.2.1.2.1.3

Oxidative Injury

Phenothiazine

Onion

Red maple leaf

Familial methemoglobinemia

12.1.3.2.1.2.1.4

Iatrogenic Conditions

Hypotonic solutions

Hypertonic saline

12.1.3.2.1.2.1.5

Miscellaneous Conditions

Hepatic disease

Hemolytic uremic syndrome

Disseminated intravascular coagulation

12.1.3.2.1.2.1.6

Other Toxicities

Intravenous dimethyl sulfoxide

Bacterial toxins *(Clostridium)*

Oak

12.1.3.2.1.2.2

Immune-Mediated Hemolytic Anemia (IMHA).

IMHA develops when an animal produces antibodies that attach to the surface of RBCs. Primary IMHA is an autoimmune process in which antibodies are directed against surface antigens and occurs when a normally suppressed B lymphocyte clone proliferates and produces an antibody directed against normal RBCs.[62] Secondary IMHA is more common than true autoimmune disease. Antibodies attach to the surface of erythrocytes for one or more reason: (1) alterations in the RBC membrane produced by a primary viral, bacterial, or neoplastic disease process; (2) antigen-antibody complex deposition on the surface of RBCs; or (3) drugs that cause immunoproteins to react indirectly with RBCs.

Drug-induced immune-mediated hemolysis may occur via three mechanisms[63]:

1. The drug may combine with RBC membranes and may be recognized as foreign by the body. An antibody to this new antigen develops and destroys the drug-coated erythrocytes. These animals have a positive direct Coombs' test.

2. The drug may complex with a carrier molecule in the blood and induce an immune response, and the drug-carrier-antibody complex attaches to RBC membranes in a complement-mediated process that leads to hemolysis.

3. Occasionally, a drug may induce true autoantibody production that leads to RBC destruction.

Antibody-coated RBCs are unable to pass through the microcirculation of the spleen, become sequestered there, and are destroyed or phagocytized. If RBC membrane is lost in excess of intracellular contents, spherocytes with an increased osmotic fragility form. Most immune-mediated hemolysis is extravascular, but if the antibody fixes and activates complement, intravascular complement-mediated hemolysis may result.[12]

IMHA may be classified as warm or cold hemagglutinin disease based on the optimal temperature at which the autoantibody agglutinates the host RBCs. Many clinical pathologists believe that these designations can be arbitrary and reflect an in vitro phenomenon rather than in vivo pathogenesis. Classically, in cold hemagglutinin disease the antibody involved is IgM. Complement is activated, and the liver is the primary site of removal of injured RBCs from the circulation. In warm hemagglutinin disease, IgG more commonly is involved, and the spleen is the primary site of RBC sequestration.[12,64,65] Warm hemagglutinins are frequently incomplete (i.e., they do not cause autoagglutination).[64] Warm[66,67] and cold[64,68] hemagglutinin anemia have been described in the horse.

Primary and secondary IMHA are uncommon in the adult horse.[42] Onset of disease is usually insidious, and many horses have fever, lethargy, and weight loss or have signs referable to a primary disease process. Routine hematologic and biochemical analyses frequently reveal decreased RBC 731 numbers, spherocytosis, increased MCV, 732 anisocytosis, and an increased total and indirect bilirubin. Most cases of immune-mediated hemolysis involve extravascular RBC destruction, but if intravascular hemolysis is occurring, hemoglobinuria will occur. One confirms the diagnosis by documenting a regenerative erythropoietic response in the bone marrow, increased erythrocyte fragility in hypotonic saline, autoagglutination, and a positive direct antiglobulin or Coombs' test. The Coombs' reagent used should contain antisera to IgG, IgM, and C3. One must differentiate autoagglutination from rouleau formation by dilution of a cell suspension with isotonic saline. Saline disperses rouleau formation but does not affect true autoagglutination.

In human beings, IMHA has been associated with lymphoreticular neoplasms, a variety of viral and bacterial diseases, inflammatory or granulomatous diseases, and generalized autoimmune disorders such as systemic lupus erythematosus. One may have difficulty distinguishing between primary AIHA, in which autoantibodies are directed against an abnormal epitope on the erythrocyte membrane, and nonspecific immune complex deposition on the surface of erythrocytes, usually occurring via attachment to Fc or complement receptors. In the horse, immune-mediated hemolysis has been reported in association with *Clostridium perfringens* septicemia,[42,65] an unclassified respiratory infection,[42] purpura hemorrhagica,[68,69] a streptococcal abscess,[68] and lymphosarcoma.[13,68,69] Regardless of the exact sequence of events leading to antibody deposition on the surface of erythrocytes, affected horses show clinical signs similar to those described for primary AIHA, and clinical pathologic data and diagnosis are similar.

IMHA after treatment with penicillin has been documented in horses.[70–73] Most affected horses have not been hemoglobinuric, indicating predominantly extravascular hemolysis. IMHA in the horse also

has been associated with administration of trimethoprim-sulfamethoxazole.[74] Although not documented as causing immune-mediated hemolysis in the horse, drugs that have caused this problem in other species include tetracycline, rifampin, cephalosporins, chlorpromazine, and various nonsteroidal antiinflammatory drugs (NSAIDs). In all reported cases of drug-induced IMHA in horses, the problem has resolved with discontinuation of administration of the offending therapeutic agent and appropriate supportive care.

Neonatal isoerythrolysis is an important form of immune-mediated hemolysis in the foal[75] and is discussed in detail elsewhere in this text.

Immune-mediated anemia resulting in intravascular and extravascular hemolysis may be present in horses with equine infectious anemia (see the following discussion). Infected horses may have a positive result on the Coombs' test or exhibit autoagglutination, especially during acute disease. Immune-mediated anemia also has been described in a horse with systemic lupus erythrematosus.[76]

Therapy of immune-mediated hemolysis is similar regardless of the initiating factor(s) involved. One should discontinue any previously administered drug and treat specifically underlying primary disease processes. If hemolysis is severe and life-threatening, the veterinarian should administer blood transfusions from a compatible donor (see the discussion on blood transfusion). If the horse has severe intravascular hemolysis with hemoglobinuria, one should initiate intravenous fluid therapy to protect against pigment-induced nephropathy.

Several cases of immune-mediated hemolysis in the horse have been at least transiently responsive to corticosteroid therapy.[13,42,66] One horse experienced complete disease remission after a 10-week regimen of glucocorticoids.[62] Parenterally administered corticosteroids are recommended initially, and dexamethasone is probably the drug of choice. One should titrate the amount and frequency of administration to the response of the individual animal, but one may try an initial dose of 20 to 30 mg dexamethasone in an adult horse. Ideally, administration of corticosteroids in the morning minimizes interference with the circadian rhythm of endogenous corticosteroid release from the adrenal gland. However, in an acute hemolytic crisis, twice daily administration of dexamethasone is recommended until RBC numbers cease to decline. After stabilization, one should decrease corticosteroid doses to once daily, then every other day, and gradually discontinue the therapy. If the horse requires long-term therapy, one may administer prednisolone orally with a declining every-other-day dosage. Potential complications of corticosteroid therapy in the horse include laminitis and secondary infections. Exacerbation of primary infectious conditions also may occur in cases of secondary autoimmune hemolysis treated with corticosteroids. Caution is recommended, especially when using corticosteroids at a high dose or for a prolonged period. One horse with IMHA refractory to corticosteroid therapy was treated successfully with cyclophosphamide at 1.1 mg/kg intramuscularly once daily and azathioprine at 1.1 mg/kg intramuscularly once daily.[77]

Oxidative injury to RBCs is evident from Heinz body formation, hemolytic anemia, and methemoglobinemia. Heinz bodies are aggregations of oxidized precipitated hemoglobin. They are small, round, blue-black, refractile granules near the cellular margin of erythrocytes stained with new methylene blue.[78] Heinz bodies damage the erythrocyte membrane, disturbing intracellular tonicity and resulting in rupture of the cell (intravascular hemolysis). Cells of the mononuclear/phagocyte system of the spleen and liver may take less damaged RBC membranes as abnormal and remove the RBCs from the peripheral circulation (extravascular hemolysis).

Oxidation of iron in the hemoglobin molecule from the normal ferrous state (Fe^{2+}) to the ferric state (Fe^{3+}) forms methemoglobin. Erythrocytes normally produce methemoglobin, which cellular enzymes rapidly convert back to hemoglobin. Also, cellular GSH acts to decrease the amount of methemoglobin formed by competing with hemoglobin for oxidizing agents.[79] Methemoglobin is incapable of carrying oxygen, and excessive accumulation results in a brownish discoloration of blood. Methemoglobin formation alone does not result in hemolysis,[80] but toxins simultaneously may oxidize the sulfhydryl groups of the globin moiety of methemoglobin. This oxidation causes methemoglobin denaturation and precipitation, Heinz body formation, and intravascular hemolysis. Pathologic methemoglobin accumulation in the blood most frequently results from exposure to an oxidative toxin that overwhelms the protective mechanisms of the body but also may result from a decreased rate of reduction of physiologically produced methemoglobin. Familial methemoglobinemia and hemolytic anemia caused by decreased erythrocyte GSH reductase and intracellular GSH have been described in two Trotter mares.[81]

Acute hemolytic anemia and methemoglobinemia can develop in horses after ingestion of wilted red maple *(Acer rubrum)* leaves.[57,82–84] Freshly harvested leaves are not dangerous but become toxic when dried and remain so for at least 30 days. Overnight freezing of the leaves does not affect toxicity. Affected horses may experience brownish discoloration of the blood (massive methemoglobinemia) and peracute death within 12 to 18 hours of exposure. A more prolonged hemolytic syndrome characterized by icterus, methemoglobinemia, hemoglobinuria, bilirubinemia, bilirubinuria, and possibly death may occur 5 days or more after ingestion of wilted leaves. Laboratory evaluation of horses with the hemolytic syndrome of red maple leaf toxicosis frequently reveals Heinz body anemia, depletion of RBC-reduced GSH, and increased RBC osmotic fragility.[83] Normal methemoglobin concentration in equine blood is 1.77% of total hemoglobin.[81] Horses with red maple leaf toxicosis may have methemoglobin concentrations of close to 50% of total hemoglobin.[84]

Treatment of horses with red maple leaf toxicity includes eliminating access to the toxic leaves, whole blood or packed cell transfusions as needed, and general nursing care. The veterinarian should treat all exposed animals with activated charcoal via nasogastric tube to decrease absorption of toxin, even if several days have elapsed since exposure. Massive intravascular hemolysis may result in pigment-induced nephropathy. Fluid diuresis is indicated to prevent or treat this complication. One should use potentially nephrotoxic drugs with caution. Some clinicians have suggested dexamethasone administration to stabilize cellular membranes.[82,85] Methylene blue therapy has been attempted in some horses without success.[57] Because methylene blue may potentiate hemolysis, its use is not advisable. Two horses with severe hemolysis and methemoglobinemia caused by ingestion of red maple leaves were treated with large doses of ascorbic acid (vitamin C).[85] Ascorbic acid is thought to convert methemoglobin to reduced hemoglobin and may supplement endogenous protective mechanisms.[86] One may give adult horses a dose of ascorbic acid at 30 mg/kg twice daily in intravenous fluids.[85]

Prognosis for horses with red maple leaf toxicosis is guarded to poor and is not correlated with PCV or methemoglobin concentration at the time of presentation. Horses are at risk for pigment nephropathy and acute anuric renal failure, colic, diarrhea, and laminitis.[84]

Outbreaks of Heinz body anemia have been reported in horses with access to unharvested onions[87] or overgrazed pasture covered with wild onions *(Allium canadense)*.[78] The toxic principle in onions is *n*-propyl disulfide. Treatment should include removal from access to the plants, activated charcoal via nasogastric tube to reduce absorption of the toxin, and blood transfusions and supportive care as indicated.

Heinz body anemia in horses also has been reported after phenothiazine administration. Toxicity appears to depend on individual susceptibility and some as yet unidentified environmental factors. Groups of animals may be affected at a dose that is generally considered safe, and animals in poor condition may be more susceptible to toxicity.[88] Although a 30-g dose for a 1000-lb horse is considered safe, one report described poisoning in six of nine horses treated with 25 g of phenothiazine.[89] Because phenothiazine is no longer widely used as an equine anthelmintic, reports of its toxicity are becoming rare.

Nitrate poisoning is associated with methemoglobin formation in horses but only rarely with intravascular hemolysis. The horse is believed to be more susceptible to nitrate poisoning than other simple-stomach species because the cecum and colon provide an optimal environment for microbial reduction of nitrate to nitrite.[57,90,91] Decreases in methemoglobin concentration have been reported after administration of methylene blue to horses with experimentally induced nitrate poisoning.[57]

Heinz body anemia has been reported in one horse with lymphosarcoma.[92] The authors could not confirm access to known oxidative toxins and presumed the anemia to be caused by the neoplastic process by an unidentified mechanism.

Equine infectious anemia virus (EIAV) is a viral disease of horses known colloquially in the United States as swamp fever because of its high prevalence in Gulf Coast states where climatic conditions are 733
favorable for transmission. The causative agent is a member of the lentivirus genus of the family 734
Retroviridae. Infected horses may have one of three clinical syndromes: acute infection, chronic infection, or inapparent carrier.[93,94]

Acutely infected horses show fever, lethargy, and anorexia within 30 days of exposure. The most consistent hematologic abnormality in these horses is thrombocytopenia, although anemia also may be present. Many acutely infected animals show few if any recognizable clinical signs, and definitive diagnosis may be difficult because some of these horses are not yet seropositive. Most horses seroconvert by 40 days after infection.

The horse chronically infected with EIAV has classic signs of recurrent fever, weight loss, ventral edema, and anemia. These animals are seropositive by the agar gel immunodiffusion (AGID or Coggins) test or by competitive enzyme-linked immunosorbent assay (C-ELISA). Each cycle of disease is associated with the emergence of a new antigenic strain of the virus, temporary evasion of the host immune response, and replication of the virus to high titer.

The anemia from which the disease derives its name results from intravascular and extravascular hemolysis of complement-coated RBCs and an impaired bone marrow response.[95–98] Each febrile episode is associated with thrombocytopenia, but platelet counts rebound rapidly as temperature

returns to normal. Thrombocytopenia may result from immune[99] or nonimmune[100] platelet destruction or decreased platelet production in the bone marrow.[101]

Most EIAV seropositive horses are clinically normal and never show any recognizable clinical signs. However, they have immune-related blood abnormalities (hyperglobulinemia, decreased percentage of $CD5^+$ and $CD4^+$ lymphocytes) consistent with ongoing viral activity.[102] These animals are inapparent carriers of the virus and remain infected for life. They have circulating infectious virus in their blood and remain a threat to other horses for the rest of their lives. Regardless of disease stage (acute, chronic, or inapparent carrier), virus is found in vivo primarily in tissue macrophages and endothelial cells.[103–105]

Transmission of EIAV occurs predominantly by the intermittent feeding of hematophagous insects such as horseflies and deerflies. Flies are strictly mechanical vectors, and virus does not appear to survive more than 30 minutes on their mouthparts. Chances of transmission are greatest when flies feed on horses undergoing a febrile, viremic episode. However, horseflies can transmit virus from inapparent carrier horses to uninfected animals under field conditions, so all seropositive horses must be considered infectious for life.

EIAV also can be transmitted iatrogenically with blood product transfusions and previously used or improperly sterilized needles, surgical instruments, tattooing instruments, dental equipment, or any other blood-contaminated materials. Virus occasionally is transmitted across the placental barrier from an infected mare to her foal. Mares experiencing a febrile episode during gestation are more likely to give birth to infected foals than are asymptomatic mares. Of foals born to chronic carrier mares, approximately 10% are virus and antibody positive.[106,107] Virus transmission to foals may occur by ingestion of colostrum and milk from infected mares.[107,108] Foals born to antibody-positive mares usually are seropositive for EIAV at 24 hours of age because of absorption of colostral immunoglobulins. This colostral immunity is usually undetectable by 6 months of age.[107,108]

The Coggins test and C-ELISA are recognized by the U.S. Department of Agriculture as valid and reliable for the diagnosis of EIAV. Good correlation has been reported between Coggins test results and C-ELISA results.[109] A few horses have been identified, however, with consistently negative or equivocal AGID results that were proved subsequently to be infected.[110,111]

No specific antiviral therapy for EIAV is available. Treatment of an animal requires supportive therapy as indicated during febrile episodes. Minimizing environmental stress may be helpful in decreasing the severity and recurrence of clinical signs. Intrastate travel of infected horses is regulated by state laws. Most state controls include options of euthanasia, some form of permanent identification with a 200-yard quarantine for the life of the horse, or shipping the animal to a recognized research facility.

Federal law prohibits interstate travel of horses that have tested positive for EIAV. Interstate movement of infected horses is allowed under three conditions: (1) back to the farm of origin; (2) to slaughter; and (3) to a diagnostic laboratory or approved research facility. Before interstate movement, reactors must be identified officially using the national uniform tag code number assigned by the U.S. Department of Agriculture to the state in which the reactor was tested, followed by the letter *A*. A hot iron, chemical brand, or freeze-marking may be used. Markings must be at least 2 inches high and applied to the left shoulder or left side of the neck. Lip tattoos should not be less than 1 inch high and ¾ inch wide and should be applied to the inner surface of the upper lip of the reactor. Veterinarians

who falsify health certificates of EIAV reactors may be reprimanded, fined up to $1,000, or lose their federal accreditation.[112]

Despite the significant decline in the incidence of EIAV in the United States since these control measures were implemented in the mid-1970s, propagating epizootics still occur.[113] Veterinarians should advise horse owners to do the following:

1. Require a negative EIAV test as part of every prepurchase examination. 734

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2. Require all new arrivals on a farm to have documentation of a recent negative EIAV test, and test all horses on the farm yearly.
3. Practice excellent fly control.
4. Encourage all events involving the congregation of horses to require documentation of a recent negative EIAV test.
5. Thoroughly disinfect any surgical items contacting equine blood before use on another horse.

Equine piroplasmosis results from infection with one or both of two species of hemoprotozoan parasite: *Babesia caballi* and *B. equi*. These intraerythrocytic parasites are found in subtropical locales and transmitted predominantly by tick vectors. Although classified in the genus *Babesia, B. equi* may be related more closely to the theilerial organisms.[114] *B. equi* undergoes some developmental stages in lymphocytes and apparently lacks transovarial tick transmission typical of most *Babesia* organisms.

Piroplasmosis is only enzootic in those areas where the tick vector can survive the winter. *B. caballi* infection has been diagnosed in horses in Florida and is spread by the tropical horse tick *Dermacentor nitens*. In the United States, *D. nitens* is found in southeastern Florida and occasionally in Texas. Only rarely has *B. equi* infection been confirmed in horses in the United States, and none of the ticks commonly found in this country are known to transmit this organism. Horses raised in *Babesia*-endemic areas frequently are infected with the organism(s) without ever showing recognizable clinical signs. Clinically recovered horses remain infected asymptomatic carriers of the organism as well, and stress may precipitate clinical relapse. Horses with *B. caballi* infection may clear the organism spontaneously after 12 to 42 months, whereas *B. equi*–infected horses do not appear to clear the organism spontaneously.[115] Infected animals develop a strong active immunity that depends on the continuing presence of the organism (premunity). They may be reinfected readily soon after the organism is eliminated from the body. No evidence of cross-protection exists between the two species of *Babesia*.[116]

Previously unexposed adult horses develop clinical signs of disease within 1 to 4 weeks of exposure. The horse may have fever, depression, dyspnea, pale or icteric mucous membranes, ecchymoses of the nictitating membrane, constipation, colic, and dependent edema. As anemia worsens, affected horses may develop diarrhea. Massive intravascular destruction of parasitized erythrocytes occasionally occurs, resulting in hemoglobinuria. Clinical disease with *B. caballi* lasts a few days to a few weeks, and mortality is usually low. Horses infected with *B. equi* generally have a more severe clinical course and may die within 24 to 48 hours of initial signs. Confirmation of diagnosis is by identification of the *Babesia* organism in blood smears stained with a Giemsa-type stain or by complement fixation test. The absence of *Babesia* organisms in the peripheral blood does not exclude the diagnosis of

piroplasmosis because parasitemia may be brief and occur before the onset of recognizable clinical signs.

Treatment of piroplasmosis varies depending on the location of the horse and the desired goal of treatment. In animals that reside in *Babesia*-endemic areas, suppressing clinical signs without eliminating the organism from the body is desirable, because premunition depends on the continued presence of the parasite at low levels. Clinical signs usually subside after one intramuscular injection of imidocarb dipropionate (Burroughs Wellcome Co., Research Triangle Park, North Carolina) at 2.2 mg/kg. Owners who wish to move their horses to or enter *Babesia*- free areas should isolate them from all tick vectors and should have them treated to eliminate the organism. *B. caballi* usually is eliminated after administration of imidocarb dipropionate at 2 mg/kg intramuscularly once daily for 2 days.[117] *B. equi* is a more difficult organism to eliminate from the horse, and four doses of imidocarb at 4 mg/kg intramuscularly at 72-hour intervals had variable efficacy in eliminating the carrier state.[118–120] In one study by Frerichs, Allen, and Holbrook,[119] the foregoing treatment regimen successfully cleared 13 of 14 horses of the infection, whereas in a more recent study by Kuttler, Zaugg, and Gipson, this treatment regimen cleared none of nine geldings of infection. This wide difference in efficacy may be because of strain differences in drug susceptibility.[118] Potential adverse effects of imidocarb administration include salivation, restlessness, colic, and gastrointestinal tract hypermotility. Donkeys appear to be sensitive to the toxic effects of imidocarb, and eight donkeys treated with the drug died.[119] Although the antitheilerial drug buparvaquone at 4 to 6 mg/kg intravenously or intramuscularly is therapeutically effective in horses acutely infected with *B. equi,* it is not consistent in clearing infection in carrier horses.[121]

12.1.3.2.1.2.3

Severe Hepatic Disease.

Acute intravascular hemolysis and anemia may develop in the terminal stages of acute or chronic hepatic failure in horses and is characterized by hemoglobinemia, hemoglobinuria, and icterus.[122] Onset and progression are rapid, and the condition is usually fatal. Affected erythrocytes have increased osmotic fragility. Hemolysis may result from decreased structural integrity of the erythrocyte membrane caused by alterations in exchangeable RBC membrane lipoproteins and the effect of bile acids on erythrocyte metabolism during liver failure.[12]

12.1.3.2.1.2.4

Microangiopathic Hemolysis.

This disorder occurs following thrombosis or fibrinoid change within the lumen of small blood vessels, 735
[123] is typical of chronic disseminated intravascular coagulation, and has been reported in horses.[59] The 736
resulting hemolyis is usually mild.

12.1.3.2.2

Nonregenerative Anemia

A variety of intrinsic or extrinsic factors may suppress normal bone marrow erythropoiesis, resulting in anemia caused by a failure to replace senescent RBCs adequately as they are removed from circulation (Box 12-4). Dietary factors often are incriminated in these abnormalities but only rarely are involved. Severe protein deprivation may result in decreased erythropoiesis as the body becomes deficient in synthesizing hemoglobin and other cellular proteins. Folic acid and cobalamin (vitamin B_{12}) deficiencies are known to

cause macrocytic hypochromic anemia in human beings, but they are associated rarely with anemia in domestic animals, including the horse. Horses do not have an absolute dietary requirement for vitamin B_{12}, which is produced by bacterial action in the gut and absorbed from the lower gastrointestinal tract.[124] Anemia associated with hypothyroidism is thought to be functional, following a lowered metabolic rate. Normocytic normochromic anemia responsive only to thyroid replacement therapy has been reported in one horse.[125] The most common disorders associated with nonregenerative anemia in the horse are iron deficiency, chronic inflammatory, endocrine, or neoplastic diseases, and generalized bone marrow failure.

12.1.3.2.2.1

BOX 12-4 DIFFERENTIAL DIAGNOSES FOR NONREGENERATIVE ANEMIA IN THE HORSE

12.1.3.2.2.1.1

Iron Deficiency

Chronic hemorrhage

Nutritional deficiency (rare)

12.1.3.2.2.1.2

Chronic Disease

Chronic infection/inflammation

- Pleuritis/pneumonia
- Peritonitis/enteritis
- Bacterial endocarditis
- Internal abscessation
- Chronic viral disease (e.g., equine infectious anemia)

Neoplasia

Endocrine disorders

12.1.3.2.2.1.3

Bone Marrow Failure

Myelophthisis

Myeloproliferative disease

Bone marrow toxins

- Phenylbutazone

Chloramphenicol

Radiation

Idiopathic pancytopenia

12.1.3.2.2.1.4

Miscellaneous Conditions

Administration of human recombinant erythropoietin

Chronic hepatic disease

Chronic renal diseae

Recent hemorrhage or hemolysis

12.1.3.2.2.2

Iron Deficiency.

Iron deficiency in the horse is associated only rarely with low dietary iron intake or absorption. Instead, iron deficiency results from chronic external blood loss. Iron deficiency can be divided into three stages, each of which varies in its laboratory abnormalities. Initially, the only detectable abnormalities reflect decreased iron storage pools: a low serum ferritin concentration and decreased stainable iron in the bone marrow. Progression of the condition affects erythropoiesis adversely. These animals have a decrease in the percentage saturation of plasma transferrin, an increased TIBC, and increased numbers of hypochromic erythrocytes (decreased MCHC). Fulminant iron deficiency anemia leads to abnormal development of erythrocytes in the bone marrow late in the maturation process. Cells continue to divide in the late rubricyte stage, without sufficient iron to continue heme synthesis. The result is the release of small cells with decreased hemoglobin concentration into the peripheral circulation (decreased MCHC and MCV).

Treatment of iron deficiency anemia should concentrate initially on the identification and elimination of the source of chronic iron loss. In the horse, occult blood loss occurs most frequently from the gastrointestinal tract and may be verified with a fecal occult blood test. Chronic gastrointestinal ulceration following phenylbutazone administration is a well-recognized phenomenon and is more frequent and severe in ponies than in horses. Gastrointestinal parasites, especially *Strongylus vulgaris* and small strongyles, occasionally may result in chronic blood loss and subsequent iron deficiency. Many iron-containing hematinics are available commercially, and oral supplementation with a product containing ferrous sulfate is probably best. Parenteral iron dextran solutions have been associated with fatal anaphylactoid reactions in horses. If parenteral administration is essential, 1 g of iron cacodylate administered intravenously for an adult horse generally is considered safe.

Serum ferritin concentration increases, and TIBC decreases over the first 24 hours of life in foals. Because serum iron concentrations and percentage saturation of transferrin are high during this period, oral iron supplementation is contraindicated within the first 2 days of life.[9,126] Administration of oral digestive inoculants containing ferrous fumarate to neonatal foals has been associated with a fatal toxic hepatopathy characterized by icterus, hepatic atrophy, bile duct hyperplasia, lobular necrosis, and intrahepatic cholestasis.[2,127]

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Over the next few weeks of life, serum ferritin decreases and then increases to normal adult values by 6 months of age. These ferritin changes occur concomitantly with opposite changes in serum TIBC, and together with a low MCV may indicate a functional iron deficiency in the foal.[126] Tissue demands for iron are high during periods of active growth, and foals may be more susceptible than adults to the development of iron deficiency anemia. In a survey of iron status in hospitalized horses, only six animals with iron deficiency were identified on the basis of low serum ferritin and serum iron concentrations. All six were foals younger than 5 weeks of age.[128] This hypothesis of marginal iron status during early life has been disputed by other researchers who saw no beneficial effect on hematologic variables when giving foals iron supplementation orally.[129]

12.1.3.2.2.3

Chronic Disease.

Chronic inflammatory conditions and neoplasms frequently are associated with a mild to moderate normocytic, normochromic, nonregenerative anemia. This anemia has been attributed to several abnormalities. A block of iron release from reticuloendothelial storage (ferritin and hemosiderin) results in an unavailability of iron for heme synthesis. One may confirm and differentiate defective iron metabolism from iron deficiency anemia by a normal to decreased TIBC, normal to elevated serum ferritin and bone marrow storage iron, and a normal to decreased percentage saturation of transferrin. In addition to altered iron mobilization, a defective response of the bone marrow to circulating erythropoietin and a decrease in RBC life span during many chronic diseases are apparent. The anemia of chronic disease rarely is associated with clinical signs of decreased tissue oxygenation. Therapy is directed at the primary disease process. Oral iron supplementation is not indicated because systemic iron stores are usually normal to increased.

12.1.3.2.2.4

Bone Marrow Suppression.

Anemia caused by selective bone marrow suppression of erythropoiesis is unusual in the horse. Selective erythroid hypoplasia has been reported as a sequela to administration of recombinant human erythropoietin to horses.[130,131] Anemia is thought to result from production of antibodies that cross-react with erythropoietin, inhibiting erythropoiesis. Treatment is with blood transfusions and corticosteroids. Prognosis for recovery is fair to guarded.

Pancytopenia (decreased circulating erythrocytes, leukocytes, and platelets) has been described in horses with aplastic bone marrow disorders[132–134] and with myelophthisic diseases. Myelophthisis is a reduction in the cellular elements of the bone marrow, frequently a result of metastatic neoplasia or myelofibrosis, a proliferation of fibrous tissue in the marrow. Myelodysplasia implies accumulation of abnormal cells in the bone marrow. These cells are not usually dysplastic in the truest sense of the word, but rather they result from myeloproliferative neoplasia. Hematopoietic neoplasia is rare in the horse and is discussed in more detail later in this chapter. These conditions usually result in concurrent anemia, leukopenia, and thrombocytopenia. Diagnosis is by identification of abnormal cells in bone marrow aspirates.

Bone marrow from horses with aplastic anemia is generally devoid of hematopoietic precursor cells because of a congenital or acquired failure of stem cell function. Acquired aplasia may be associated with bacterial and viral infections, chronic renal or hepatic disease, neoplasia, irradiation, or drug therapy. In most cases, a predisposing factor is not identified.[132–137] Because of the shorter life spans of the cells,

leukopenia and thrombocytopenia usually precede anemia.[11] Diagnosis depends on bone marrow biopsy. If one cannot obtain adequate marrow by serial aspirates, one must obtain a core biopsy sample. Hypoplastic marrow is yellow because of fatty infiltration with a mixture of cell types, but the number of erythroid, myeloid, and megakaryocytoid cells is decreased.[11] Aplastic anemia is suspected to be an autoimmune disorder in some cases, and some horses have apparently benefited from therapy with corticosteroids and anabolic steroids.[132] These drugs stimulate erythropoietin production and increase sensitivity of the stem cell receptors to erythropoietin.

Temporary or permanent bone marrow aplasia has been associated with the administration of a variety of drugs in other species. Transient hypoplastic anemia following phenylbutazone administration has been reported in one horse.[138] Aplastic anemia has been induced experimentally by feeding of trichlorethylene extracted soybean oil meal to horses or by irradiation.[139] Chloramphenicol produces transient and irreversible forms of bone marrow dysfunction in human beings, and similar reactions are possible in other species. One always should handle chloramphenicol with caution because human toxic reactions appear to be idiosyncractic and may be associated with absorption of low amounts of the drug. One should discontinue any ongoing drug therapy in horses with aplastic anemia and should administer supportive therapy. Most cases of drug-induced bone marrow failure are temporary, and hematopoietic function returns to normal with time if secondary complications are not severe. One may adiminster compatible whole blood transfusions to horses with severe anemia or thrombocytopenia, and one may consider systemic antibiotics and strict isolation from other animals for horses with severe leukopenia.

Bone marrow erythropoiesis frequently is suppressed in horses with chronic renal disease. Suppression generally is attributed to a decrease in erythropoietin production by the kidneys. Recombinant 737
erythropoietin has been used successfully in cats and human beings with severe anemia associated with 738
renal disease.

12.2 Leukon

The leukon consists of circulating leukocytes, their precursor cells, and the tissues that produce them. This includes the granulocytes (neutrophils, eosinophils, and basophils), monocytes/macrophages, and lymphocytes. The leukon provides the primary effector cells for immune surveillance and clearance. This discussion provides a brief description of the production of these cells and their quantitative abnormalities in peripheral blood. Excellent pictures of the various developmental stages of equine leukocytes in the bone marrow and peripheral blood are available in other texts, and the reader is referred to these for identification of cell types.[11,12]

12.2.1 NEUTROPHILS

12.2.1.1 Physiology

Granulocytes and mononuclear phagocytes originate from a common committed progenitor cell in the bone marrow. Under the influence of cytokines—including granulocyte-macrophage colony-stimulating factor, monocyte colony-stimulating factor, granulocyte colony-stimulating factor, and various interleukins—this stem cell proceeds along a path to granulocyte or monocyte production. Cytokine signals for constitutive leukocyte production differ from those during periods of increased demand for specific cell types. The progenitor cell undergoes successive mitotic divisions from myeloblast (the first recognizable precursor of neutrophils) to promyelocyte and myelocyte. Mitosis does not occur as the cell matures from metamyelocyte

to band cell to mature neutrophil. Mature neutrophils are stored in the bone marrow until needed. The peripheral neutrophil pool is equally divided between circulating cells and cells adhered to the endothelium of small vessels (the marginated pool).[140] Circulating neutrophils have a half-life of 10½ hours in the horse[141] and eventually migrate into peripheral tissues, where they live several more days.

Neutrophils alter their distribution between storage, circulating, and marginating pools in response to various endogenous and exogenous stimuli. The marginating neutrophils are mobilized into the circulating pool in response to exercise, epinephrine, or stress. Glucocorticoids increase the rate of neutrophil egress from the bone marrow storage pool and decrease egress from the circulation.[142,143]

Neutrophils are attracted to sites of infection and inflammation by soluble chemotactic factors released during proinflammatory reactions. Neutrophils ingest and kill invading microorganisms and release additional factors that further propagate inflammation at the site of tissue injury.

The morphology of neutrophils on a stained smear of blood or body fluids (e.g., peritoneal or pleural fluid) aids in interpretation of the significance and severity of many disorders. Toxic changes in circulating neutrophils occur in response to inflammation and include cytoplasmic basophilia, cytoplasmic granulation and vacuolation, and appearance of Döhle's bodies (slate gray inclusions caused by retention and aggregation of rough endoplasmic reticulum).[12] Degenerative changes result from altered cell membrane permeability and include hydropic degeneration of the nucleus and a spreading out of the nuclear chromatin so that it more completely fills up the cytoplasm of the cell. Aged neutrophils may exhibit hypersegmented, pyknotic nuclei with round, tightly clumped chromatin. Aged neutrophils most commonly are visible in tissue fluids. Idiopathic hypersegmentation of blood neutrophils of one Quarter Horse, unrelated to any clinical disease, has been described.[144]

12.2.1.2 Disorders of Neutrophils

12.2.1.2.1 Neutropenia

Neutropenia is a decrease in the number of circulating neutrophils and may be acute, occurring transiently over 24 to 48 hours, or chronic, lasting several days to months. Acute neutropenia most commonly results from a shift of neutrophils from circulating to marginating pools. Endotoxin is a potent stimulus for margination of circulating neutrophils with sequestration in pulmonary capillaries.[140] Experimentally, administration of endotoxin to horses results in neutropenia within 90 minutes and return to baseline numbers within 6 to 18 hours.[145] Endotoxemia is probably the common denominator in the neutropenia associated with various equine gastrointestinal disturbances including strangulating obstruction, peritonitis, enteritis, and salmonellosis. Neutropenia is a common finding in acute septicemia in the adult and is considered an aid for diagnosis of sepsis in the neonate.[146] A variety of bacterial, rickettsial, and viral disorders also may be associated with neutropenia in the horse.

Chronic neutropenia may result from increased peripheral use of neutrophils or decreased bone marrow production. Neutropenia may accompany severe infectious or inflammatory diseases such as pleuritis, pneumonia, peritonitis, internal abscessation, enteritis, burns, vasculitis, or immune-mediated diseases. Neutropenia caused by increased use often is accompanied by appearance of immature cells in circulation. As tissue demand increases, the bone marrow releases progressively more immature band cells into circulation.[11] This regenerative left shift is an appropriate response to high tissue demand for neutrophils. In 738

animals with a degenerative left shift, the number of immature band cells or metamyelocytes in circulation exceeds the number of mature neutrophils because tissue use exceeds the capacity of the bone marrow to increase production.[12] A degenerative left shift is considered a poor prognostic indicator.

Neutropenia caused by bone marrow suppression may occur in horses with pancytopenia from a variety of causes (discussed previously). Myeloproliferative disorders including granulocytic leukemia also may result in chronic neutropenia.[147] Immune-mediated neutropenia has been described in human beings and in foals.[148,149] A syndrome of neutropenia and thrombocytopenia is described in related Standardbreds. A heritable cyclic neutropenia was suspected. Most affected horses died from complications of infectious diseases and/or thrombocytopenia.[150]

Administration of canine or bovine recombinant granulocyte colony-stimulating factor to normal newborn foals results in a profound increase in blood neutrophil count.[151,152] Anecdotally, administration of the factor has induced the same response in foals with neutropenia following septicemia or alloimmune disease. Recombinant granulocyte colony-stimulating factor is reported to improve recovery of adult horses when administered after experimental bowel resection.[153]

12.2.1.2.2 Neutrophilia

Many of the same disorders associated with neutropenia may be associated alternatively with neutrophilia. Endogenous or exogenous glucocorticoids or epinephrine, excitement, exercise, or stress may result in neutrophilia.[12,154] Any infection or inflammation in any part of the body may result in neutrophilia. Many neoplastic conditions also are accompanied by peripheral neutrophilia. A rebound neutrophilia is common in later stages of endotoxemia.

The magnitude of a neutrophilic response is determined by the balance between bone marrow production and tissue use. A regenerative left shift is not uncommon in animals with neutrophilia because of excessive tissue demand. A severe neutrophilia with significant left shift including metamyelocytes and myelocytes indicates serious inflammatory disease and is termed a *leukemoid response* because of its similarity to granulocytic leukemia.[11]

12.2.2 EOSINOPHILS

Eosinophils arise from the same bone marrow precursor as neutrophils and mononuclear phagocytes. They may be distinguished first from neutrophils at the early myelocyte stage and eventually develop characteristic bright red staining cytoplasmic granules. IL-5 is critical in the differentiation and maturation of eosinophils. The prominent cytoplasmic granules of eosinophils contain a variety of substances, including major basic protein, peroxidase, and various hydrolytic enzymes. Eosinophils are important in parasite immunity and are involved in some hypersensitivity reactions. Eosinopenia may result from acute infections, corticosteroid or epinephrine administration or release, or stress. Peripheral eosinophilia most commonly results from parasitic infections, including habronemiasis, stronglylosis, and pediculosis. Occasionally, allergic reactions also may result in peripheral eosinophilia. Eosinophilic myeloproliferative disease has been described in the horse.[155] A marked eosinophilia also has been seen in horses with lymphosarcoma and transitional cell carcinoma.[156]

12.2.3 BASOPHILS

Basophils also originate from the common granulocyte/macrophage precursor cell and mature into cells with basophilic cytoplasmic granules. They are the least common of the circulating granulocytes, and basopenia is not clinically significant. Basophils live in the blood approximately 6 hours and then migrate into tissues, where they exist another 10 to 12 days.[12] Basophils are involved in mediating some hypersensitivity reactions. Increased numbers of circulating basophils may occur with allergic, inflammatory, or neoplastic diseases or in association with lipemia.

12.2.4 MONONUCLEAR PHAGOCYTES

Monoblasts are the first recognizable bone marrow precursors of peripheral blood monocytes, originating from a pluripotent marrow precursor. After progressing through the promonocyte stage, monocytes are released into the peripheral blood where they circulate for a few days before migrating into tissues to mature into tissue macrophages. The role of mononuclear phagocytes in orchestrating immune and inflammatory reactions has long been underplayed. These cells are critical in regulating inflammation through release of proinflammatory cytokines such as tumor necrosis factor, IL-l, and platelet-activating factor. Mononuclear phagocytes phagocytose microbial organisms, particulate debris, and possibly neoplastic cells. They process these foreign antigens and present them to T lymphocytes in a form that initiates specific immune responses. They produce cytokines, including granulocyte-macrophage colony-stimulating factor, that are critical for regulation of hematopoiesis. They are responsible for removal of senescent cells and activated coagulation factors from circulation. Quantitative abnormalities of monocytes rarely are encountered in equine medicine, but monocytosis may be observed in some cases of chronic inflammation.

12.2.5 LYMPHOCYTES

Lymphocytes are the primary mediators of humoral and cell-mediated immune responses. They originate from an uncommitted bone marrow progenitor cell that is capable of differentiating into a committed lymphopoietic precursor or a granulocyte/macrophage precursor cell. Committed lymphopoietic progenitor cells differentiate 739
740 into T lymphocytes that mature during migration through the thymus or B cells that appear to differentiate in the marrow and then migrate to lymph nodes. Circulating lymphocytes are predominantly T cells and represent only a small fraction of the total lymphocyte pool. Most lymphocytes reside in the spleen, lymph nodes, and other lymphoid tissue of the body.

The spleen contains the greatest number of lymphocytes in the adult horse. The spleen plays a major role in immune defense. The large number of phagocytic cells in the spleen facilitates filtering of senescent blood cells, particulate debris, and microorganisms from the blood. Hemoglobin is degraded and iron is stored in splenic phagocytes pending reuse for erythropoiesis. The spleen is also important as a reservoir of RBCs and platelets, as discussed previously.

Lymphopenia is associated with glucocorticoid administration, stress, many viral infections, and combined immunodeficiency of Arabian foals. Lymphocytosis is associated with epinephrine administration, excitement, exercise, lymphocytic leukemia, and chronic immune stimulation. As horses age, the number of lymphocytes in their peripheral blood gradually decreases.[10,157]

12.2.6 HEMATOPOIETIC NEOPLASIA

12.2.6.1 Myeloid Neoplasia

Myeloid neoplasia is characterized by the unregulated proliferation of a bone marrow–derived blood cell line. The eventual outcome is severe myelophthisis with loss of normal marrow elements. Forms of myeloid neoplasia described in horses include granulocytic leukemia,[147] myelomonocytic leukemia,[158–161] monocytic leukemia,[162] and eosinophilic myeloproliferative disorder.[155]

No age predilection is apparent for equine myeloid neoplasia, for affected horses have ranged from 10 months[155] to 9 years of age.[147] Predominant clinical signs include depression, weight loss, edema, anemia, and mucosal petechial hemorrhages. Fever,[155,158,160,161] peripheral lymphadenopathy,[158,161] hemorrhagic diathesis,[155] and oral ulcers[158,159] were identified in some of the horses. Severe destruction of normal marrow architecture by the neoplastic cell line results in myelophthisic disease characterized by inadequate production of erythrocytes, platelets, and normal leukocytes. Most horses are anemic and have thrombocytopenia. The total white blood cell count of affected horses may be elevated, normal, or reduced, but abnormal leukocytes invariably occur in the peripheral blood. Abnormal leukocytes dominate in bone marrow aspirates.

The cause of myeloid neoplasia remains undefined. Clinical signs are caused by the loss of normal blood cells, which predisposes horses to hemorrhagic diathesis, infections, and inadequate oxygenation of tissues. The diagnosis in all reported cases was based on abnormal morphology of the circulating neoplastic leukocytes; the type of cell was delineated by cytochemistry or special stains. Treatment with cytotoxic agents in one case of acute myelomonocytic leukemia was unsuccessful.[161] All horses died or were destroyed humanely because of the effects of myelophthisic disease. Postmortem examination revealed abnormal leukocytes in various organs in addition to the bone marrow, most commonly in the lymph nodes, spleen, and liver.

12.2.6.2 Lymphoid Neoplasia

The most common type of neoplasia to involve the equine hemolymphatic system is lymphosarcoma.[163] Four anatomic forms have been described (generalized, intestinal, mediastinal, and cutaneous) based on the major site of tumor involvement[163,164]; however, the forms overlap substantially clinically and pathologically. The typical age of onset is between 5 and 10 years,[165] although lymphosarcoma has been documented in horses ranging in age from birth through 25 years.[166–168] No sex predilection exists. The incidence of lymphosarcoma is unknown, but the reported prevalence of affected horses at postmortem examination is 2% to 5%.[169,170]

12.2.6.2.1 Cause and Pathogenesis

A viral cause of equine lymphosarcoma has not been documented[171,172]; however, one report describes viruslike particles in the lymph node of a foal with lymphosarcoma that died shortly after birth.[168] Clinical signs and laboratory changes of lymphosarcoma generally are caused by loss of normal organ and tissue function following infiltration by lymphocytes or physical obstruction by tumor masses or the excessive generation of tumor cell products. Early reports suggested that some neoplastic lymphocytes were of the T

lymphocyte lineage.[173,174] However, more recent reports suggest that equine malignant lymphomata are composed of a heterogeneous cell population. Immunohistochemical analysis of biopsy specimens from 31 horses with lymphosarcoma revealed that 13 horses had diffuse lymphomata derived primarily from B lymphocytes, 11 additional horses had diffuse large B cell lymphomata containing 40% to 80% nonneoplastic T lymphocytes (T cell–rich, large B cell lymphoma), and 6 tumors were derived from neoplastic T lymphocytes. One diffuse large cell lymphoma did not react with B or T cell markers.[175]

Decreased serum concentrations of IgM and other indications of immunosuppression have been noted in horses with lymphosarcoma.[174,176–178] Other neoplastic lymphocytes may arise from autoreactive B cell clones that produce antibodies responsible for gammopathies and immune-mediated cytopenias. Neoplastic proliferation of large granular lymphocytes with natural killer cell activity was described in one horse.[179] 740

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12.2.6.2.2 Clinical Signs and Laboratory Findings

The most common clinical signs of lymphosarcoma are chronic weight loss, ventral subcutaneous edema, and regional lymphadenopathy.[180,181] Peripheral lymphadenopathy is not observed frequently. Other clinical manifestations are highly variable depending on the organs involved and the duration of the disease process. Most clinical signs are progressive over weeks or months, although they may have a sudden onset.

Lymphosarcoma involving the thoracic cavity may cause tachypnea, dyspnea, cough, and pleural effusion.[166,182,183] If pleural effusion is present, one frequently identifies neoplastic lymphocytes by routine cytologic evaluation of fluid obtained by thoracocentesis.[183]

Extensive intestinal infiltration by neoplastic lymphocytes typically produces intestinal malabsorption, which contributes to hypoalbuminemia and weight loss. Affected horses frequently have abnormal glucose or xylose absorption indicative of extensive small intestinal malabsorption. In addition to weight loss, signs of gastrointestinal involvement may include colic or diarrhea.[184–186]

One may palpate splenic enlargement, internal lymphadenopathy, or abdominal masses on rectal examination. One may define the character of these masses further by ultrasonography.[187] Abdominocentesis may reveal inflammatory changes in the fluid (increased nucleated cell count and protein concentration), but identifying neoplastic cells in abdominal fluid of horses with alimentary or abdominal lymphosarcoma is not common.

The regional or generalized occurrence of multiple subcutaneous nodules with histopathologic characteristics of lymphosarcoma, unassociated with other lesions of lymphosarcoma, has been reported in horses.[163,188] Nodules may appear suddenly, grow slowly, remain static or regress, and recur at a later time. Hormonal factors including estrous cycle, pregnancy, lactation, and foaling may influence this cutaneous form of lymphosarcoma. In one horse, cutaneous lymphosarcoma cells were positive for progesterone receptors, and lesions regressed completely after surgical removal of an ovarian tumor.[189]

Tumor masses in localized areas may result in clinical signs referable to dysfunction of involved and adjacent tissues and organs.[171,180,182,190–195] Pharyngeal and laryngeal masses may result in dysphagia and unresponsive nasal discharge. Ocular lymphosarcoma may manifest as uveitis or as infiltrates in palpebral conjunctiva, eyelids, third eyelid, at the corneoscleral junction, or in the retrobulbar area.[196] Lymphosarcoma of the central nervous system may result in ataxia and cranial nerve deficits.[171,195,197]

The laboratory findings in horses with lymphosarcoma are highly variable. Hematologic indications of chronic inflammatory disease are common, including neutrophilic leukocytosis, nonresponsive anemia, hyperfibrinogenemia, and hypergammaglobulinemia.[181,182] Lymphocytic leukemia with peripheral lymphocytosis and large numbers of circulating neoplastic lymphocytes is rare and usually is associated with bone marrow involvement.[173,186,198–200] Although the lymphocyte count is usually normal or reduced, the presence of atypical or obviously neoplastic lymphocytes on a peripheral blood smear occurs in 30% to 50% of cases.[*] The total plasma protein concentration may be low, normal, or elevated, but the albumin:globulin ratio often is reduced, particularly with gastrointestinal involvement.[186,203] Polyclonal gammopathy is not unusual, and monoclonal gammopathy associated with serum hyperviscosity and a hemorrhagic diathesis has been described.[204] A mild elevation in liver-derived serum enzymes may occur following hepatic involvement. Hyperbilirubinemia usually is caused by anorexia or hemolysis.

A variety of paraneoplastic syndromes have been identified in association with equine lymphosarcoma. Hypercalcemia occasionally is reported.[192,205–207] Pseudohyperparathyroidism, resulting in hypercalcemic nephropathy and polyuria or polydypsia, has been reported in a horse with splenic lymphosarcoma.[208] In some patients, Coombs'-positive IMHA, immune mediated thrombocytopenia, or both may occur.[13,69,209] Hypereosinophilia has been reported in one pony with intestinal lymphosarcoma.[156] Concurrent intestinal lymphosarcoma and eosinophilic epitheliotrophic disease have been reported in a Paso Fino mare.[210] These two cases suggest the possibility that clonal proliferation of lymphocytes occasionally may result in hypersecretion of IL-5 with subsequent eosinophil activation. Paraneoplastic pruritus and alopecia also has been described in a horse with lymphosarcoma.[205]

* References 13, 173, 174, 181, 201, 202.

12.2.6.2.3

Diagnosis

The diagnosis of lymphosarcoma is by demonstration of neoplastic lymphocytes in affected tissue. Histologic examination of a biopsy from a tumor mass or affected lymph node is the most reliable method, and excisional biopsies are much preferred over needle biopsies or aspirates. Without lymph nodes or other masses accessible for biopsy, antemortem diagnosis is often difficult.[13,166,194,211] Diagnosis is only rarely possible on a peripheral blood smear[173,199] and generally requires careful cytologic evaluation of bone marrow, pleural effusion, or peritoneal fluid.[182,212,213] One rarely may find neoplastic lymphocytes by transcutaneous liver biopsy[202] and may use laparoscopy to see a mass better for biopsy.[166] Radiography and ultrasonography may be useful in locating and perhaps enabling biopsies of masses in the thorax or 741
abdomen. Often, diagnosis of lymphosarcoma is possible only by exploratory laparotomy or postmortem 742
examination.[13,184,194,211]

The morphology of neoplastic lymphocytes in horses is highly variable.[167] On cytologic preparations they often appear as large lymphoid cells with a variable nucleus-to-cytoplasm ratio, multiple nucleoli, nuclear chromatic clumping, cytoplasmic basophilia, and vacuolation. Mitotic figures and binucleate cells may be visible. The cytologic diagnosis of lymphosarcoma is best left to those experienced in evaluation of equine fluid specimens, because normal reactive lymphocytes and mesothelial cells may be difficult to distinguish from well-differentiated neoplastic cells. Histologically, the neoplastic cellular morphology also varies, but destruction of normal tissue architecture by a population of lymphoid cells aids the diagnosis.

12.2.6.2.4 Treatment

Experience in treating horses with lymphosarcoma is limited. Horses generally are debilitated greatly by the time of a diagnosis. Transient improvement in generalized forms has occurred following use of cytotoxic drugs, immunomodulators, and corticosteroids[180,211]; however, the long-term response is poor. Rarely, resection of localized tumors may be curative or significantly prolong survival.[214] The cutaneous form of lymphosarcoma may be responsive to corticosteroid and progestin therapy.[215] However, abrupt discontinuation or insufficient duration of therapy may result in recurrence of cutaneous lesions in a more aggressive and rapidly progressive form.

The prognosis for equine lymphosarcoma is grave. Most horses die or are destroyed humanely within 6 months of the onset of signs. Survival for a number of years rarely occurs. Survival times are longer for horses with cutaneous lymphosarcoma than for horses with internal organ involvement.

12.2.6.3 Plasma Cell Myeloma

Plasma cell myeloma (multiple myeloma), characterized by proliferation of neoplastic plasma cells in the bone marrow, spleen, liver, and lymph nodes, is rare in horses. Clinical signs include weight loss, weakness, recurrent fever, ventral edema, hemorrhage, lameness, and posterior paralysis.[216–220] Renal failure and infections are not uncommon. In human beings and dogs, skeletal pain and pathologic fractures result from neoplastic invasion of bone that causes osteolytic “punched-out” lesions[221,222]; osteolysis, however, does not occur consistently in horses.

Laboratory findings include anemia, hypercalcemia, azotemia, and hyperproteinemia with monoclonal gammopathy. Light-chain proteinuria (Bence Jones proteins) is variable. Criteria for diagnosis of plasma cell myeloma include plasmacytosis in the bone marrow or a soft tissue lesion, evidence of invasiveness, and the presence of monoclonal gammopathy or light-chain proteinuria.[221] Because of the overlap in histologic and clinicopathologic findings with some cases of lymphosarcoma, definitive diagnosis of plasma cell myeloma can be difficult.[204,223] Successful treatment of plasma cell myeloma in horses has not been reported.

12.3 Platelets

Platelets are circulating anucleate fragments of bone marrow megakaryocytes that are essential for the formation of primary hemostatic plugs. Platelets adhere to injured vascular endothelium, release substances that initiate and propagate hemostatic events, and provide a phospholipid surface for activation of several coagulation factors.[224] Platelets also are important proinflammatory elements that interact with endothelium, mononuclear phagocytes, neutrophils, and fibroblasts in initiation and propagation of inflammation.

12.3.1 PHYSIOLOGY

Platelets ultimately originate from the same pluripotent stem cell as erythrocytes and leukocytes. This stem cell undergoes successive divisions, becoming progressively more differentiated. The colony-forming unit megakaryocytoid cell is committed fully to platelet production. Polyploid megakaryoblasts double their DNA content by a process of endomitosis, resulting in megakaryocytes, the largest of the hematopoietic cells of the

bone marrow, within 5 days. The highly compartmentalized cytoplasm of the megakaryocyte ultimately disintegrates to release up to 8000 platelets into the peripheral circulation. Various cytokines, including IL-3, thrombopoietin, and IL-6, are essential for this process of differentiation, maturation, and release.

The mature platelet is a complex anucleate cell fragment that accumulates many substances in a variety of secretory granules. These include dense bodies containing adenosine diphosphate (ADP), adenosine triphosphate, calcium, and serotonin; α-granules that accumulate various procoagulant factors, albumin, platelet-derived growth factor, thrombospondin, and platelet factor 4; and lysosomal granules with acid hydrolases.[224] Glycoprotein receptor molecules are embedded in the external lipid bilayer membrane and interact with extracellular proteins that mediate platelet adhesion and aggregation. The external lipid membrane of the platelet invaginates into the interior to form a complex canalicular system important for secretory function. A second internal membrane system, the dense tubular system, supplies enzymes for arachidonic acid metabolism and is involved in sequestration and release of calcium.[225]

When a circulating platelet contacts exposed vascular subendothelial collagen, platelet surface receptors 742
recognize and attach to the damaged endothelium in a process known as adhesion. Normal adhesion requires von 743
Willebrand's factor (vWF), a glycoprotein found in vascular endothelium and platelets and present in plasma as a multimolecular complex with coagulation factor VIII. (The role of vWF in platelet adhesion is separate from the role of factor VIII in secondary hemostasis, which is discussed later. The two activities are referred to as VIII:vWF for the platelet adhesion mediated by vWF and as VIII:C for the coagulative protein.) Adhesion of platelets attracts additional platelets in a process known as aggregation. Primary aggregation is reversible and not associated with platelet degranulation. Irreversible secondary aggregation begins with release of ADP from platelet granules and production of thromboxane A_2 (TXA_2) via metabolism of arachidonic acid in the platelet membrane. Substances that promote aggregation usually do so by decreasing the concentration of intracellular cyclic adenosine monophosphate by inhibiting adenylate cyclase or by stimulating phosphodiesterase.[224] Equine platelets exhibit reversible aggregation when exposed to serotonin and arachidonic acid and exhibit irreversible aggregation when exposed to ADP.[226] Platelet-platelet bridging requires fibrinogen attachment to a newly exposed binding site on activated platelets.[225] Thrombospondin, a glycoprotein released from platelet α-granules, is also integral in the platelet aggregation response. Platelet aggregation is responsible for formation of the initial hemostatic plug (primary hemostatsis) at any site of vascular injury.

Arachidonic acid release from the membranes of the platelet-dense tubular network and its subsequent metabolism are critical to platelet aggregation. Arachidonic acid is a polyunsaturated fatty acid normally attached to the second glycerol carbon atom of many membrane glycerophospholipids. Arachidonic acid may be released by the direct action of phospholipase A_2 or by the sequential actions of phospholipase C and diglyceride lipase. Free arachidonic acid is metabolized via a cyclooxygenase pathway to form prostaglandins and TXA_2 or via a lipoxygenase pathway to form various eicosanoids. TXA_2 is a potent platelet activator, stimulating platelet aggregation and vasoconstriction. Although platelets preferentially produce TXA_2 via the cyclooxygenase pathway, vascular endothelial cells produce prostacyclin (prostaglandin I_2), a potent inhibitor of platelet aggregation and a strong vasodilator. The balance between TXA_2 production by platelets and prostacyclin production by vascular endothelium appears to regulate platelet aggregation in vivo.[224,225]

As with erythrocytes, platelets can be sequestered in the spleen and splenic contraction can increase the number of circulating platelets by 30% to 50%. In horses, splenectomy results in substantial and persistent increases in platelet counts.[12] Normal equine platelets have a life span of 4 to 5 days.[227] As platelets age, tissue macrophages of the spleen, liver, and bone marrow remove them from circulation.[228]

12.3.2

DISORDERS OF PLATELETS

12.3.2.1

Thrombocytopenia

Thrombocytopenia is a decrease in the number of circulating platelets. Normal platelet counts in the horse are slightly less than in other species, with higher counts in horses younger than 3 years of age and in male horses.[229] Most laboratories define thrombocytopenia in the horse as a peripheral platelet count of less than 100,000 per microliter. Clinical signs of thrombocytopenia in the horse reflect abnormal primary hemostasis and include petechial and ecchymotic hemorrhages of mucosal membranes, epistaxis, increased bleeding after venipuncture, melena, or hyphema.[230–235] Clinical bleeding usually is associated with platelet counts of less than 30,000 per microliter. Decreased numbers of circulating platelets can result from decreased production of platelets in the bone marrow, increased destruction of platelets, sequestration of platelets, or increased use of platelets during processes of coagulation (Box 12-5).

Proper sample collection and platelet counting is essential before one can make a diagnosis of true thrombocytopenia in a horse.[228] One can obtain an accurate platelet count in most horses from a blood sample obtained with EDTA as the anticoagulant. However, the platelets of some horses consistently clump in EDTA, resulting in an innacurate count or pseudothrombocytopenia.[236] An experienced technician always should examine a stained blood smear visually to assess the presence of platelet clumps. Platelet clumping may occur as a result of in vivo or in vitro platelet activation.[228] When in doubt, reassessing the platelet count in a blood sample anticoagulated with sodium citrate instead of EDTA is advisable. Platelet clumping and pseudothrombocytopenia also have been described in equine blood samples collected using low-molecular-weight heparin as an anticoagulant.[237]

12.3.2.1.1

Decreased Platelet Production

Thrombocytopenia only infrequently results from decreased platelet production.[238] One diagnoses primary bone marrow disease by bone marrow analysis and must exercise caution in interpreting megakaryocyte numbers from equine bone marrow aspirates. Megakaryocytes may be absent or present in low numbers in aspirates, even though adequate numbers are present in the intact marrow, possibly because of trapping of these large cells within the subendothelial layer of marrow sinuses. Bone marrow core biopsies are preferred for adequate evaluation of megakaryocyte numbers in the horse. Flow cytometric enumeration of thiazole orange–positive platelets in peripheral blood may be useful as a noninvasive test to assess platelet production.[239] 743

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12.3.2.1.1.1

BOX 12-5 DIFFERENTIAL DIAGNOSES FOR THROMBOCYTOPENIA IN THE HORSE

12.3.2.1.1.1.1

Decreased Platelet Production

Hereditary defects

Myelophthisis

Myeloproliferative disease

Idiopathic pancytopenia

Myelosuppressive drugs

- Phenylbutazone
- Chloramphenicol
- Estrogens
- Trichlorethylene extracted soybean meal

Irradiation

12.3.2.1.1.1.2

Increased Platelet Use

Intravascular coagulation

- Disseminated intravascular coagulation
- Localized
    - Hemolytic uremic syndrome
    - Hemangioma

Hemorrhage

Thrombosis

12.3.2.1.1.1.3

Platelet Sequestration

Splenomegaly

12.3.2.1.1.1.4

Increased Platelet Destruction

Infectious diseases

- Equine infectious anemia
- *Ehrlichia equi*

Immune-mediated diseases

- Autoimmune disease
  - Systemic lupus erythematosus
- Idiopathic disease
- Secondary disease
  - Neoplasia (lymphosarcoma)
  - Bacterial infection
  - Viral infection
  - Drugs
- Neonatal alloimmune disease

Drugs or toxins

Snake bites

12.3.2.1.1.1.5

Laboratory Error

Because of the short life span of platelets in the peripheral circulation, thrombocytopenia is a common abnormality in animals with generalized bone marrow suppression of any cause. Thrombocytopenia with dramatically decreased bone marrow megakaryocyte numbers has been reported in horses with a variety of myeloproliferative disorders.[147,155,158,218] Thrombocytopenia also is recognized in horses with idiopathic pancytopenia[132,136] and drug- or toxin-induced myelosuppression.

A syndrome of neutropenia and thrombocytopenia has been described in related Standardbreds. A heritable cyclic neutropenia was suspected. Most affected horses died from complications of infectious diseases and/or thrombocytopenia.[150]

12.3.2.1.2

Increased Platelet Destruction

A variety of pathophysiologic events may increase the rate at which platelets are removed from circulation. Most of these disorders are accompanied by a compensatory bone marrow megakaryocytosis best identified with core marrow biopsies. Immunoglobulin deposition on the surface of platelets is the most common cause of accelerated platelet destruction. Cells of the mononuclear phagocyte system, especially splenic

macrophages and hepatic Kupffer's cells, remove antibody-coated platelets from circulation. Nonimmune mechanisms of accelerated platelet destruction include drug- or toxin-induced platelet damage and snake bites. Thrombocytopenia of controversial cause has been described in human beings and horses receiving heparin therapy.[240,241]

12.3.2.1.2.1 Immune-Mediated Thrombocytopenia.

Most cases of idiopathic thrombocytopenia in the horse likely are the result of an immune-mediated increase in platelet destruction. The initiating factors of this condition often are not identified, but immune-mediated destruction of platelets may be associated with viral or bacterial infections, neoplastic conditions such as lymphosarcoma, and with other autoimmune disorders, including autoimmune hemolytic anemia, glomerulonephritis, and systemic vasculitis.[230] Circulating platelets from human beings with immune-mediated thrombocytopenia (IMTP) have increased quantities of surface-bound IgG, IgM, or complement.[242–244] The mechanics of antibody attachment to platelets are similar to those described for IMHA. True autoantibodies may be produced against normal platelet surface antigens or against novel platelet antigens that have developed in response to a primary disease process. Circulating immune complexes may attach to platelets nonspecifically via Fc or complement receptors. Antibodies also may be directed against foreign molecules, such as drugs, that have attached themselves to platelet membranes. Regardless of the source of the antibody, mononuclear phagocytes remove immunoglobulin-coated platelets from the circulation, principally in the spleen and liver.[245]

Horses with IMTP may have petechial and ecchymotic hemorrhages of mucosal membranes, epistaxis, increased bleeding after venipuncture, melena, or hyphema.[230–235] Clinical bleeding usually is associated with platelet counts of less than 30,000 per microliter. Patient history and a careful physical examination 744
are critical for identifying underlying disease. An AGID or Coggins test for equine infectious anemia 745
(EIA) is indicated. One should evaluate other coagulation parameters, including prothrombin time (PT) and partial thromboplastin time (PTT), to identify complex coagulative defects such as those seen with disseminated intravascular coagulation (discussed in a subsequent section). A bone marrow analysis usually reveals normal to increased numbers of megakaryocytes. However, if antibodies against platelet membranes cross-react with megakaryocyte membrane antigens, megakaryocytes may be decreased or absent in the bone marrow.[246]

One can confirm a diagnosis of IMTP only with the demonstration of increased quantities of platelet-bound antibody, which may be measured indirectly with the platelet factor 3 test. Platelet factor 3 is a platelet membrane phospholipid released when platelets are injured. Addition of patient plasma containing released platelet factor 3 to platelet-rich plasma from a normal horse results in accelerated clotting of the sample. Unfortunately, this test is difficult to perform consistently, results vary, and most veterinary laboratories do not offer the assay. Direct fluorescent antibody testing and flow cytometric assay for platelet surface IgG and IgM have been described in horses with IMTP following EIAV infection.[99] Direct and indirect immunoradiometric assays and an indirect enzyme-linked immunosorbent assay for detection of platelet-bound antibody also have been described.[247,248] These tests are not widely available, however, and require careful control and interpretation. In the absence of an easy, accurate, and reliable method for definitive diagnosis of IMTP in the horse, one may make a presumptive diagnosis in horses with a low platelet count, normal coagulation parameters (PT and PTT), and no evidence of excessive consumption of platelets (disseminated intravascular coagulation).

One immediately should discontinue any current medication being administered to horses suspected of having IMTP. If life-threatening hemorrhage is occurring, transfusions of fresh whole blood or platelet-rich plasma are indicated. Most horses respond to therapy with parenterally administered corticosteroids.[230,234] Corticosteroids appear to act by suppressing the phagocytic activity of mononuclear phagocytes, inhibiting antibody synthesis, increasing effective platelet production, and reducing capillary fragility. One should tailor the corticosteroid dose and route of administration to the individual patient to achieve the best results with the lowest possible dose. Horses with IMTP have been reported to respond to dexamethasone at 0.1 mg/kg intravenously or intramuscularly twice daily.[231,234] One always should taper corticosteroid administration gradually to once-daily morning administration to decrease interference with endogenous corticosteroid release. If long-term maintenance therapy is necessary, every-other-day administration of the lowest effective dose is indicated. Maintenance therapy may be oral or parenteral. Potential complications associated with corticosteroid administration in the horse include laminitis, secondary infections, and iatrogenic hyperadrenocorticism.

Occasionally, horses with IMTP fail to respond to parenteral corticosteroid therapy. Two such horses responded favorably to azathioprine at 3.0 mg/kg orally once daily.[235] Azathioprine has been used in human beings and dogs with IMTP and acts as an immunosuppressive agent interfering with humoral and cell-mediated immune function. Vincristine is a vinca alkaloid used to treat refractory IMTP in human beings. Vincristine therapy was successful in treating one horse with idiopathic IMTP[249] and unsuccessful in treating another.[235] Alternative treatments for IMTP that have been effective in other species include danazol (a synthetic analog of androgenic steroids and progesterone), gammaglobulin infusions, and splenectomy.[250]

Neonatal alloimmune thrombocytopenia has been described in horse and mule foals.[247,248,251] A syndrome of presumptive neonatal alloimmune thrombocytopenia, mucosal erosions, and skin lesions has been described in foals of several breeds. These foals respond to supportive care with judicious administration of corticosteroids and transfusions of platelet-rich plasma. Long-term prognosis is good, but platelet counts can take 30 days or longer to stabilize above 10,000 per microliter.

12.3.2.1.2.2

Equine Infectious Anemia Virus.

Acute febrile episodes of EIAV are accompanied by a sharp decline in platelet count followed by a rapid rebound as fever and viremia resolve. This thrombocytopenia is probably multifactorial in origin. Circulating platelets have increased quantities of IgG and IgM on the surface[99] and show evidence of in vivo activation, possibly indicating enhanced non–immune-mediated destruction of platelets.[100] Bone marrow megakaryocytes are not infected during acute disease[99]; however, evidence exists of decreased bone marrow production of platelets in horses with EIA, possibly because of altered cytokine production in the bone marrow.[101,252,253] The thrombocytopenia of EIAV may contribute to petechia and ecchymoses but rarely is associated with a severe bleeding diathesis. EIAV is discussed in detail in previous sections.

12.3.2.1.2.3

Equine Granulocytic Ehrlichiosis.

Infection with the rickettsia *Anaplasma phagocytophilum* (formerly *Ehrlichia equi*) results in a syndrome of fever, anorexia, depression, petechia and ecchymoses, icterus, ventral edema, and ataxia lasting 3 to 16

days. Consistent hematologic abnormalities include thrombocytopenia, leukopenia, and mild anemia.[254,255] Clinical signs are more severe in adult horses than in those younger than 4 years of age. Foals younger than 1 year of age often experience only fever.[255,256] Granular inclusion bodies may be visible 745
in the cytoplasm of neutrophils and eosinophils during routine cytologic examination using any Wright's 746
or Giemsa type of stain. One may see inclusion bodies best under oil immersion, and they appear as pleomorphic, blue-gray to dark blue spoke-wheel shapes.[255] Inclusion bodies represent a cluster of coccobacillary organisms, varying in size from 0.2 to 5 μm in diameter, within cytoplasmic membrane-bound vacuoles. The appearance of cytoplasmic inclusion bodies correlates closely with the onset of fever, and they remain visible for approximately 10 days.[254]

Mortality is rare in horses with ehrlichiosis; most untreated animals recover over 2 weeks and acquire a sound immunity against reinfection for at least 2 years.[254,255] Treatment with oxytetracycline at 7 mg/kg intravenously once or twice daily for up to 7 days may hasten recovery. One should provide supportive therapy, including intravenous fluids, leg wraps, and stall confinement for the severely ataxic horse, as indicated.[254,255,257] In animals that die or are killed, necropsy lesions include petechial and ecchymotic hemorrhages, edema, and icterus. Histologically, small arteries and veins are inflamed, and mild inflammatory vascular or interstitial lesions may be present in kidneys, heart, brain, and lungs.[254] Most infected horses originate from the foothills of northern California, but infections also have been diagnosed in horses from other parts of the country.[256,257] Most cases of ehrlichiosis occur in late fall, winter, and spring. The disease is thought to be transmitted by ticks, but an exact vector has not been identified. Tick control is recommended for prevention of disease in endemic areas. Asymptomatic or mild infections occur in sheep, goats, dogs, cats, monkeys, and baboons.[258]

12.3.2.1.3 Increased Platelet Use

Rapid use of circulating platelets occasionally occurs and may be appropriate, as in the case of massive trauma or external hemorrhage, or inappropriate, as in some cases of systemic or localized activation of hemostatic mechanisms. Thrombocytopenia accompanying hemorrhage or trauma is usually mild to moderate and rapidly reversible. Disseminated intravascular coagulation (DIC) is a complex disorder of hemostasis resulting from widespread systemic activation of coagulation mechanisms. This microvascular coagulation rapidly uses circulating platelets, and thrombocytopenia is a common result. Affected animals have a variety of other abnormalities detectable by routine coagulation testing. Horses with DIC and normal platelet counts may have abnormal platelet function, inhibiting the use of those platelets. In one study of horses presented to a veterinary referral hospital, DIC following gastrointestinal or inflammatory diseases was the most common cause of thrombocytopenia.[238] The section on DIC provides more information on the pathophysiology, diagnosis, and treatment of the disease.

Localized activation of coagulation may occur in some disease conditions, including vascular tumors and renal disease. Thrombocytopenia has been reported accompanying hemangiosarcoma in the horse[259,260] and is associated with vascular tumors in human beings and dogs.[261,262] Hemolytic uremic syndrome is an unusual condition in the horse characterized by acute renal failure with microangiopathic intravascular hemolysis and disseminated or renal intravascular coagulation. Thrombocytopenia is a common finding in affected horses.[59,60]

12.3.3 THROMBOCYTOSIS

Thrombocytosis is an increase in the number of circulating platelets. Increased bone marrow production of megakaryocytes may occur as a primary myeloproliferative disorder or in association with other neoplastic conditions, including polycythemia vera. Thrombocytosis more commonly is associated with acute or chronic inflammatory disorders or follows acute hemorrhage.[263] Mild thrombocytosis also may occur during and immediately after exercise or excitement caused by splenic contraction and release of sequestered platelets into the peripheral circulation. Thrombocytosis is usually asymptomatic, and specific therapy is not indicated. Thrombocytosis rarely may be associated with venous thrombotic tendencies, and one may consider anticoagulative therapy.

12.3.4 FUNCTIONAL DEFECTS OF PLATELETS

Functional defects of platelets can result in clinical signs similar to those of thrombocytopenic patients, despite a normal to increased platelet count. The most widely used laboratory tests to assess platelet function are bleeding time and platelet aggregation studies. Bleeding time depends on the number and function of platelets, the level of vWF, and vascular integrity. One performs the test by inverting the lower lip and making a vertical incision 1 mm deep by 5 mm long, with a template bleeding time device (Surgicutt, International Technidyne Corp., Edison, New Jersey). After 30 seconds, one removes blood with Whatman filter paper; repeats the previous step every 15 seconds until one no longer detects blood on the filter paper; and records the total time.[264] Alternatively, one may make incisions on the caudolateral aspects of the forelegs with proximal venostasis using a sphygmomanometer cuff above the carpus.[265,266] One should shave the site before testing. Normal bleeding time varies, depending on the technique and site of incision, and one must establish the time for each technique. The veterinarian should evaluate a normal control horse concurrently with the patient. One may use a platelet aggregometer to measure the aggregation of platelets in response to ADP, serotonin, epinephrine, arachidonic acid, and collagen.[267,268] One should collect whole blood samples with sodium citrate as an anticoagulant and prepare platelet-rich plasma by centrifugation. 746

747

To the author's knowledge, congenital defects in platelet function have not been reported in the horse, but they are recognized in other species and should be considered in young animals. Von Willebrand's disease has been described in the horse.[269] Because the disease is not an inherent platelet defect, it is discussed later in this chapter, with hereditary defects of coagulation. Abnormal responses to platelet aggregating agents and increased bleeding time have been described in normal human and equine neonates,[264,268] and neonatal foals may have an increased susceptibility to platelet-associated hemorrhagic diathesis. Acquired platelet functional defects may be associated with uremia, acute myelodysplastic diseases, and dysproteinemias and following administration of various drugs.

Many human beings and animals with uremia exhibit abnormal hemostasis, most of which is attributable to abnormal platelet function and likely is caused by abnormalities in the biochemical processes necessary for platelet aggregation and granule release. Human beings with myeloproliferative and lymphoproliferative disorders have been reported to have abnormal platelet shapes and decreased aggregation and secretion.[270] Dysproteinemia in these patients may result in platelet dysfunction when abnormal proteins coat platelets or endothelium and interfere with normal adhesion and aggregation. A hemorrhagic diathesis caused by platelet dysfunction has been described in one horse with monoclonal gammopathy and lymphoproliferative disease.[204]

Drugs commonly used in equine medicine and associated with abnormalities of platelet function in human beings include penicillins, cephalosporins, quinidine, chlorpromazine, halothane, and NSAIDs.[270] NSAIDs act by inhibiting cyclooxygenase, an enzyme critical for the formation of prostaglandins, prostacyclins, and thromboxane from arachidonic acid. As previously discussed, TXA_2 is a strong stimulus for platelet aggregation and release reactions and is a potent vasoconstrictor. Aspirin covalently acetylates cyclooxygenase, and because platelets are anucleate cells, they are incapable of producing new enzyme. The result is an irreversible inhibition of platelet cyclooxygenase that can be corrected only when the body clears aspirin from the circulation and the bone marrow releases new platelets. In contrast, other NSAIDs produce only a temporary inhibition of cyclooxygenase by reversibly binding to the enzyme. Template bleeding time is prolonged in horses that have received aspirin, phenylbutazone, or flunixin meglumine.[266,267,271,272] The effect is most pronounced after aspirin therapy, and a dosage of 17 mg/kg once daily for 3 days increases bleeding time for at least 3 days after discontinuation of therapy.[266] The minimal effective dose of aspirin to decrease thromboxane generation is 5 mg/kg; the duration of decrease is dose dependent.[273]

12.4 Hemostasis

The vascular system provides a closed conduit for the circulation of fluid and cellular components of blood. Exchange of substances across the vessel surfaces is controlled carefully. The body must repair any disruption in the closed network of blood vessels rapidly and efficiently to prevent excessive loss of critical blood elements into the extravascular environment. This repair process requires interaction between the injured vessel wall, platelets, and soluble proteins and accessory molecules in the blood. The end result is a stable fibrin mesh that prevents loss of blood while the vascular endothelium regenerates. When endothelium has been repaired, fibrinolytic reactions occur to remove fibrin and reestablish vessel patency. A careful balance of coagulative and fibrinolytic processes must occur to maintain optimal circulatory capacity without loss of blood. The body also must control procoagulative forces carefully to prevent inappropriate activation that might result in thrombosis of normal vessels with subsequent tissue and organ ischemia.

12.4.1 PHYSIOLOGY

Primary hemostasis is the process of platelet interaction with the vessel wall to achieve a temporary plug of the vascular defect. Secondary hemostasis involves the interaction of soluble coagulation factors to produce a stable fibrin mesh that reinforces the platelet plug. The body carefully regulates hemostatic mechanisms via an intricate network of positive and negative feedback with inhibitors and potentiators of coagulation and fibrinolysis.

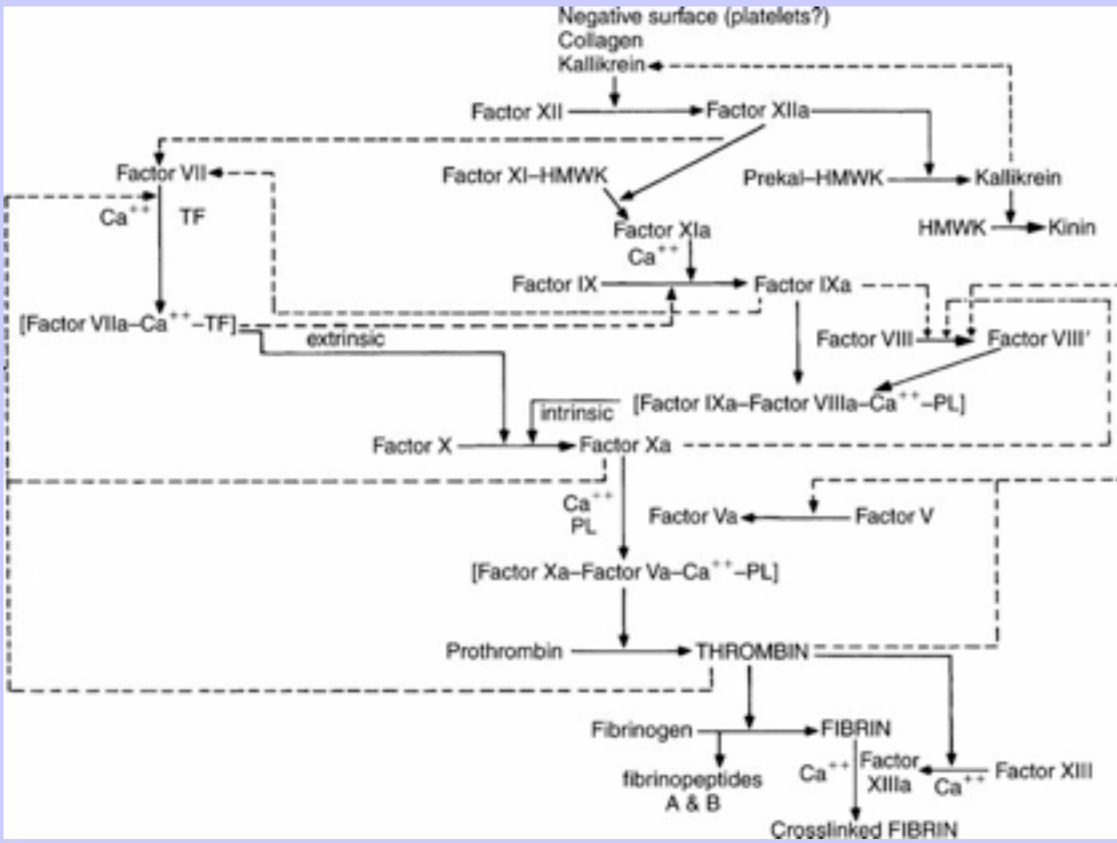
The initial vascular response to injury is intense vasoconstriction to divert blood from the site. Anticoagulative processes associated with vascular endothelium, including synthesis of prostacyclin and plasminogen activators and uptake and degradation of proaggregating molecules,[12] prevent the interaction of normal intact vascular endothelium with circulating platelets. Exposure of subendothelial collagen at the site of vascular injury triggers platelet adherence with interaction between endothelial vWF and platelet receptors. The function of platelets in primary hemostasis has been discussed in detail already. Platelet adhesion stimulates a release reaction involving platelet secretory granules. Contents of these granules (including ADP, adenosine triphosphate, serotonin, platelet factors 3 and 4, TXA_2, and acid hydrolases) attract other platelets in a process of irreversible platelet aggregation at the site of vascular injury. Platelet aggregation provides a favorable environment for accumulation and activation of the intrinsic and extrinsic coagulation systems.

The soluble coagulation factors (Table 12-3) participate in a cascade system that involves sequential activation of inactive precursor factors by limited proteolysis (Figure 12-2). Activated factors are indicated by the letter *a* following their numerical designation. Most coagulation factors are classified as serine proteases because of a critical serine residue at the active site of the enzyme. This serine is exposed following cleavage of a short peptide during activation of factor precursors. Although commonly discussed as separate pathways, intrinsic and extrinsic coagulation cascades interact at many levels and are considered more accurately as part of one highly complex system. For the sake of simplicity, however, they are discussed separately. The liver produces most soluble coagulation factors. Mononuclear phagocytes are responsible for clearing activated clotting factors from the circulation.

TABLE 12-3 Coagulation Factors

FACTOR	SYNONYM
I	Fibrinogen
II	Prothrombin
III	Tissue factor, tissue thromboplastin
IV	Calcium
V	Proaccelerin, labile factor
VI	Not assigned
VII	Proconvertin, stable factor
VIII	Antihemophilic factor
IX	Christmas factor
X	Stuart-Prower factor
XI	Plasma thromboplastin antecedent
XII	Hageman factor
XIII	Fibrin-stabilizing factor
Prekallikrein	Fletcher factor
High-molecular-weight kininogen	Fitzgerald factor

Figure 12-2 Schematic representation of blood coagulation showing the numerous interactions between coagulation factors in the classic extrinsic and intrinsic pathways. *Arrow,* Primary action; *dashed arrow,* actions of secondary importance; *a,* activated form; *HMWK,* high-molecular-weight kininogen; *TF,* tissue factor; *PL,* phospholipids; *Prekal,* prekallikrein. (From Morris DD: Recognition and management of disseminated intravascular coagulation in horses, *Vet Clin North Am Equine Pract* 4:115-143, 1988.)



Contact of soluble coagulation factors with negatively charged subendothelial collagen and with the various products of the platelet release reaction initiates the intrinsic coagulation system. This initial contact phase (see Table 12-3) requires interaction of coagulation factor XII, high-molecular-weight kininogen (HMWK), and prekallikrein to accelerate production of the activated form of factor XII (designated XIIa). Factor XIIa in turn catalyzes the limited proteolysis of factor XI to XIa. Elements of the contact phase reaction are also capable of inducing parallel proinflammatory reactions by initiating kinin and complement cascades. The importance of this contact phase reaction to in vivo coagulation is questionable. Human beings and animals with hereditary deficiencies of the proteins involved in this reaction (factor XII, HMWK, or prekallikrein) rarely show any 748 749

clinical bleeding tendencies. In contrast, deficiency of almost any other coagulation factor, including factor XI, results in profound hemorrhagic tendencies.

Factor XIa, in the presence of calcium, activates factor IX. Factor IXa forms a molecular complex with factor VIIIa and calcium on a phospholipid membrane surface to catalyze the conversion of factor X to Xa. Platelet membranes are thought to provide the phospholipid surface for this reaction. Activation of factor X is the point of interaction between the extrinsic and intrinsic systems.

The extrinsic coagulation system begins with formation of a molecular complex between tissue factor (thromboplastin), factor VIIa, and calcium ions on a platelet phospholipid surface to catalyze the conversion of factor X to Xa. Factor Xa is capable of activating factor VII to VIIa in a feedback loop that increases the production of both. In a slower reaction that appears to be important in vivo, the tissue factor VIIa–calcium complex is also capable of activating factor IX.[274] Tissue factor is present in virtually every tissue of the body, and a variety of insults can initiate the extrinsic coagulation system, including vascular endothelial damage, endotoxin, antigen-antibody complexes, RBC hemolysis, and local areas of tissue necrosis.

Formation of factor Xa marks the beginning of what classically is termed the *common pathway of coagulation*. Factor Xa complexes with factor Va and calcium on a phospholipid membrane to convert prothrombin to thrombin enzymatically. Thrombin in turn is the catalyst for formation of fibrin from fibrinogen. Thrombin is also important in the activation of factors V, VIII:C, and XIII, forming positive feedback loops that potentiate the coagulative process and promote platelet aggregation. Fibrin monomers aggregate, and factor XIIIa catalyzes the formation of covalent cross-linkages between the monomers to stabilize the clot.

Inhibitors of procoagulative elements in part maintain a balance between clot formation at sites of vascular injury and pathologic thrombosis. Two physiologically important systems for in vivo procoagulant inhibition have been described. The most important of these is plasma antithrombin III (AT III), responsible for 50% to 75% of plasma antithrombin activity. AT III inhibits all serine proteases generated during the coagulation process, including thrombin; factors Xa, IXa, XIa, and XIIa; plasmin; kallikrein; and the anticoagulants protein C and protein S. Heparin interacts with AT III, causing a conformational change that increases the activity of AT III 1000-fold.[275] The second main anticoagulative system involves protein C and protein S, two vitamin K–dependent serine proteases that inhibit coagulation at the level of factors Va and VIIIa. Protein S is a cofactor for protein C, required for binding of activated protein C to platelet and endothelial cell surfaces.

The fibrinolytic system is responsible for eventual remodeling and removal of a stable fibrin clot to restore blood flow (Figure 12-3). Because small amounts of fibrin production and subsequent fibrinolysis occur continuously in vivo, the body must maintain a delicate balance between procoagulant and fibrinolytic processes to prevent widespread thrombosis or hemorrhage. The principal enzyme of the fibrinolytic system is plasmin, normally present in plasma as an inactive precursor, plasminogen. Plasminogen is converted to plasmin proteolytically by endothelial tissue plasminogen activator, an enzyme present in most normal and neoplastic tissue. Endothelial cells adjacent to clot formations release tissue plasminogen activator in response to vascular stasis and a high local concentration of thrombin. Released tissue plasminogen activator has a high affinity for fibrin, localizing it to the adjacent clot for plasminogen activation. Circulating antiplasmins, the most important of which is α_2-antiplasmin, rapidly inactivate circulating plasmin. Plasmin bound to fibrin is much less accessible for inactivation because plasmin interactions with fibrin obscure binding sites for inhibitors.[276]

Plasminogen also may be activated during the contact phase of the intrinsic coagulation system. Factor XIIa and HMWK interact with prekallikrein to produce kallikrein. Kallikrein activates urokinase, which in turn converts

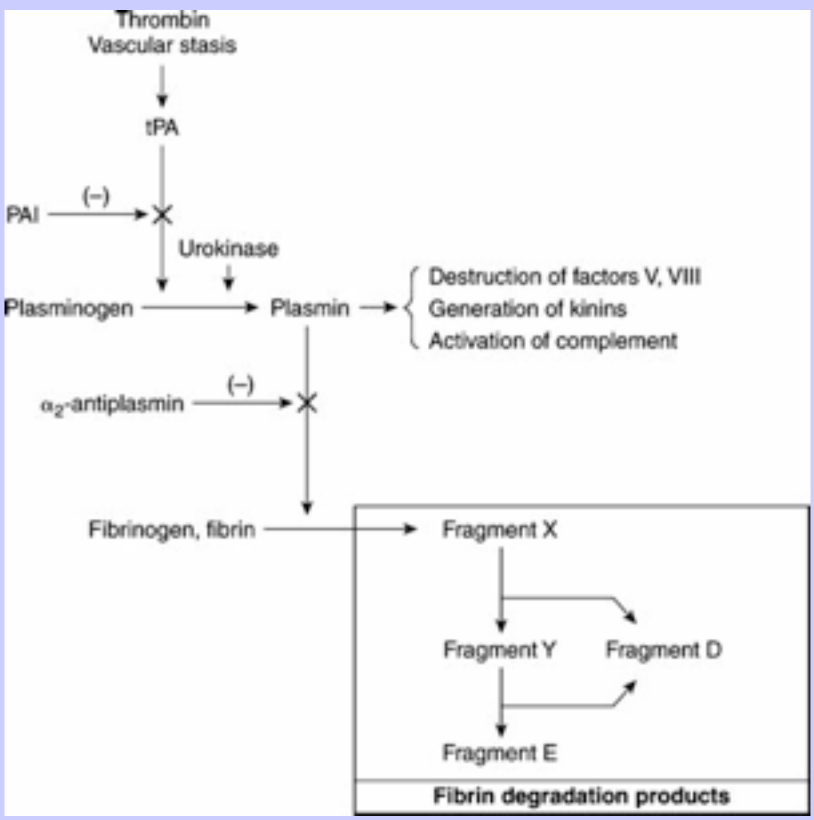
plasminogen to plasmin.[277] Because plasmin can activate factor XII, a potential feedback loop amplification exists. The importance of contact phase activation in vivo is questionable, however.

Plasmin is a serine protease with a high affinity for fibrinogen and fibrin, degrading them into fibrin degradation products (FDPs). Plasmin also may aid in degradation of factors V, VIII, IX, and XI. Fibrin and fibrinogen are split into the clinically significant FDPs, fragments X, Y, O, and E. The mononuclear phagocytes of the liver clear FDPs from the circulation.

12.4.2 EVALUATION OF HEMOSTASIS

Disorders of hemostasis usually may be classified based on clinical signs as abnormalities of primary or 749
secondary hemostasis. Abnormalities of primary hemostasis include vascular diseases and changes in the number 750
or function of platelets. Affected horses usually have signs of mucosal bleeding, petechia and ecchymoses, epistaxis, hyphema, or melena. Platelet function tests were discussed previously. Diagnosis of the vasculitides is discussed in another section.

Figure 12-3 Major fibrinolytic reactions (see text for details). A negative sign (–) indicates inhibition of a specific reaction. *TPA,* Tissue plasminogen activator; *PAI,* plasminogen activator inhibitor. (From Kobluk CN, Ames TR, Geor RJ: *The horse: diseases and clinical management,* Philadelphia, 1995, WB Saunders.)



Disorders of secondary hemostasis usually involve abnormalities in quantity or function of the soluble coagulation factors and present as spontaneous or excessive hemorrhage in response to surgery or trauma. These horses may have hemorrhage into body cavities, hematoma formation, hemarthroses, prolonged bleeding after venipuncture, or external hemorrhage. Most coagulation tests measure the time necessary for clot formation under various circumstances. Coagulation times tend to be longer in horses than in human beings or other domestic animals.[278] Table 12-4 lists some laboratory tests used to assess equine hemostatic function with normal reference values.

Activated clotting time measures the time to clot formation on activation of whole blood by contact with 750
diatomaceous earth. This process bypasses the contact phase of the intrinsic cascade and is useful in evaluating 751
deficiencies of factors VIII and IX, prothrombin, and fibrinogen. Abnormalities of factor VII and platelets do not appear to alter the activated clotting time.[278] One collects blood directly into a tube containing diatomaceous earth, mixes, incubates the contents at 37° or 38° C, and measures the time until clot formation.

TABLE 12-4 Reference Ranges for Equine Hemostatic Values*

PARAMETER	UNITS	VALUE	REFERENCE
Platelet count	per µl	75,000–300,000	NCSU
Fibrinogen	g/dl	<400	NCSU
Prothrombin time	seconds	8.5–9.9	NCSU
APTT	seconds	30–44	NCSU
FDPs	mg/dl	<20	NCSU
AT III	% PNEP	63–131	279
AT III	% PNHP	218±18	280
Plasminogen	% PNEP	64.6–155.9	281
Protein C	% PNEP	104.5±13.8	282

NCSU, North Carolina State University College of Veterinary Medicine clinical pathology laboratory normal equine reference values; *APTT,* activated partial thromboplastin time; *FDPs,* fibrin degradation products; *AT III,* antithrombin III; *PNEP,* pooled normal equine plasma; *PNHP,* pooled normal human plasma.

* These numbers are for reference only. Each laboratory should establish its own normal equine values.

PTT and activated PTT (APTT) measure the function of the intrinsic and common coagulation pathways, including factors XII, XI, X, IX, VIII, and V, prothrombin, and fibrinogen. One performs the PTT test by adding platelet-poor plasma to a glass tube containing phospholipid emulsion and calcium and determining the time to clot formation. One performs the APTT test similarly, except one adds an activating agent. PT, also known as one-stage PT, measures the function of the extrinsic and common coagulation pathways, including factors V, VII, and X, prothrombin, and fibrinogen. One mixes platelet-poor plasma with thromboplastin and calcium and determines the time to clot formation. One may use citrated whole blood or plasma for accurate measurement of PT up to 3 days after collection. A control sample from a clinically normal horse should accompany the patient's sample.[283] Thrombin time measures the time to clot formation after addition of thrombin to citrated plasma. Thrombin time is only prolonged with abnormalities in fibrinogen quantity or function or in the presence of thrombin inhibitors.

One most commonly measures FDPs in the serum using antibody-coated latex beads and monitoring macroscopic agglutination. This test reflects only the presence of D and E fragments. One must collect blood into a special tube containing thrombin and a protease inhibitor (ThromboWellcotest, Burroughs Wellcome Co., Research Triangle Park, North Carolina). One usually determines fibrinogen concentration by heat precipitation and determines plasma protein concentration before and after heating to 57° to 68° C for 3 minutes and centrifugation to remove precipitated fibrinogen. The difference in plasma protein concentration after heating and centrifugation approximates the plasma fibrinogen concentration.

Tests for plasma AT III measure the presence or the activity of the protein. Because inactive AT III antigen may be present, functional tests are preferable. Assays of AT III activity are clotting or chromogenic tests that measure residual thrombin activity after one adds the patient sample to a known quantity of thrombin. Normal AT III activity in the horse has been reported as 218% of normal human pooled plasma[280] and 63% to 131% of normal equine pooled plasma.[279,284] Widespread in vivo activation of coagulation is the most common cause of decreased plasma AT III activity. Decreased activity also is associated with protein-losing enteropathies or nephropathies. Increased activity has been reported in horses with hepatic disease.[284] AT III may act as an acute phase reactant, increasing in animals with acute inflammatory reactions.

Specialized assays for various components of the equine procoagulative and fibrinolytic systems have been described but are not yet widely available. These include assays for protein C,[282] plasminogen,[281] α_2-antitrypsin,[285] vWF,[269] and factor VIII:C.[286]

12.4.3 HEREDITARY DISORDERS OF HEMOSTASIS

Inherited disorders of hemostasis are most common in purebred animals that have been highly inbred. Many of these disorders are inherited in an autosomal recessive fashion; both parents must be carriers of the disorder to produce clinically affected offspring. For practitioners to be able to differentiate between inherited and acquired disorders of hemostasis is essential to advise owners appropriately. Inherited disorders are potentially treatable, but they are incurable. Owners should not use affected animals and their carrier parents as breeding stock. Practitioners see most animals with inherited bleeding problems when they are still young. Owners frequently complain of inappropriate bleeding in response to trauma or surgery or apparently spontaneous external hemorrhage or hematoma formation. As with any inherited defect, one should obtain a careful history and examine the patient and any close relatives, including siblings and parents, if possible. Most inherited defects result from a single factor abnormality. Routine coagulation studies may narrow the differential diagnoses for these bleeding disorders, but confirmation of diagnosis usually requires specialized tests. In contrast, most acquired defects of hemostasis are characterized by multiple factor abnormalities, frequently with a severe underlying systemic disorder.

12.4.3.1 von Willebrand's Disease

vWF is a glycoprotein of plasma, platelets, and endothelium required for platelet adhesion to exposed subendothelium and subsequent normal platelet plug formation. In plasma, vWF is bound in a multimolecular complex to coagulation factor VIII. Quantitative or qualitative abnormalities of vWF result in spontaneous bleeding from mucosal surfaces and excessive bleeding after surgery or trauma. Von Willebrand's disease has been reported in one Quarter Horse filly[269] presented for episodes of oral bleeding, conjunctival hemorrhage, and prolonged bleeding at injection sites and in a Thoroughbred mare and foal presented for episodes of

epistaxis. Routine complete blood count, including platelet count and coagulation profile, was within normal limits. In the related Thoroughbreds with von Willebrand's disease the APTT was prolonged because of decreased factor VIII:C concentrations, whereas values for PT were within reference ranges.[287] Assays for vWF quantity and function were abnormal. This condition is heritable in human beings and dogs, and owners should not use affected animals for breeding. 751 752

12.4.3.2 Abnormalities of the Contact Phase of Coagulation

The initiation of the intrinsic coagulation system involves interaction of factor XII, prekallikrein, and HMWK ultimately to activate factor XI. This process usually begins with contact between factor XII and negatively charged molecules such as subendothelium or collagen. Prekallikrein deficiency has been diagnosed in families of Belgian horses[288] and American Miniature Horses.[289] Plasma prekallikrein deficiency is characterized by prolonged APTT with a normal PT. Affected horses may or may not show clinical signs of a bleeding tendency.[288,289] Confirmation of diagnosis is by demonstrating low prekallikrein activity with normal activity of other intrinsic coagulation factors. One also can correct the prolonged APTT by extended incubation with a contact activator.[288,289] In human beings, prekallikrein deficiency is inherited as an autosomal recessive or autosomal dominant trait.[290] The mode of inheritance in horses has not been confirmed, but appearance of the defect in multiple members of some equine families strongly suggests that it is an inherited disorder in this species as well. A similar defect in the contact phase of coagulation, not thought to result from prekallikrein deficiency, was diagnosed in one Quarter Horse gelding with prolonged APTT and normal PT.[291] Researchers did not observe a clinical bleeding problem in association with this defect.

12.4.3.3 Factor VIII Deficiency

Classic hemophilia (factor VIII deficiency, hemophilia A) is the most commonly reported inherited defect of hemostasis in the horse. Hemophilia has been diagnosed in Thoroughbred, Standardbred, Quarter Horse, and Arabian colts[286,292–299] with severe spontaneous hemorrhage or excessive hemorrhage related to surgery or mild trauma. Coagulation testing reveals abnormal function of the intrinsic coagulation system, with a prolonged APTT and a normal PT. Confirmation of diagnosis is by measuring factor VIII:C activity in plasma. In human beings the severity of clinical signs varies inversely with this value, and the same appears to be true for affected horses. In severely affected horses, factor VIII activity is less than 10% of normal equine plasma.[286,294,296] A less severely affected colt that survived castration and was undiagnosed until 3 years of age had a factor VIII activity of 20% to 30%.[298] As in human, canine, and feline hemophilia, equine factor VIII deficiency is transmitted as an X-linked recessive trait.[293] Carrier mares transmit the defect to 50% of their male offspring on average. Carrier female horses usually have a factor VIII:C activity of approximately 50% of normal, although this value may vary widely and even overlap normal values. No cure exists for hemophilia, and the disease is progressively debilitating and potentially lethal. Affected human beings are treated with repeated transfusions of fresh plasma and factor concentrates. Factor IX and XI deficiencies have been reported in one Arabian foal with concurrent factor VIII deficiency.[295]

12.4.4 ACQUIRED DISORDERS OF HEMOSTASIS

12.4.4.1 Vasculitis

Vasculitis, inflammation of blood vessels, may occur as a primary disease process but more commonly is encountered as a secondary complication of infectious, immunologic, toxic, or neoplastic disorders.[300] Hypersensitivity vasculitides are characterized by predominant involvement of small blood vessels in the skin. In most cases the postcapillary venules are involved. The most common inflammatory pattern is leukocytoclasis, defined by the presence of neutrophilic nuclear debris in and around the involved vessels. Vessel wall necrosis and fibrinoid changes occur.

Clinical signs depend on the type of vessel affected and the severity of the inflammatory response. Vascular occlusion may result in tissue ischemia and necrosis. Increased vascular permeability results in hemorrhage and edema as fluid and cellular constituents of the blood escape into the extravascular space. In horses, vasculitis is characterized by well-demarcated areas of cutaneous edema that may involve any portion of the body.[301] The distal extremities and ventral body wall are affected most commonly, and the edematous area is often hot and painful. Affected horses generally are depressed and reluctant to move. Hyperemia, petechial and ecchymotic hemorrhages, and ulcerations are commonly present on mucous membranes of the mouth, nose, vulva, etc. Other clinical signs may reflect edema, hemorrhage, and infarction in other body systems. Lameness, colic, diarrhea, dyspnea, or ataxia can result from involvement of vessels in joints, muscles, gastrointestinal tract, respiratory tract, and central nervous system. Subclinical renal disease may occur. Secondary complications such as laminitis, thrombophlebitis, and localized infections are common. Affected skin often weeps serum and eventually sloughs.

The underlying disease, length of illness, other organ involvement, and secondary complications usually determine hematologic and serum biochemical findings in vasculitis; none are characteristic. Neutrophilia, mild anemia, hyperglobulinemia, and hyperfibrinogenemia usually accompany chronic inflammation. The 752
platelet count is generally normal unless concomitant consumptive coagulopathy or IMTP exists. With renal 753
involvement, serum creatinine may be elevated or urinalysis may reveal hematuria or proteinuria or both.

12.4.4.2 Infectious Diseases

Infectious diseases associated with vasculitis and subsequent petechiation, ecchymoses, and dependent edema include EIAV, equine granulocytic ehrlichiosis, and equine viral arteritis (EVA). All three diseases may result in thrombocytopenia, although this is somewhat less common with EVA. The first two are discussed in detail elsewhere in this chapter; a discussion of EVA follows.

Equine viral arteritis is characterized by fever, chemosis, lacrimation, rhinitis, focal dermatitis, ventral edema, and occasionally abortion.[302,303] Many infected horses do not show recognizable clinical signs. Equine arteritis virus officially is classified in the *Arterivirus* genus of the Arteriviridae family.

Horses become infected by aerosol or venereal contact with an infected animal. The virus replicates in the intima and media of most arteries, resulting in an inflammatory reaction and cellular influx.[304] Stallions may become asymptomatic carriers of the disease, harboring virus in the accessory sex glands[302,305] and transmitting infection to previously unexposed mares. Infected mares make an uneventful recovery from EVA

and eliminate the virus from their body. Abortion may occur during or shortly after acute illness or asymptomatic infection as a result of myometrial necrosis and edema leading to placental separation and fetal death.[306] Not all infected mares abort, however, and the incidence of abortion may be strain dependent.[307]

One confirms diagnosis of EVA in mares by serologic assay, demonstrating a fourfold or greater rise in titer between acute and convalescent serum samples. EVA titer may remain elevated for a long time after natural infection, and recovered mares are considered immune to further infection. Diagnosis of persistently infected asymptomatic stallions depends on isolation of the virus from semen samples or demonstration of seroconversion of an otherwise unexposed mare within 2 weeks of breeding to the suspect stallion.[308] Diagnosis of abortion caused by EVA requires virus isolation from the fetus or the placenta. Recent seroconversion in the mare is highly supportive of a diagnosis of EVA abortion. One may offer supportive care to acutely infected animals and should isolate them from uninfected animals, especially pregnant mares. A modified live virus vaccine is available.[307]

12.4.4.3 Immunologic Diseases

Most noninfectious cases of vasculitis in the horse are thought to be immune-mediated. Small cutaneous vessels most commonly are affected, resulting in edema that may progress to skin infarctions, necrosis, and exudation.[300] The best characterized of these disorders is purpura hemorrhagica following *Streptococcus equi* subsp. *equi* infection. However, vasculitides of undetermined cause also have been described in the horse.[233,309] Most of these cases appear to have an immune-mediated cause. The antigenic stimulus of hypersensitivity vasculitis is usually a microbe, drug, toxin, or foreign or endogenous protein. The deposition of circulating immune complexes with subsequent vessel wall damage is probably the major event in the pathogenesis of these vasculitic syndromes. Soluble immune complexes, formed in moderate antigen excess, become deposited in blood vessel walls in areas of increased permeability. Deposited immune complexes activate complement with resulting formation of C5a, a potent chemotactic factor for neutrophils. Infiltrating neutrophils release lysosomal and other cytoplasmic enzymes such as elastase and collagenase that directly damage vessel walls. The net effect is vessel wall leakage and luminal compromise, resulting in edema, hemorrhage, thrombosis, and ischemic changes in supplied tissues.

Aside from vessel wall necrosis, vasculitis results in vascular dysfunction characterized by a net increase in vasoconstriction and platelet aggregation, which contribute to tissue ischemia.[310] The diseased endothelium releases endothelin, a polypeptide that causes contraction of underlying smooth muscle[311] and produces fewer dilator substances such as endothelial-derived relaxing factor.[312] Injury to the vessel wall also results in diminished prostacyclin production, which usually serves to maintain vasodilation and platelet nonreactivity.[313] Thus endothelial dysfunction contributes ultimately to vessel failure following vasculitis.

The factors that determine which individuals develop hypersensitivity vasculitis remain undefined. Genetic predisposition, altered immunoregulatory mechanisms, and the amount, relative size, and type of complement components in circulating immune complexes are important in determining the potential risk of developing vasculitis, because these factors determine how quickly the mononuclear phagocyte system clears immune complexes.[314] Perhaps even more significant in this potential risk are genetic and acquired differences in the number and activity of receptors for complement and IgG on erythrocytes and macrophages. The difference can affect immune complex handling and distribution greatly.[315,316] Receptors for complement and the Fc of IgG mediate immune complex transport to and removal by the macrophages of the mononuclear phagocyte system. Physical factors such as turbulence of blood flow, hydrostatic pressure within vessels, and previous

endothelial damage likely determine the size, type, and location of blood vessels involved in vasculitis. The propensity for lesion formation in skin of dependent body portions likely is caused by the hydrostatic pressure in affected postcapillary venules in these areas.[313] 753 754

12.4.4.4 Diagnosis

The diagnosis of vasculitis depends on demonstration of typical histologic changes (e.g., leukocytoclasis and fibrinoid necrosis) in involved vessels. One should obtain full-thickness punch biopsies (6 mm in diameter) of skin in an affected area and preserve it in 10% formalin for histopathologic study. One can process the samples saved in Michel's transport medium subsequently for immunofluorescence analysis to detect immune complexes.[317] Multiple biopsies from various sites are optimal in reaching a diagnosis, because distribution of histologic lesions and immune complexes is patchy; however, affected skin is often on the distal limbs where risk increases for cellulitis or exuberant granulation tissue. The irregular distribution of histologic lesions, coupled with difficulty in obtaining biopsies, makes the definitive diagnosis of vasculitis difficult. Often a diagnosis of vasculitis is based on history, clinical signs, and response to therapy.

Purpura hemorrhagica is a syndrome of cutaneous vasculitis that frequently develops several weeks after infection with *S. equi* subsp. *equi, S. equi* subsp. *zooepidemicus,* influenza virus, or equine herpesvirus. Purpura hemorrhagica also has been described as an adverse side effect of antistreptococcal vaccination in the horse. Purpura hemorrhagica after infection with *S. equi* subsp. *equi* has been attributed to deposition of circulating immune complexes consisting of IgA and *S. equi* subsp. *equi* M protein in small subcutaneous vessels.[318] Horses are often febrile with ventral edema, especially of the distal limbs. Affected animals frequently have great pain and are reluctant to move. Petechia and ecchymoses may be present on oral, nasal, and conjunctival mucous membranes. Extensive skin necrosis and sloughing often follow severe swelling. Horses with purpura hemorrhagica are not usually thrombocytopenic but frequently have laboratory evidence of chronic inflammatory disease, including increased fibrinogen and total plasma protein, and neutrophilia.[300] Cutaneous biopsy of affected areas may confirm the diagnosis. Histopathologically, a leukocytoclastic vasculitis characterized by neutrophilic accumulation in and around small dermal and subcutaneous vessels occurs.

12.4.4.5 Treatment

The aims in the therapy of equine vasculitic syndromes are (1) to remove the antigenic stimulus if possible, (2) to reduce vessel wall inflammation, (3) to normalize the immune response, and (4) to provide supportive care. Regardless of the cause, vasculitis in horses generally warrants aggressive nursing care, which one should institute immediately. Hydrotherapy can minimize edema, and pressure wraps are useful on the limbs. Animals that become severely depressed and fail to drink or those with dysphagia caused by pharyngeal edema require fluids, intravenously or via nasogastric tube. A tracheostomy may be necessary in horses with stridor and dyspnea following edema of the upper respiratory tract. Phenylbutazone, flunixin meglumine, or other NSAIDs are indicated to reduce vascular inflammation and provide analgesia. The disease also may warrant corticosteroid administration. Antimicrobial therapy may reduce the incidence or severity of cellulitis or other septic sequelae.

Because no treatment effectively eliminates the viruses of EIA or equine arteritis virus from the body, neither disease warrants specific therapy. Although equine ehrlichiosis spontaneously resolves, elimination of the organism by oxytetracycline therapy considerably shortens the clinical course of disease.

When the cause is unknown, vasculitis can be difficult to treat because the antigenic stimulus remains undefined and may not be eliminated easily. One should discontinue administration of any drug being used at the time that signs occur. A thorough search for an underlying infection or neoplasia is warranted. Horses with a recent history of strangles or that have been exposed to the disease should receive procaine penicillin G at 22,000 U/kg intramuscularly twice daily or potassium penicillin G intravenously 4 times daily for at least 2 weeks. One should drain accessible abscesses. Even though the sensitizing infection usually has resolved by the time signs of purpura hemorrhagica occur, ongoing sepsis and antigen production prolong the allergic vasculitis. The use of corticosteroids for treating hypersensitivity vasculitis remains controversial[313]; however, clinical experience suggests that horses with purpura hemorrhagica or idiopathic vasculitic syndromes respond favorably to corticosteroid therapy.[300]

Mild cases of vasculitis may resolve without immunosuppressive therapy, but life-threatening edema involving the upper respiratory tract or causing organ system dysfunction necessitates early aggressive corticosteroid treatment. One should administer dexamethasone (0.05 to 0.2 mg/kg intravenously or intramuscularly once or twice daily) at the dose and rate necessary to reduce edema. One may substitute prednisone 0.5 to 1.0 mg/kg orally twice daily for dexamethasone, but prednisone often is not as effective, possibly because of poor oral absorption of the drug. After edema and hemorrhages start to resolve, one may reduce the corticosteroid dose gradually (by 10% to 15% every 1 to 2 days) while carefully monitoring the horse for relapse. For horses with purpura hemorrhagica to require corticosteroid therapy for a period of 4 to 6 weeks before edema permanently resolves is not uncommon. 754

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Occasionally, horses suffer disease flare-ups that require the corticosteroid dose to be increased over the previously efficacious level. In these instances, flunixin meglumine may enhance steroid efficacy. Antimicrobials are indicated throughout the period of systemic corticosteroid administration to reduce the incidence and severity of secondary sepsis.

12.4.4.6 Prognosis

The prognosis for vasculitis depends on the initiating disease process. With early aggressive therapy and supportive care, most horses recover from purpura hemorrhagica within 4 weeks, although numerous sequelae may prolong the convalescence. Dermal infarction in the distal limbs often leads to skin sloughing followed by exuberant granulation tissue and may require excision followed by skin grafting. Laminitis and various infections such as cellulitis, pneumonia, colitis, and thrombophlebitis are common and may be related to long-term corticosteroid therapy.

EVA and equine ehrlichiosis carry a good prognosis, and disease confers some immunity to reinfection. Horses with EIA are infected persistently and may suffer recurrent clinical relapses. Horses with idiopathic vasculitic syndromes have an unpredictable response to therapy, and some have a poor prognosis. Inadequate resolution of hypersensitivity vasculitis in human beings usually is caused by failure to identify the antigenic stimulus or completely eliminate it from the body.[313] Although in most horses remission is spontaneous, some have debilitating cutaneous disease or develop systemic necrotizing vasculitis with a poor prognosis.

12.4.5 THROMBOCYTOPENIA

When circulating platelet counts decrease to less than 30,000 per microliter, a bleeding diathesis frequently occurs. Affected horses have petechial and ecchymotic hemorrhages of mucosal membranes, epistaxis, increased

bleeding after venipuncture, melena, or hyphema.[230–235] Similar signs may occur in horses with platelet function defects. Disorders involving alterations in platelet number or function are described in foregoing sections.

12.4.6 VITAMIN K DEFICIENCY

Vitamin K is a fat-soluble vitamin essential for a final step, γ-carboxylation, in the hepatic synthesis of many coagulation factors. The serine proteases, including factors II, VII, IX, and X, require γ-carboxylation of glutamate before they are biologically functional. Vitamin K deficiency results in a gradual decrease in functional coagulation factors in circulation that ultimately can end in a clinical syndrome of inappropriate bleeding. The rapidity with which coagulation factor concentrations in circulation decline depends largely on the half-life of those factors in circulation. Factor VII has a plasma half-life in the dog of 4 to 6 hours (compared with 41, 14, and 16 hours for factors II, IX, and X, respectively) and reaches a critical concentration in the circulation more rapidly than other vitamin K-dependent factors.[319] As a result, coagulation studies early in the course of vitamin K deficiency may reveal abnormalities in the extrinsic coagulation system (prolonged PT) with a normal intrinsic system (normal APTT). As the condition progresses, however, extrinsic and intrinsic coagulation parameters are abnormal.

All green leafy feeds, including hay and fresh pasture, contain high concentrations of vitamin K. Vitamin K also is thought to be synthesized by the intestinal microflora of many species. As a result, vitamin K deficiency only rarely results from an absolute deficiency of the nutrient in the diet. More commonly, deficiency results from an inability of the horse to absorb or use dietary vitamin K. Chronic intestinal malabsorption of lipids can result in clinical bleeding problems. One may assess dietary fat absorption by feeding 150,000 IU of vitamin A (another fat-soluble vitamin) in corn oil with grain and measuring increases in serum vitamin A concentration.[320] Concentrations should double over 12 to 24 hours. Fat absorption depends on bile acid secretion from the liver, and chronic cholestatic disorders also can result in decreased vitamin K absorption.

Antagonism of vitamin K by warfarin or other dicumarol derivatives is a more common clinical problem than is absolute vitamin K deficiency. The dicumarol derivative warfarin has been advocated as an oral anticoagulant for use in horses with navicular disease[321] or jugular thrombophlebitis.[322] Warfarin also is the active ingredient in many rodenticides, and accidental exposure to toxic doses of the compound is possible. Warfarin is an effective antagonist of vitamin K and inhibits the production of functional coagulation factors II, VII, IX, and X. Warfarin therapy may begin with a daily dose of 0.018 mg/kg orally and increase in increments of 20% to achieve the desired therapeutic effect,[321] which usually results in a final dose of 0.012 to 0.75 mg/kg daily.

Concurrent administration of other drugs, especially phenylbutazone, may increase the risk of warfarin-induced hemorrhage. Phenylbutazone acts by competing with warfarin for binding sites on plasma albumin, increasing the concentration of free warfarin in circulation. Similarly, hypoalbuminemia can increase the risk of warfarin toxicosis. Thyroxin and corticosteroids lower the necessary therapeutic dose of warfarin by enhancing receptor affinity and increasing clotting factor catabolism. Rapid withdrawal of drugs that induce hepatic microsomal enzyme metabolism, such as rifampin, chloramphenicol, and barbiturates, also can potentiate toxicosis because these drugs enhance warfarin metabolism. 755

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Because factor VII has the shortest half-life of the vitamin K–dependent factors, warfarin affects the extrinsic coagulation system more rapidly than the intrinsic system, and determination of PT is the most commonly used method for monitoring the effectiveness of warfarin anticoagulative therapy. An increase in PT of 1.5 to 2.5 times baseline (15% to 25% of normal activity) is recommended as a therapeutic goal of warfarin anticoagulation.[322,323]

Toxic doses of warfarin result in spontaneous hemorrhage that may be life-threatening. One makes a diagnosis of warfarin toxicity based on a history of exposure, clinical evidence of hemorrhage (bleeding from body orifices or into body cavities, hematoma formation, or clinical pathologic indications of blood loss anemia), prolonged PT or PTT with normal platelet count and fibrinogen and FDP concentrations, and response to exogenous vitamin K administration.[324]

Because warfarin interferes with the final stages of clotting factor synthesis, administration of vitamin K_1 at up to 1.0 mg/kg subcutaneously rapidly reverses toxicity.[322] One may repeat this dose every 4 to 6 hours until PT returns to normal, followed by daily monitoring for 3 to 4 days.[325] PT returns to normal approximately 5 days after the last dose of warfarin without administration of supportive therapy; administration of vitamin K_1 results in a return of PT to baseline within 24 hours.[325,326] In horses with severe bleeding, one should administer whole blood transfusions concurrently to replace plasma clotting factors. The horse should improve within 1 to 2 hours. One may administer smaller doses of vitamin K_1 orally, concurrent with warfarin, to titrate the anticoagulant effects of the latter.[326]

Second-generation anticoagulant rodenticides such as brodifacoum have a prolonged half-life (1.2 days) and lower median lethal dose compared with warfarin.[327] As a result, their potential for adverse effects are greater. Accidental exposure to these agents necessitates more prolonged antidotal therapy.[328]

Toxicosis caused by ingestion of moldy sweet clover (*Melilotus* spp.) hay is reported in all herbivores.[329] White sweet clover *(Melilotus alba)* and yellow sweet clover *(Melilotus officinalis)* contain coumarin. When sweet clover hay is cured improperly, numerous fungi (*Penicillium, Mucor,* etc.) propagate; these fungi convert coumarin to 4-hydroxycoumarin, which condenses to dicumarol.[330] Animals that ingest sufficient quantities of moldy hay develop severe coagulation dysfunction within 2 to 7 days with internal and external hemorrhaging.[331] Continued ingestion of moldy hay can result in a lethal hemorrhagic crisis. One should suspect toxicity as a diagnosis in horses with history of access to moldy sweet clover hay and can confirm the diagnosis with coagulation tests described previously for warfarin toxicosis. Suspect hay should be destroyed because toxic concentrations of dicumarol may remain for up to 4 years.[330]

One may treat clinical bleeding episodes resulting from inadequate vitamin K and subsequent deficiency in the levels of vitamin K–dependent coagulation factors in circulation with parenteral vitamin K_1 (phylloquinone) injections and may administer a dose of 0.3 to 0.5 mg/kg. Subcutaneous injection is recommended because intravenous administration has resulted in a high incidence of adverse reactions in other species. One ultimately must address underlying gastrointestinal or hepatic disorders as well. Parenterally administered vitamin K_3 (menadione sodium bisulfite) is nephrotoxic in horses at the manufacturer's recommended dose and should not be administered.[332] Horses that receive vitamin K_3 may show signs of colic, hematuria, azotemia, and electrolyte abnormalities consistent with acute renal failure.

12.4.7 HEPATIC DISEASES

Severe hepatic disease often is associated with hemorrhagic tendencies caused by decreased production of coagulation factors II, V, VII, IX, and X and fibrinogen. Impairment of bile acid secretion may impair absorption of the fat-soluble vitamins, resulting in decreased production of the vitamin K–dependent clotting factors. Hepatic Kupffer's cells also are responsible for removal of many activated coagulation factors and FDPs from the

circulation. Increased circulating FDPs inhibit normal platelet function and generation of fibrin. These abnormalities may combine to initiate DIC. A severe bleeding diathesis is a poor prognostic indicator in horses with hepatic disease.

12.4.8 DRUG-INDUCED ALTERATIONS IN HEMOSTASIS

A variety of pharmaceutic agents can alter hemostatic mechanisms. Incorrect or poorly monitored administration of these agents may result in hemorrhagic tendencies in the horse.

12.4.8.1 Aspirin

Aspirin causes an irreversible inhibition of platelet function. The mechanisms and implications of this effect were discussed previously.

12.4.8.2 Heparin

Heparin is a mucopolysaccharide complex of variable molecular weight that potentiates the activity of AT III in neutralizing coagulation factor X and increasing the rate of inactivation of other serine proteases (factors II, IX, XI, and XII).[333,334] Potentiation of AT III activity is attributed primarily to lower molecular weight forms 756
of the molecule that inhibit factors X and XII, with minimal inhibitory effects on thrombin and factors IX and 757
XI.[334] Heparin has been advocated for maintenance of the patency of indwelling catheters, prophylaxis for venous thrombosis and laminitis, anticoagulation of whole blood transfusions, prevention of postoperative peritoneal adhesions, and treatment and prevention of DIC.[333,335–337] Heparin efficacy in most of these conditions has not been confirmed.[338,339] Bleeding complications associated with heparin therapy are uncommon, but administration of protamine sulfate at up to 1 mg for every 90 to 100 U of heparin has been recommended in horses with heparin-associated hemorrhage.[333] Protamine sulfate forms a stable salt with heparin and with soluble fibrinogen monomers. If the FDP concentration increases in a patient, precipitation of these salts may result in intravascular infarction, and use of protamine sulfate is contraindicated.

The most pronounced adverse effect associated with heparin administration in the horse is a significant reduction in the circulating RBC mass.[240,340] Doses of 160 to 320 U/kg subcutaneously twice daily for nine doses resulted in a significant decrease in RBC numbers, PCV, and total hemoglobin, and an increase in MCV, with a rapid return of values to baseline after discontinuation of therapy.[240] Because heparin is known to increase the activity of mononuclear phagocytes, decreased RBC numbers originally were hypothesized to result from an enhanced phagocytosis of erythrocytes.[340] However, decreased RBC numbers now are thought to result primarily from in vivo erythrocyte agglutination.[341,342] One can reverse this agglutination in vitro by adding a trypsin solution to the RBC suspension. Decreases in RBC numbers and increases in MCV associated with heparin administration are most likely artifactual, resulting from the measurement of multiple agglutinated cells as single units.[342] Heparin administration in horses (160 U/kg subcutaneously twice daily) also has been associated with development of large plaques of painful edema.[343]

12.4.9 DISSEMINATED INTRAVASCULAR COAGULATION

DIC is a condition of pathologic activation of coagulative and fibrinolytic systems, ultimately leading to microvascular ischemia and secondary organ dysfunction. DIC may be acute or chronic, systemic or localized,

and frequently is characterized by severe hemorrhage caused by consumption of coagulation factors but also may be manifest as a pronounced thrombotic tendency. The type and severity of clinical signs depend on the initial stimulus for pathologic systemic coagulation activation, the duration and extent of activation, the availability of coagulation factors, and the relative strengths of activation of procoagulant and fibrinolytic forces. The final result in horses with severe DIC is often widespread ischemic organ failure, severe hemorrhage, and death.

DIC is not considered a primary diagnosis and occurs following a variety of disorders triggered by many different mechanisms. In the horse, DIC most commonly is associated with gastrointestinal disease or sepsis.[344–354] The common initiating factor in most cases is endotoxin, the external lipopolysaccharide cell wall of gram-negative bacteria. Endotoxin can activate factor XII directly to initiate the intrinsic coagulation pathway and may cause widespread damage to vascular endothelium, exposing subendothelial collagen and releasing tissue thromboplastin to trigger the intrinsic and extrinsic coagulation pathways. Endotoxin damages platelets, inducing a platelet release reaction and exposing platelet factor 3. Other conditions associated with DIC include intravascular hemolysis, bacteremia (gram positive or gram negative), viremia, neoplasia, circulating immune complexes, immune thrombocytopenia, fetal death in utero, burns, hepatic disease, renal disease, and vasculitides. Virtually any primary disease process may initiate DIC by injury to blood cells, increasing the availability of phospholipid surfaces, injury to vascular endothelium, or tissue injury and subsequent release of thromboplastin.[355]

In addition to coagulative abnormalities, widespread activation of proinflammatory pathways occurs, including generation of kinins and stimulation of the complement cascade.[355] Factor XIIa is responsible for initiating kinin formation by activating kallikrein, which in turn proteolytically converts HMWK to kinins. Bradykinin, the most physiologically important of the kinins, enhances vascular permeability, dilates some blood vessels, and stimulates migration of leukocytes into the extravascular space.[356] Plasmin activates the complement cascade and induces neutrophil chemotaxis, increases vascular permeability, and destroys RBCs and platelets, releasing membrane phospholipoprotein and ADP to act as procoagulants. Complement products can activate factor XII.

Tissue mononuclear phagocytes become activated during disseminated coagulation and inflammation. In response to endotoxin, these cells release procoagulant substances including tissue factor, platelet activating factor, tumor necrosis factor, and IL-l.[357] These phagocytic cells are responsible for removal of activated clotting factors and FDPs from the circulation. Massive concentrations of activated clotting factors or FDPs, or impairment of phagocytic function, can lead to abnormally high levels of either element in the blood. Activated clotting factors then remain available for continued coagulative events. Elevated FDP levels in the peripheral circulation can inhibit fibrin monomer polymerization and thrombin formation and also coat platelet membranes, interfering with aggregation.

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The clinical signs associated with DIC in the horse vary widely and reflect the primary disease process, the duration and severity of coagulation activation, and the balance between procoagulant and fibrinolytic forces. Horses seldom show severe bleeding diathesis following DIC. Careful clinical examination may reveal petechia and ecchymoses of mucous membranes and prolonged bleeding from venipuncture sites. Many horses exhibit an increased tendency to venous thrombosis after venipuncture.[348] Other clinical signs may reflect organ dysfunction following microvascular thrombosis and tissue ischemia. In the kidney, organ dysfunction may occur as tubulointerstitial disease and acute renal failure. In the foot, DIC may contribute to digital ischemia and laminitis[335,336,344,348]; however, experimentally induced laminitis was not associated with significant changes in hemostatic parameters.[285] Many horses with DIC have clinical signs reflecting poor peripheral perfusion and shock, including prolonged capillary refill time, cool extremities, and tachycardia, which may result from the primary disease process or occur following DIC-induced microvascular occlusion and inflammation.

Diagnosis of DIC depends on the results of a variety of tests assessing the quantity and function of coagulative substances. The most commonly evaluated parameters in horses are platelet count and function, PT, PTT, activated clotting time, and FDPs. Platelet counts usually are decreased in horses with DIC.[345–348,350,353] In horses with normal platelet counts, platelet function frequently is impaired.[344] Although early in the disease coagulation times may be shorter, rarely does one examine horses at this time. Most horses have reached a stage of hemorrhagic tendency, largely because of consumption of coagulative elements, by the time DIC is recognizable. These patients have increased PT, PTT, and activated clotting time. Because DIC accelerates fibrinolytic processes, elevations in circulating FDPs occur commonly.[344,348,350,353] In contrast to other species, horses with DIC commonly have normal to increased fibrinogen concentrations.[344,348,353] Fibrinogen acts as an acute phase reactant protein in the horse, and inflammation, which accompanies most cases of DIC in the horse, stimulates accelerated hepatic production.

Several other laboratory tests that may aid in diagnosis of DIC in the horse are not yet as widely available. Plasma AT III concentrations are considered a sensitive indicator of DIC in human beings.[358] Because AT III is bound irreversibly to serine proteases in the plasma, AT III is consumed rapidly during pathologic intravascular coagulation. AT III activity has been shown to decline in horses with experimentally induced large colon torsion.[351] This decline was reversible in horses that survived and persisted in horses that died. In horses with naturally occurring colic or acute colitis, plasma AT III activity is decreased significantly.[279,345,346] One does not always find decreases in AT III activity, however, and normal activity has been reported in ponies with coagulopathy following equine ehrlichial colitis.[349] Because AT III is a small protein, it may be lost from circulation in most disease conditions associated with hypoalbuminemia, including protein-losing enteropathies and nephropathies.[359] Increased plasma AT III activity has been associated with hepatic disease in the horse.[279]

Assays for equine α_2-antiplasmin, plasminogen, and protein C have been described, and normal values have been reported.[281,282,285] Plasma α_2-antiplasmin concentrations were decreased in ponies with coagulopathy following equine ehrlichial colitis. Plasminogen concentrations were increased in these ponies, possibly indicating a role of plasminogen as an acute phase reactant.[349] Plasma protein C concentrations may be decreased in human beings with DIC.[360] One may determine individual coagulation factor levels, but such determination rarely provides more information than PT or PTT.

Diagnostic criteria for DIC in the horse have not been defined rigidly. One must assess each patient in the light of the severity and duration of underlying disease processes, clinical appearance, and laboratory hemostatic values. One may document subclinical DIC with laboratory evaluation of animals that have no overt clinical signs of pathologic coagulopathy. Multiple hemostatic abnormalities in an animal with thrombotic or hemorrhagic tendencies strongly suggest DIC.

The therapeutic plan for horses with DIC must focus on treatment of the primary underlying disease process. Only by removing the pathologic stimulus for coagulation can procoagulant and fibrinolytic mechanisms be rebalanced effectively. One should administer supportive therapy for shock, decreased tissue perfusion, and acid-base/electrolyte abnormalities. If endotoxemia contributes to the disease process, one should administer NSAIDs. Low-dose flunixin meglumine therapy (0.25 mg/kg intravenously 3 times daily) is effective in inhibiting endotoxin-induced cyclooxygenase activity.[361] One should treat animals with sepsis with appropriate antimicrobial agents. One may adminstered fresh platelet-rich plasma intravenously to horses with life-threatening hemorrhage following DIC. Fresh plasma resupplies soluble coagulation factors, and normal platelets may restore primary hemostatic mechanisms. Administration of whole blood or blood products remains

controversial in treatment of patients with DIC because of the potential for worsening the coagulopathy on administration of fresh coagulation factors. One must consider this possibility, but in the face of life-threatening hemorrhage, benefits may outweigh risks.

Heparin therapy has been advocated for DIC in other species. Because heparin acts as an anticoagulant by complexing with AT III to inhibit serine proteases, AT III levels must be normal to achieve any benefits from 758
heparin therapy. Heparin administration is controversial in the horse because of a tendency to cause erythrocyte 759
agglutination and anemia in vivo.[240,342] Heparin has been advocated to decrease the incidence and severity of laminitis after gastrointestinal surgery, but no benefit has been documented.[338] Similarly, no significant difference in survival was apparent with heparin therapy after exploratory celiotomy in the horse.[339]

Early recognition of DIC and aggressive therapy of underlying disease conditions remain the most effective treatments for the horse with pathologic coagulopathy. Understanding the pathogenesis and diagnosis of DIC will aid the practitioner in this process.

12.4.10 THROMBOSIS AND THROMBOPHLEBITIS

A thrombus is an intravascular accumulation of fibrin, platelets, and leukocytes that usually occurs at a site of endothelial damage. Three factors may predispose a horse to vascular thrombosis: vascular endothelial injury, vascular stasis or slowing of blood flow, and abnormalities of coagulation processes. After vascular damage, thrombus formation is the normal end result of procoagulative forces. However, pathologic or abnormal thrombus formation may occur in patients with an imbalance of procoagulative and fibrinolytic forces, as in DIC.

The horse appears to be particularly prone to superficial vein thrombosis during periods of systemic activation of coagulation. Jugular vein thrombosis may occur in these animals after a single, apparently atraumatic, venipuncture. Clinical impressions suggest that endotoxemic/septicemic horses have an increased susceptibility to thrombosis at the site of indwelling venous catheters. Bilateral jugular vein thrombosis leads to increased intravascular pressures proximal to the thrombus formation and severe edema of the head. This edema may impair respiratory function, necessitating tracheostomy. If one suspects that sepsis is contributing to thrombus formation, one should initiate appropriate antibiotic therapy. If one suspects hypercoagulation as a predisposing factor for thrombosis, aspirin administration at 20 mg/kg every other day may be beneficial to inhibit platelet aggregation and release reactions.[271,273] Hot compresses and hydrotherapy may improve collateral blood flow and decrease inflammation at the site of thrombosis. The thrombus eventually should recanalize and venous flow should return. One may assess recanalization and the size of the thrombus with ultrasound examination of blood flow through the affected vessel.[362] If an underlying disease process is present, one always should direct primary care at correcting this disorder. In horses with unilateral jugular vein thrombosis, one should protect patency of the opposite jugular vein by minimizing prothrombotic trauma to that vessel, including venipuncture or catheter placement.

12.5 Blood and Blood Component Therapy

Treatment of a variety of equine disorders involves administration of whole blood, plasma, or other blood constituents. One must base therapeutic decisions on clinical signs, laboratory parameters, availability of suitable donor services, and economic considerations.

12.5.1 WHOLE BLOOD TRANSFUSION

One most commonly administers blood transfusions to horses with anemia severe enough to impair tissue oxygenation. Tachycardia, tachypnea, lethargy, weakness, cool extremities, and pale mucous membranes indicate significant tissue hypoxia and may occur at a PCV as high as 18 or as low as 10, depending on the rapidity of erythrocyte loss. Chronic anemias allow for physiologic adaptation to the low circulating RBC numbers, and severe clinical signs may not be apparent until the PCV is extremely low. Fresh whole blood transfusions also may be indicated in horses with severe hemorrhage associated with DIC to renew supplies of soluble coagulation factors and platelets.

Donor animals should lack detectable circulating alloantibodies against erythrocyte antigens that might be present in the recipient.[363,364] One can use crossmatching to detect existing antibody before transfusion. One should collect EDTA-anticoagulated blood and serum from the recipient and from several potential donor horses. The major crossmatch checks for compatibility between donor RBCs and any alloantibody that might be present in patient serum. The minor crossmatch assesses compatibility between alloantibody that might be present in donor serum and patient RBCs.[12] Both tests are important before transfusion of whole blood; the minor crossmatch is most critical when one contemplates plasma transfusion.

Transfusion of RBCs may sensitize the recipient to produce alloantibody against any incompatible erythrocyte antigens present in donor blood. For this reason, one should consider routine use of donors that are negative for factors Aa and Qa to prevent the inadvertent sensitization of brood mares against the two most common alloantigens involved in neonatal isoerythrolysis, Aa and Qa.

If crossmatching is not possible, one may consider unmatched transfusion for critical patients. One must weigh the potential adverse effects of a transfusion reaction against the potential benefits to the patient. Most horses without previous transfusions may safely receive a single transfusion or multiple transfusions over 3 to 4 days from an unmatched donor. Chances of an adverse reaction increase when either donors or recipients are mares 759
that have been bred previously. For this reason, male horses or female horses that have never been pregnant are 760
preferred as donor animals for unmatched transfusions.

One safely may remove 5 to 10 L of blood from most adult donor horses. All equipment used for blood or blood product collection and administration should be sterile, and one should perform all procedures with strict attention to aseptic technique. Stock solutions of acid citrate dextrose anticoagulant are available commercially. Alternatively, one may use sodium citrate as an anticoagulant, mixing one part of a 3.2% sodium citrate solution to nine parts whole blood. Plastic blood collection bags with premeasured anticoagulant are convenient and effective for use with horses. Little information is available about the effects of storage on equine whole blood, and use of donor blood immediately after collection is preferable.

The volume of blood administered to a patient depends on the severity of RBC depletion and the total blood volume. A recommended formula for calculating this is as follows[365]:

$$\text{body mass (kg)} \times \text{blood volume (ml/kg)} \times \frac{(\text{PCV desired} - \text{PCV observed})}{\text{PCV of donor blood}}$$

Normal blood volume in adult horses is approximately 72 ml/kg.[366] In neonates, blood volume is 151 ml/kg, decreasing to 93 ml/kg at 4 weeks, 82 ml/kg at 12 weeks, and adult values by 4 to 6 months of age.[367]

Transfused RBCs survive less than 1 week in the horse, sometimes less than 2 days, and multiple transfusions may be necessary to maintain a patient until bone marrow production can exceed the rate of peripheral RBC destruction or loss.[368]

One should administer blood products through an intravenous system with an in-line filter. Even with crossmatching of donor and recipient, one should administer the initial 25 to 50 ml of transfused blood slowly over 15 to 30 minutes with close monitoring of patient heart rate, respiratory rate, and behavior. If these parameters remain stable, one may administer the remainder of the transfusion at a rate of 15 to 25 ml/kg/hr. One should give transfusions to horses with suspected or confirmed endotoxemia or septicemia more cautiously.

Several adverse reactions, immediate or delayed, may be associated with administration of blood or blood components and may be characterized by increases in heart and respiratory rates, dyspnea, fever, trembling, weakness, hypotension, diarrhea, abdominal pain, anaphylaxis, shock, or pulmonary edema. Acute hemolytic reactions with hemoglobinemia and hemoglobinuria result from transfusions from a donor with an incompatible blood type. DIC may accompany severe acute hemolysis. One should discontinue the transfusion immediately and administer supportive therapy. Intravenous crystalline fluids improve peripheral circulation and maintain renal perfusion. NSAIDs may decrease inflammatory reactions.

Febrile reactions are not uncommon during or immediately after transfusions and may result from incompatible leukocyte or platelet antigens or presence of pyrogens in the transfused blood. One should discontinue transfusions if clinical signs are severe. NSAIDs may help in decreasing the febrile response.

Occasionally, blood products become contaminated with bacteria or bacterial products, and their administration can result in severe septicemia/endotoxemia. Affected animals develop uncontrollable shaking, hypotension, weakness, tachycardia, tachypnea, and collapse. One should discontinue the transfusion immediately and administer supportive care and should culture the blood being administered. A Gram stain may reveal bacteria if contamination is severe. One should institute appropriate antibiotic therapy. NSAIDs may help in alleviating the adverse effects of endotoxin.

More delayed reactions to transfusion include transmission of infectious diseases and allosensitization of the recipient. One should test all donor horses regularly for EIAV. Other infectious agents potentially transmittable via blood transfusions include *Anaplasma phagocytophilum* and the equine herpesviruses. Mares that receive blood transfusions are at risk for producing foals that develop neonatal isoerythrolysis later in life.

12.5.2 PLASMA TRANSFUSION

Plasma transfusion is indicated in horses with hypoproteinemia sufficient to result in significant fluid loss from the blood to the extravascular space and also is indicated for foals with failure of passive transfer (discussed previously in the text), replacement of some coagulation factors (II, V, VII, X, XIII), reversal of warfarin toxicity, endotoxemia/septicemia, and in some cases of DIC. Selection of donor animals, administration of plasma, and potential adverse reactions are similar to those described for whole blood transfusions. One may estimate the volume (in milliliters) to be administered to a hypoproteinemic horse from the following formula:

$$\text{body mass (kg)} \times \text{blood volume (ml/kg)} \times \frac{(\text{albumin desired} - \text{albumin observed})}{\text{albumin concentration of donor}}$$

Plasma volume in adult horses is estimated as 48 ml/kg.[366] In neonates, plasma volume is approximately 95 ml/kg, decreasing to 62 ml/kg at 4 weeks, 53 ml/kg at 12 weeks, and adult values by 4 to 6 months of age.[367] The observed increase in plasma protein after plasma transfusions is often not as great as would be predicted from this formula and most likely is caused by equilibration of administered blood proteins between intravascular and extravascular spaces. 760

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Plasma from suitable donors is commercially available. Large equine practices may consider the purchase of plasmapheresis equipment, capable of economically preparing large quantities of pure plasma. One also may prepare plasma by allowing erythrocytes in whole blood to settle by gravity or, preferably, by centrifugation. One then removes plasma in an aseptic manner and administers it through an intravenous system with an in-line filter.

12.5.3 BLOOD COMPONENT THERAPY

Continuous-flow centrifugation hemapheresis has been used extensively in human medicine to produce relatively pure blood components for administration to patients needing replacement of one specific blood constituent. This technique is being used increasingly in veterinary medicine and is the preferred method for equine plasma collection. Blood removed from one jugular vein of a donor horse circulates in a closed loop through the blood separation device with collection of desired blood components and return of unwanted components to the donor via the opposite jugular vein.[369,370] By altering the centrifugal force of the collection device, one may collect plasma, RBCs, leukocytes, or platelets preferentially.[371]

Collection of leukocyte and platelet-rich fractions from horses has been described.[369,371] Platelet transfusions may be indicated in selected horses with thrombocytopenia. Leukocyte transfusions have been beneficial in septic human neonates, and the feasibility and efficacy of granulocyte transfusions in the equine neonate have been investigated.[371,372]

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13 CHAPTER 13 DISORDERS OF THE GASTROINTESTINAL SYSTEM

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13.1 13.1—Examination for Disorders of the Gastrointestinal Tract

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13.1.1 Physical Examination

Examination of patients with disease of the gastrointestinal tract must include evaluation of the metabolic and cardio vascular status of the patient, because acute conditions of the proximal or distal intestinal tract have the potential to lead to endotoxemia and sepsis. Examination of the cardiovascular system (heart, peripheral pulse, and mucous membranes), lungs, and abdomen is essential to detect clinical signs of systemic inflammation from endotoxemia, coagulation disorders, dehydration, ileus, shock, and other abnormalities resulting from injury to the small or large intestine. Chapter 13.7 covers clinical signs of systemic inflammation from endotoxemia and sepsis.

One performs the physical examination of the abdomen primarily by auscultation, transabdominal ballottement, and transrectal palpation. Abdominal distention often indicates distention of the large intestine; however, small intestinal distention also can cause visible abdominal distention if a large proportion of the small intestine is involved. One can perform abdominal palpation in neonatal foals; after several weeks of age, however, the abdominal wall is too rigid to allow effective palpation of intraabdominal structures.

Abdominal auscultation is particularly useful for assessing the motility of the large intestine. Progressive motility of the small intestine, conversely, is difficult to distinguish by auscultation from nonprogressive motility. The distinct character of the borborygmi produced during propulsive contractions of the cecum and ascending colon allow evaluation of the frequency and strength of retropulsion and propulsion. Propulsive contractions of the cecum and ventral colon occur every 3 to 4 minutes and give rise to prolonged rushing sounds heard over long segments of intestine. Retropulsive sounds presumably are similar to propulsive sounds, but they occur less frequently. The distinction of propulsion from retropulsion is not important clinically because both types of contractions signify normal motility. Inter- and intrahaustral mixing contractions produce nonspecific sounds of fluid and ingesta movement that are difficult to distinguish from other borborygmi, such as small intestinal contractions or spasmodic contractions.[1]

Auscultation over the right flank and proceeding along the caudal edge of the costal margin toward the xiphoid allows evaluation of the cecal borborygmi. Auscultation over a similar area on the left side allows evaluation of the pelvic flexure and ascending colon. Typical progressive borborygmi heard every 3 to 4 minutes on both sides of the abdomen indicate normal motility of the cecum and ascending colon. Less frequent progressive sounds may indicate a pathologic condition of the large intestine or may result from anorexia, nervousness (sympathetic 769
tone), or pharmacologic inhibition of motility (i.e., α_2-adrenergic agonists such as xylazine).[2-4] Absolute absence 770
of any auscultable borborygmi suggests abnormal motility and indicates ileus resulting from a serious pathologic

condition but is not specific to any segment of the intestine.[3,5] If borborygmi are audible but progressive sounds are not detectable, determining whether a significant abnormality exists is difficult.[5]

Borborygmi heard more frequently than normal may result from increased motility following feeding; from excessive stimulation from irritation, distention, or inflammation; or after administration of parasympathomimetic drugs such as neostigmine. Large intestinal motility increases in the early stages of intestinal distention regardless of the site.[6] Mild inflammation or irritation of the large intestinal mucosa also can stimulate motility.[3] Parasympathomimetic drugs stimulate contractions and auscultable borborygmi in the large intestine; however, an increase in parasympathetic tone may result in segmental contractions, which actually inhibit progressive motility.[2]

One can detect sand or gravel in the large intestinal ingesta by auscultation behind the xiphoid process. One can hear sand or gravel particles grinding together during progressive contractions of the ascending colon. The presence of sand in the ingesta becomes clinically detectable by auscultation or fecal sedimentation before the amount of sand is enough to produce clinical signs of pain or irritation (diarrhea).[7] If progressive contractions are audible without hearing sand sounds, clinically important quantities of sand likely are not present. If the frequency of progressive contractions is low or absent, detecting sand by auscultation is difficult.

Percussion of the abdomen during auscultation can reveal gas in the large intestine. The characteristic ping produced by simultaneous digital percussion and auscultation over a gas-filled viscus often is associated with abnormal accumulation of gas under pressure. This technique is particularly useful in foals, ponies, and Miniature horses because of the limitations of palpation per rectum.

One can use transabdominal ballottement to detect large, firm masses or an abnormal volume of peritoneal fluid. The usefulness of this technique is usually limited to animals too small to palpate per rectum. One can detect soft tissue masses or fetuses by bumping the structures with a hand or fist. If excessive peritoneal fluid is present, one can generate a fluid wave by ballottement; however, this technique is not as useful in horses older than 4 weeks because the abdominal wall is rigid.

Transrectal palpation is the most specific physical examination technique for investigation of intestinal disease and is particularly valuable when evaluating obstructive diseases.[8,9] The primary objectives of transrectal palpation are to assess the size, consistency, and position of the segments of the large intestine; to determine the presence of any small intestinal distention; and to detect intraabdominal masses. Evaluation of the wall thickness and texture and the mesenteric structures (blood and lymphatic vessels and lymph nodes) also may aid in diagnosis of large intestinal disease. The interpretation of transrectal palpation findings in light of clinical signs and laboratory results is an important diagnostic aid for developing appropriate treatment strategies for intestinal diseases manifested by abdominal pain.

Enlargement of one or more segments of large intestine detected by transrectal palpation provides evidence of obstruction at or distal to the enlarged segment. By systematically evaluating each segment, one may determine the site of obstruction. Obstruction of the pelvic flexure, for instance, results in enlargement of the pelvic flexure and ventral colon, but the dorsal and descending colons are of normal size. Enlargement of a segment of the large intestine usually is accompanied by abnormal consistency of the contents. One may distinguish accumulation of gas, fluid, or ingesta and may detect foreign bodies in palpable segments. Accumulation of gas and fluid infers complete and acute obstruction, whereas accumulation of ingesta infers chronic and incomplete obstruction. Accumulation of fluid usually indicates ileus. One must evaluate the consistency of the contents in light of the size of the segment; ingesta in the ventral colon of a dehydrated patient may be firm, but the size of

the ventral colon will be normal. Conversely, if the ingesta is firm because of a distal obstruction, the ventral colon will be enlarged.

Displacement of a segment of the large intestine may create an obstruction detectable by enlargement of the segment and accumulation of gas and fluid, even if the site of obstruction is not palpable. Torsion of the ascending colon at the sternal and diaphragmatic flexures results in acute accumulation of gas and fluid proximal to the torsion, causing distention of the left dorsal and ventral colons. Depending on the degree of torsion, the position of the ventral and dorsal colons may not be significantly abnormal. Displacement of a segment of large intestine often results in incomplete obstruction, and the diagnosis relies solely on detection of the displaced segment in an abnormal position. The position of the displaced segment may not be palpable, and the diagnosis then relies on the inability to find the segment in a normal position. One must take care to ensure that the segment that appears to be displaced is not in a normal position but has become too small to palpate from a decrease in the volume of ingesta. The cecum, right dorsal and ventral colons, pelvic flexure, and descending colon are palpable in most horses. One should palpate the nephrosplenic space to detect the presence of intestine, usually pelvic flexure, entrapped within the ligament.

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Small intestine is not normally palpable in the horse. Distention is an indicator of ileus with gas or fluid retention, usually following a strangulating or nonstrangulating obstruction. Strangulating obstructions result from conditions such as volvulus or torsion, lipoma, or entrapments. Such conditions often are accompanied by severe pain, dehydration, peritoneal fluid changes, and a varying degree of gastric fluid accumulation. The small intestine in these cases is turgid and firm on palpation. One should assess the mesentery and wall thickness as for large intestinal disorders. Careful palpation of the inguinal rings in stallions with small intestinal distention is crucial for determining inguinal herniation.

Evaluation of the wall thickness and mesenteric vessels can reveal venous congestion (mural edema and enlarged blood and lymphatic vessels) or inflammation (mural edema with normal vessels). Disruption of arterial blood flow does not cause venous congestion, but the arterial pulse is not detectable. Mesenteric tears may not be palpable, but the entrapped ischemic intestinal segment may be thickened with edema. One may detect acute or chronic inflammation with cellular infiltration of the intestinal wall as thickening of the wall without edema and also may note enlargement of mesenteric lymph nodes. One should interpret abnormalities in the wall or vessels in light of the size, consistency, and position of the segment of intestine and the clinical signs.

Several conditions involving small intestinal strangulating lesions do not necessarily cause abnormal rectal examination findings until the disease has been present for an extended time. These conditions include diaphragmatic herniae and epiploic foramen entrapments. Peritoneal fluid analysis may be normal in these cases as well because the fluid is trapped in the thorax or in the cranial abdomen. Surgery is usually necessary for diagnosis.

Nonstrangulating causes of small intestinal distention can be divided further into intraluminal and extraluminal obstructions. Ileal impactions are probably the most common cause of intraluminal obstructions, and on rare occasions one can palpate the impaction in the upper right quadrant, near the ileocecal opening. Intraluminal masses caused by lymphoma, eosinophilic enteritis, foreign bodies or ascarid impactions often lead to small intestinal distention and are usually indistinguishable from one another based on palpation alone. Small intestine in these cases can be moderately to severely distended, depending on the degree of obstruction. Extraluminal obstructions include abdominal masses, abscesses or tumors, and large colon displacement. One always should palpate the rest of the abdomen carefully to help rule out these causes.

Some cases of small intestinal distention result from a physiologic rather than a mechanical obstruction. Ileus may result postoperatively or following inflammatory diseases of the bowel (proximal enteritis) or peritoneal

cavity (peritonitis). The bowel is usually mildly to moderately distended and almost always is accompanied by significant amounts of accumulated gastric fluid.

The small colon is easily distinguishable by the presence of normal fecal balls and an antimesenteric band. In cases of impaction of the small colon, a long, hard, tubelike structure is present in the caudal abdomen, and the band is palpable along the length. Fluid stool is often present in the rectum in these cases, as is tenesmus.

One can detect and carefully evaluate rectal tears by palpation. One can detect mural masses in palpable segments of intestine or mesentery; however, if a mass causes obstruction, one can detect the result of the obstruction in proximal segments of intestine even if the mass is unreachable. Palpation of the mesenteric vessels may reveal thickening and thrombosis, which can lead to ischemia or infarction.

One can perform visual inspection of the mucosa of the rectum and descending colon with a speculum or flexible endoscope and also can evaluate rectal tears or perforations, mural masses, strictures, or mucosal inflammation. One also can perform guided biopsy of the mucosa or masses. The obvious limitations are the amount of fecal material interfering with the examination and the distance of the lesion of interest from the anus. These techniques offer little advantage over palpation in many cases unless the patient is too small to palpate.

Examination of the oral cavity in cases of dysphagia or weight loss is a necessary part of the physical examination. One should adequately sedate the horse and should use a full-mouth speculum to allow palpation and visualization of all parts of the oral cavity. One should examine the area for abnormal dentition, foreign bodies, fractures, abscesses, and ulceration.

The presence of fluid accumulation in the stomach indicates a decrease or absence in propulsive motions of the small intestine or obstruction of gastric outflow. Decreased small intestinal motility may result from a functional or mechanical blockage. Masses, feed impactions, or strictures in the pylorus or in the proximal duodenum may obstruct gastric outflow. One routinely assesses fluid accumulation in the stomach by siphoning off the gastric contents with a nasogastric tube and examining the fluid for amount, color, and any particular odor. Normal fluid is green and may contain foamy saliva. The volume obtained by gastric lavage is usually less than 4 L.

Large volumes of fluid (>8 to 10 L) accumulate in the stomach of horses with proximal enteritis, and the fluid is foul smelling and often has an orange to yellow discoloration. If one suspects proximal enteritis, one can submit the fluid for culture and Gram staining. *Salmonella* sp. and *Clostridium* sp. have been cultured in some cases. Patients with postoperative ileus also frequently accumulate large amounts of gastric fluid. Horses with 771
strangulating obstructions or luminal obstructions often accumulate moderate amounts of gastric fluid, but the 772
amount is generally less than in horses with proximal enteritis or postoperative ileus. Hemorrhage in the gastric fluid usually indicates devitalized small intestine, stomach wall, or severe gastric ulceration. Fluid with large amounts of food material often indicates a gastric impaction, and one should lavage the stomach until obtaining no more ingesta. Horses and foals with chronic gastric ulceration in the glandular mucosa of the stomach or in the duodenum may develop strictures and have fluid accumulate in the stomach. Endoscopy or contrast radiography aids in diagnosing gastric outflow obstruction.

13.1.2 Clinical Pathology

Evaluation of the hemogram is essential when one assesses conditions of the gastrointestinal tract. However, hematologic alterations associated with diseases of the gastrointestinal tract are often nonspecific, reflecting systemic response to inflammation, endotoxemia, or sepsis. Neutrophilic leukocytosis and normochromic, normocytic anemia with or without hyperfibrinogenemia commonly are associated with chronic inflammatory conditions of the intestine. Anemia from chronic blood loss occurs infre quently in adult horses because of the

large iron stores and high concentrations of iron in their diet; usually anemia follows chronic inflammation, as do alterations in the leukon and plasma fibrinogen concentrations. Plasma protein concentrations vary depending on gastrointestinal losses of albumin and globulin and elevation of globulin concentration from antigenic stimulation. Protein-losing enteropathies may manifest predominantly as a hypoalbuminemia or may have a concurrent hypoglobulinemia. Immunoglobulin quantification can be useful in selected cases; immunosuppression with low immunoglobulin M concentration has been shown to occur in some cases of lymphosarcoma.[10] Parasitic infections, especially strongylosis, may be characterized by elevated serum immunoglobulin G(T) concentration.[11]

Significant alterations of the hemogram do not accompany acute disease of the intestine unless severe inflammation, dehydration, endotoxemia, or sepsis is present. During the early stages of endotoxemia, elevations in circulating concentrations of inflammatory mediators, epinephrine, and cortisol produce characteristic changes in the hemogram. Leukopenia, with neutropenia and a left shift, toxic changes in the neutrophil cytoplasm, and lymphopenia occur commonly.[12] Hemoconcentration and hyperfibrinogenemia are also common. Thrombocytopenia and other coagulopathies are also features of endotoxemia. Indeed, thrombocytopenia may be the earliest indicator of sepsis.[13] Endotoxemia and circulating mediators of inflammation activate the coagulation cascade, causing a hypercoagulable state that can lead to consumption of coagulation factors and coagulation defects manifested as elevated prothrombin time, partial thromboplastin time, fibrin degradation products, and bleeding time, and reduced activity of antithrombin III.[14–16] Neutrophilic leukocytosis occurs during the later stages of endotoxemia.[14]

The most common serum biochemical abnormalities with diseases of the large or small intestine are electrolyte imbalances. Serum calcium concentrations are often low with strangulating obstructions and acute inflammatory diseases.[17] Inflammation of the mucosa can disrupt electrolyte fluxes severely. Diarrhea or gastric reflux greatly exacerbates the loss of sodium, potassium, calcium, magnesium and bicarbonate. Ischemia of the intestine causing hypoxia and cellular damage may be reflected by an elevated serum phosphate concentration resulting from phosphate leakage from damaged cells.[18] Ischemia and cellular hypoxia in any segment of the intestine also causes a shift in energy metabolism to anaerobic glycolysis, resulting in increased production of lactate and elevated serum lactate concentration. Reduced perfusion of peripheral tissues from hypotensive shock and intestinal ischemia can cause elevations in serum lactate. However, obstruction of the intestine during ischemia may result in absorption of lactate from the lumen.[19,20] Anion gap is an indirect measurement of organic acid production during states of tissue hypoxia and is a reasonable estimate of serum lactate concentration.[20] Metabolic acidosis may accompany lactic acidemia, but an inconsistent association exists between the two, especially when mixed acid-base imbalances are present.[20,21] Elevations of hepatic enzymes, specifically γ-glutamyltransferase, may occur with large colon displacements, duodenal strictures, or anterior enteritis.

Relative polycythemia from hemoconcentration or splenic contraction and changes in red blood cell deform ability from hypoxia or hypocalcemia may increase blood viscosity. Blood viscosity increases in patients with acute obstructive disease. Hyperviscosity reduces perfusion of capillary beds, thereby exacerbating ischemia and tissue hypoxia.[22] Hyperviscosity is one manifestation (along with lactic acidemia, coagulopathies, and clinical signs of shock) of the pathophysiologic events that take place during acute inflammatory or vascular injury to the large intestine. Laboratory tests designed to reflect the systemic effects of endotoxemia, ischemia, sepsis, and shock are important to design therapeutic strategies, and monitor response to therapy.

13.1.2.1 EXAMINATION OF PERITONEAL FLUID

Abdominocentesis and analysis of peritoneal fluid (PF) is a diagnostic technique performed on many patients with disease of the gastrointestinal tract. One can quantitate cytologic examination of PF; white blood cell and red blood cell counts; protein, fibrinogen, lactate, phosphate, and glucose concentrations; lactate dehydrogenase, creatine kinase, and alkaline phosphatase activity; and pH. The results of PF analysis may help establish a specific diagnosis but more importantly may reflect inflammatory, vascular, or ischemic injury to the intestine requiring surgical intervention. 772 773

PF reflects a sequence of events that takes place during acute vascular injury to the intestine. The PF protein concentration first increases, followed by an increase in the red blood cell count and fibrinogen concentration. A transudative process resulting from vascular congestion and increased endothelial permeability allows small macromolecules (albumin) to escape into the PF, followed by larger macromolecules (globulin and fibrinogen), and finally diapedesis of cells (red blood cells, then white blood cells).[23,24] If ischemic inflammation of the intestine and visceral peritonitis occur, an exudative process ensues. Severe inflammation of the intestine and visceral peritoneum causes large quantities of protein and white blood cells, primarily neutrophils, to escape into the PF.[24] As damage to the bowel progresses, the protein concentration and red blood cell and white blood cell counts continue to rise. As the degree of irreversible damage to the intestine increases, the PF characteristics become more exudative.[23,24] Eventually, bacteria begin to translocate across the intestinal wall and appear in the PF as the mucosal barrier breaks down. Neutrophils predominate, and their cytoplasm becomes granulated, and Döhle bodies often are visible. If perforation occurs, bacteria and particles of ingesta appear in the PF, and the neutrophils become degenerate, that is, pyknotic, with karyorrhexis, karyolysis, and smudge cells.[23]

Elevated PF protein concentration is a sensitive indicator of early inflammation, whereas elevated red blood cell counts in the presence of normal white blood cell counts suggest vascular damage without significant tissue ischemia.[24] Elevation of the white blood cell count usually indicates severe tissue inflammation or intestinal injury.[25] The gross color of the PF can be helpful in detecting injury and necrosis of the intestine. A serosanguinous appearance indicates vascular injury, whereas orange or brown-red indicates necrosis with the release of pigments such as hemosiderin. Serial samples of PF are most useful in determining the nature and extent of damage to the intestine, but in many cases of ischemia, irreversible tissue damage has occurred by the time PF abnormalities appear.

Tissue hypoxia and ischemia cause a rapid elevation of PF lactate dehydrogenase, creatine kinase, and alkaline phosphatase activity and lactate concentration.[19,20,26] Phosphate concentration increases when cellular disruption occurs.[18] PF enzyme activities, phosphate, and lactate concentration increase faster and higher than serum activities.[18–20,26] PF pH and glucose concentration tend to decrease during intestinal ischemia, but not as low as in septic peritonitis.[27] Although biochemical alterations may provide early indicators of intestinal ischemia and necrosis, they are nonspecific and offer no advantage over conventional methods of PF analysis in many cases. PF alkaline phosphatase has been shown to arise predominantly from degenerating white blood cells, and elevations of other enzyme activities may occur with many inflammatory diseases.[26] Thus the specificity of many tests run on PF is questionable. However, in selected cases in which conventional PF analysis and physical examination do not provide sufficient information to develop a treatment plan, biochemical analysis of the PF may be useful.

Cytologically examined cells of the PF may reflect chronic inflammatory conditions of the large intestine, especially eosinophilic or lymphocytic processes.[28] Infectious and inflammatory conditions often cause increases in the neutrophil count and may be indistinguishable unless bacteria are visible. One also may detect neoplastic diseases by PF examination. Chronic infection and inflam mation may be associated with elevated PF protein and fibrinogen concentrations. Culture of PF usually is required to distinguish bacterial infections from noninfectious inflammation unless bacteria are visible on cytologic examination. However, culture of PF is often unrewarding because factors that are found in inflammatory PF inhibit bacterial growth, and leukocytes phagocytose many bacteria in the PF.[29] Decreases in PF glucose concentrations (<30 mg/dl) and pH (<7.3) are early indicators of a septic process. The glucose concentration and pH in the PF should approximately equal the blood glucose concentration and pH. A PF fibrinogen concentration greater than 200 mg/dl also indicates bacterial infection.[30]

13.1.2.2 FECAL EXAMINATION

Gross examination of the feces can provide information about digestion and transit time in the large intestine. Large fiber particles in the feces represent poor mastication or poor digestion in the large intestine. Small, mucus-covered, hard fecal balls indicate prolonged transit through the descending colon, whereas increased fluidity implies decreased transit time. Feces containing sand or gravel are not necessarily abnormal. However, a significant amount of sand implies that large quantities are present in the colon. Frank blood indicates substantial bleeding into the distal colon (right dorsal colon and/or small colon) from mucosal damage.

Laboratory analysis of the feces is performed frequently in cases of diarrhea. Fecal cytologic examination and 773
tests for occult blood detect mucosal inflammation, erosion, or ulceration. Severe inflammatory diseases in 774
human beings, invasive bacterial infections in particular, have been shown to increase the shedding of leukocytes in the feces. A higher percentage of horses with salmonellosis and diarrhea have fecal leukocyte counts greater than 10 cells per high power field than horses with negative fecal cultures for *Salmonella*. These results suggest that high fecal leukocyte counts indicate salmonellosis in horses with diarrhea. However, the specificity of this test is probably low. Low fecal leukocyte counts do not rule out salmonellosis.[31]

Fecal occult blood tests detect blood in the feces, presumably from erosion or ulceration of the mucosa, but do not distinguish the source of the blood. Large volumes of blood (1 to 2 L) given by nasogastric tube were required to produce a positive test for occult blood in the feces, but the amount of blood originating from the large intestine required to produce a positive test is unknown. A positive test implies significant hemorrhage into the gastrointestinal tract. Newer, more sensitive tests detect not only occult blood but also degraded blood and may be useful to determine the site and quantity of blood loss.[32] A positive test implies significant hemorrhage into the gastrointestinal tract.

Bacteriologic examination of the fecal flora has been used to quantitate specific bacterial species in the feces of horses with diarrhea. Quantitation of clostridial species may be beneficial in diagnosing clostridial infection of the large intestine.[33] Tests to detect clostridial toxins in intestinal contents or feces are important to determine whether clostridia cultured from the feces are causing disease. The most common bacterial pathogens isolated from the feces of horses are *Salmonella* and *Clostridium*. The number of *Salmonella* organisms isolated from the feces of horses with clinical salmonellosis is usually higher than from horses with asymptomatic infections. However, the volume of feces in many cases of acute diarrhea is high, and the concentration of *Salmonella* organisms may be lower than would be expected, accounting for many false-

negative fecal cultures. The sensitivity of fecal cultures for detecting *Salmonella* infection may be as low as 20%. Culture of five consecutive daily fecal samples is recommended to increase the sensitivity of the test. Because salmonellae are intracellular organisms, culture of rectal scrapings or a rectal biopsy sample, along with fecal material, may increase the sensitivity of culture for detecting *Salmonella* infection to 50%.[34] One can perform a polymerase chain reaction assay on fecal samples to detect DNA from *Salmonella* sp. The polymerase chain reaction test is more sensitive than culture and is frequently positive in clinically normal horses that continuously shed small amounts of bacteria. Polymerase chain reaction or immunologic tests also may detect *Clostridium perfringens* and *C. difficile* exotoxins in the feces.

Qualitative fecal examination is a technique to detect nematode and cestode ova, protozoan oocysts, parasitic larvae, and protozoan trophozoites. A direct smear of fecal material is a rapid method to screen feces for ova and oocysts, to detect parasite larvae and trophozoites, and to observe motility of ciliates and parasite larvae. Fecal flotation is a more sensitive technique for isolating and detecting ova and oocysts because the eggs are concentrated from the sample. Zinc sulfate and sucrose solutions are often used to concentrate less dense ova and oocysts. Zinc sulfate produces less distortion of trophozoites and larvae than sucrose solutions. Fecal sedimentation is particularly appropriate for ciliates, *Giardia,* and trichomonads. Quantitative techniques such as the Cornell-McMaster method allow one to estimate the number of eggs per gram of feces and are most appropriate in monitoring parasite control programs.[35]

13.1.3 Radiography

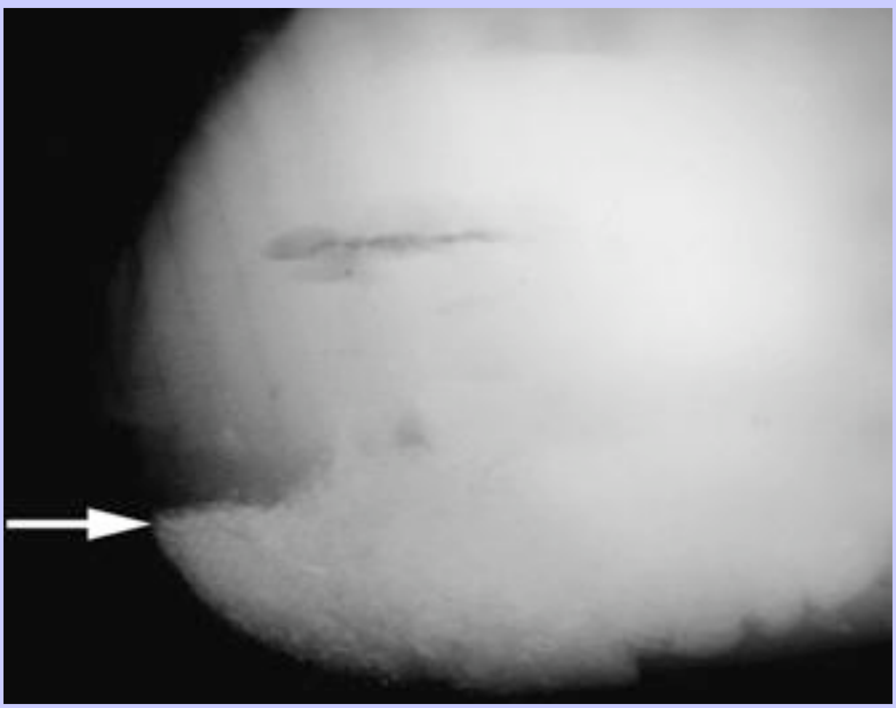
Survey radiography of the normal esophagus is usually unrewarding but may be useful in horses with esophageal obstructions to determine the extent and location of the obstruction. One may detect foreign bodies or soft tissue masses, and in cases of esophageal rupture, one may see free air and ingesta in the tissues surrounding the esophagus and may observe pneumomediastinum.[36] Thoracic radiographs may be necessary to detect intrathoracic esophageal obstructions, megaesophagus, or cranial mediastinal masses causing extraluminal obstruction. One may use barium swallows or double-contrast esophagrams after resolution of the obstruction to determine whether a stricture or diverticulum or other underlying disorder is present.[37] Barium sulfate is the usual contrast medium and can be administered orally via a dose syringe or by nasogastric tube (50 to 100 ml of a 40% barium sulfate suspension or barium paste). Oral administration is preferred for evaluation of swallowing and lesions in the proximal esophagus. Administration of contrast using a nasogastric tube (preferably cuffed) allows for delivery of larger volumes of barium (up to 500 ml) but should be performed without sedation if possible. One can follow administration of contrast material with air insufflation to create a double-contrast effect. If one suspects a rupture of the esophagus or if the likelihood of aspiration of the contrast material is high, one should use iodinated organic compounds in an aqueous solution as contrast material.[36] Contrast radiography may be the most definitive method for the diagnosis of primary megaesophagus or other functional disorders such as autonomic dysautonomia (grass sickness) affecting the esophagus.[37] One should take care when interpreting esophageal radiographs if the horse is sedated. Acepromazine or detomidine administration causes 774
esophageal dilation in normal horses, especially after passage of a nasogastric tube.[38] 775

Radiography of the adult equine abdomen is an effective technique in detecting radiodense material in the large intestine, such as enteroliths, sand, and metallic objects.[39,40] One survey demonstrated that radiography has 76.9% sensitivity and 94.4% specificity for diagnosing enterolithiasis.[39] Radiography also can be a useful tool for detecting sand accumulation in the colon that causes diarrhea or impactions (Figure 13.1-1) and for monitoring resolution in medically treated horses.[41] The large size and density of the adult abdomen precludes evaluation of soft tissue structures because the detail and contrast of the radiographs are usually poor. One is

more likely to obtain diagnostically useful abdominal radiographs from small ponies and miniature horses than from full-size adult horses. Accumulation of gas is visible on radiographs of adult horses, but distinguishing normal intestinal gas from obstruction is often difficult. Horses should be fasted for 24 to 48 hours to reduce the amount of ingesta in the large intestine before radiography.

Abdominal radiography is more useful in foals than in adult horses. Radiographs are more detailed and contrast can be good. Radiographic evidence of gas distention in the large intestine may indicate large intestinal obstruction, and radiographic signs of displacement are often diagnostic. One may diagnose impactions, intussusceptions, foreign bodies, and other disorders with the aid of radiography. Functional ileus may be difficult to distinguish from mechanical obstruction.[42,43] Administration of contrast (barium sulfate 30% at 5 ml/kg) via nasogastric tube increases the diagnostic capabilities of radiography.[44] Gastric ulceration also is recognizable with contrast radiography in the foal, although this is not as accurate a method as endoscopy.[45] Contrast administered retrograde via a 24-F Foley catheter inserted into the rectum at a dose of up to 20 ml/kg has excellent potential for diagnosing disorders of the small colon, transverse colon, and large colon in foals.[46]

Figure 13.1-1 Radiograph of the cranioventral abdominal region of a weanling colt with diarrhea. The radiopaque accumulation of sand *(arrow)* in the sternal flexure of the ventral colon is notable.



13.1.4 Ultrasonography

Ultrasonographic evaluation of the abdomen can add valuable information in cases of acute or chronic gastrointestinal disease. Examination of the adult horse requires a 2.5- to 5.0-MHz transducer at minimum. One

may use sector, linear, or curved linear transducers. Clipping of the hair over the area to be examined, along with the application of isopropyl alcohol and ultrasound coupling gel, enhances evaluation.

To evaluate the abdomen adequately, one must know the anatomic location and normal appearance of the individual organs. In the left cranial abdomen, one can assess the greater curvature of the stomach between the eleventh and thirteenth intercostal space, and one can use the spleen and the large splenic vein as landmarks. Cases of gastric dilation from gas or impaction appear as an enlargement of the viewing area to cover greater than five rib spaces.[47] One also can evaluate the stomach for intramural or extramural masses such as abscesses or for squamous cell carcinoma.[48] The lesser curvature is not routinely visible.

Assessment of the small intestine should include evaluation for changes in thickness, motility, location, and visibility. One may find small intestinal loops easily in the left lower quadrant of the abdomen, but these normally are visible in other locations. One can visualize the duodenum consistently on the right side of the abdomen deep to the liver in the tenth to twelfth intercostal space or deep to the right kidney at the fifteenth to sixteenth intercostal space. Mural thickening (>4 mm) may occur in cases of infiltrative or proliferative diseases, postoperative cases, enteritis, and paralytic or mechanical ileus. Thickening of the small intestinal wall in foals, with or without the presence of gas shadows within the wall, should raise suspicions of clostridial enteritis.

One can assess motility by monitoring a specific area for contractions over time. Ultrasonography is an accurate method of distinguishing strangulating versus nonstrangulating disorders of the small intestine. Strangulated small intestine has thicker small intestinal walls and larger intestinal diameter than in nonstrangulating disorders. Strangulating lesions have decreased motility in the incarcerated segments with normal motility elsewhere.[49] Cases of paralytic ileus or nonstrangulating obstruction have a diffusely decreased peristalsis, but not to the 775

776 degree observed with strangulating lesions.[47,49] One may diagnose some specific lesions of the small intestinal tract using ultrasonography. One may see ascarids in foals in cases of ascarid impaction[47] and epiploic foramen entrapments as edematous loops of small intestine found in the right cranial abdomen.[50] One may note small intestinal intussusceptions as targetlike lesions when viewed in cross sections.[51] The presence of bowel loops, stomach, or liver in the thoracic cavity indicates the presence of herniation through the diaphragm and should be confirmed using radiography or surgical exploration.

Evaluation of the large intestine may be difficult because of the large amounts of gas within the lumen. However, certain disorders are readily identifiable via ultrasonography. One can assess the nephrosplenic ligament area for bowel entrapment in the left paralumbar fossa. In cases of entrapment, the spleen will be pulled away from the body wall and fluid or gas shadows will be observable dorsal to the spleen,[52] obscuring the kidney, which is normally adjacent and abaxial to the spleen. Small colon, small intestine, or pneumoperitoneum also may produce a gas shadow and obscure the kidney from view.[47]

Sand impactions may appear as hyperechoic bands on the ventral abdominal wall.[47] One may see ileocecal and cecocolic intussusceptions in the upper right paralumbar fossa.[53] In cases of colitis, large, fluid-filled colons may be visible with or without intramural edema. One can find the right dorsal colon consistently abaxial to the liver, within the right thirteenth to fifteenth intercostal space and may be thickened (>5 mm) in cases of right dorsal colitis.

Evaluation of the abdomen always should include assessment of the peritoneal space for any evidence of an increased amount of PF or increased cellularity of the fluid as indicated by an increase in echogenicity. Ultrasonography also can be useful in determining the ideal location for abdominocentesis. One also should evaluate the liver, kidneys, and spleen. One may detect choleliths, nephroliths, masses, abscesses, or enlargement

of any of these organs. Abscesses or tumors not associated with visceral organs may be difficult to visualize and interpret via ultrasonography.

13.1.5 Nuclear Scintigraphy

Although more commonly used to diagnose lameness and musculoskeletal problems, nuclear scintigraphy has several uses for evaluation of the gastrointestinal tract. Scintigraphy is now available at most universities and many private referral hospitals. One must use proper isolation protocols and waste disposal techniques strictly.

The procedure requires special gamma cameras and the injection of radioactive materials into the bloodstream. One can use one of two methods: injection of technetium-99m methylene diphosphonate (^{99m}Tc-MDP) directly into the blood or injection of ^{99m}Tc-labeled leukocytes. Labeling of leukocytes involves aseptically collecting heparinized blood samples, isolating the buffy coat, and mixing those leukocytes with a radioactive dye (^{99m}Tc hexamethylpropyleneamine oxime, or ^{99m}Tc-HMPAO) in vitro.[54] One then reinjects the labeled leukocytes and obtains images. The principle of nuclear scintigraphy then lies in increased uptake of the dye or the white blood cells into areas of inflammation.

One of the most common uses of nuclear scintigraphy in evaluating the gastrointestinal tract is diagnosis of dental disease. Scintigraphy using ^{99m}Tc-MDP proved to be more sensitive in cases of dental disease than was radiography. Scintigraphy was slightly less specific, however, and therefore should be used with radiography or computed tomography for ultimate accuracy.[55] Scintigraphy using radiolabeled white blood cells can support a diagnosis of right dorsal colitis in the horse.[40] Images taken of the abdomen 20 hours after injection showed an increased linear uptake of leukocytes in the region of the right dorsal colon in horses with right dorsal colitis compared with normal horses. This technique also may prove useful for diagnosing intraabdominal abscesses in the horse. Other uses of nuclear scintigraphy include evaluation of metastasis of abdominal tumors to bony areas, assessment of biliary kinetics, and determination of gastric emptying times.[56–58]

13.1.6 Endoscopy

Endoscopic examination of the gastrointestinal tract begins with evaluation of the pharyngeal area by examination for any signs of collapse or dysfunction. One should evaluate the ability of the horse to swallow. The floor of the pharynx should be clean and free of feed material and foreign bodies. One can examine the oral cavity with the horse under heavy sedation or anesthesia and with the help of a full-mouth speculum. One can examine the teeth for any irregularities, obvious cavities, sharp points, or hooks and the hard and soft palpate for completeness and any evidence of ulceration, masses, or foreign bodies.

One should use a 3-m flexible fiberoptic endoscope to examine the esophagus, which is accomplished best by passing the endoscope into the stomach and viewing the esophagus as one withdraws the endoscope while dilating the lumen with air. The esophageal mucosa normally should be a glistening, light pink color. Ulceration can occur with cases of choke, reflux esophagitis or in horses that have had an indwelling nasogastric tube. 776
Erosions may be punctate, linear, or circumferential. One should evaluate carefully for any ulcers to ensure that 777
no areas of perforation through the entire thickness of the esophageal wall exist. Distinguishing normal peristaltic contractions from areas of stricture requires observation of the area and its motility over time. One also may note diverticula as outpouchings of the mucosa, sometimes associated with a stricture distally. Megaesophagus, although rare, appears as a generalized dilation of the esophagus. One may detect food or foreign body impactions of the esophagus via endoscopy. One always should reevaluate the esophagus after

removing any obstruction to detect the presence of complications (ulceration, rupture) or initiating causes (strictures, diverticula, and masses).

A 3-m flexible endoscope also allows examination of the stomach. The horse should be fasted for at least 12 hours before endoscopy. One can examine the cardia and fundus easily, as well as the margo plicatus. The squamous mucosa should resemble the esophageal mucosa. The glandular mucosa should be glistening red and may have a reticulated pattern. One should carefully examine for evidence of ulceration or masses. One can obtain transendoscopic biopsy material easily from esophageal, pharyngeal, or gastric masses, and because the biopsy size will be small, one should take several samples for histopathologic examination. Pharmacologic agents (bethanechol) to empty the stomach and provide complete visualization of the entire fundic region, the pylorus, and the duodenum may be useful. For a complete description of gastroscopy and evaluation of gastric and gastroduodenal ulceration, please refer to Chapter 13.10.

13.1.7 Tests of Absorption and Digestion

D-Glucose or D-xylose absorption tests are useful in determining malabsorption of carbohydrates from the small intestine in horses. The protocol for absorption tests using either carbohydrate is similar. The horse should be fasted for 18 to 24 hours before testing. Increased periods of fasting actually have been shown to decrease absorption of d-xylose and interfere with results.[59] One administers a dosage of 0.5 to 1 g/kg of D-glucose or D-xylose via a nasogastric tube. Administration of sedatives may increase the blood glucose levels falsely and interfere with gastrointestinal transit times. One then collects blood samples to measure glucose or xylose concentrations at 0, 30, 60, 90, 120, 150, 180, 210, and 240 minutes after administration. One may take additional samples up to 6 hours after dosing if the results are questionable. One should measure glucose in blood samples collected with sodium fluoride as an anticoagulant and measure xylose in samples collected in heparinized plasma.

A normal D-glucose absorption test, also known as an oral glucose tolerance test, should have a peak between 90 and 120 minutes, and this peak should be greater than 85% above the resting glucose value.[60] *Complete malabsorption* is defined as a peak less than 15% above the resting levels, and *partial malabsorption* is defined as a peak between 15% and 85% above the resting level. One must keep in mind that gastric emptying, gastrointestinal transit time, length of fasting, cellular uptake and metabolism, and endocrine function influence glucose absorption curves. Malabsorption demonstrated by the oral glucose tolerance test is sensitive but not specific. Diseases that may cause a lowered or delayed peak include infiltrative lymphosarcoma, inflammatory bowel disease (lymphocytic-plasmacytic or eosinophilic), cyathostomiasis, chronic colitis (*Salmonella* sp.), multisystemic eosinophilic epitheliotropic disease, food allergies, and small intestinal bacterial overgrowth.[61]

D-Xylose absorption tests have some advantages over the oral glucose tolerance test because xylose is not metabolized in the small intestinal mucosa and insulin does not influence its absorption. Gastric and intestinal motility, intraluminal bacterial overgrowth, and renal function still influence xylose absorption, because the kidneys clear xylose.[61] The other main drawback to d-xylose is that it is generally available only in research settings. However, xylose measurements are available at most major universities. A normal d-xylose absorption curve should peak between 20 and 25 mg/dl at 60 to 120 minutes after dosing.[62] Decreased xylose absorption can occur in horses with inflammatory bowel disease, lymphosarcoma, multisystemic eosinophilic epitheliotropic disease, cyathostomiasis, extensive small intestinal resections, and any cause of villous atrophy.[61]

Maldigestion is a common occurrence in foals with diarrhea. Bacteria (especially *Clostridium* sp.) and viruses (especially rotavirus or coronavirus) may invade and destroy the villous epithelial cells that manufacture lactase

and other disaccharidases, resulting in an inability to digest lactose. In this case, continued ingestion of the mare's milk may cause an osmotic diarrhea, which may exacerbate the underlying enterocolitis. One can perform lactose tolerance testing to assess the degree of maldigestion. One administers d-lactose at 1 g/kg as a 20% solution via nasogastric tube and measures glucose concentrations in the blood at 0, 30, 60, 90, 120, 150, 180, 210, and 240 minutes. A normal curve shows doubling of glucose levels compared with baseline by 60 minutes after administration.[63]

13.1.8 Evaluation of Gastric Emptying

Assessment of gastric emptying may be useful in evaluating delayed emptying of feed or fluids from the stomach in cases of gastric and esophageal ulceration, pyloric stenosis, proximal enteritis, and postoperative ileus. 777
However, accurate measurement of gastric emptying can be difficult to assess. Several methods are currently 778
available.

Multiple diagnostic imaging techniques have been used to study gastric emptying times. One can use contrast radiography to assess gastric emptying in foals. In the normal foal, barium remains in the stomach for varying amounts of time, but a significant amount should be gone within 2 hours.[44] Gastric emptying of solid, nondigestible, radiopaque markers also has been used in adult horses and ponies, but the results were variable and unpredictable even in the normal horse.[64] Nuclear scintigraphy is used commonly in human beings to measure gastric emptying and can be used in horses where available. This technique requires oral administration of ^{99m}Tc pentenate (10 mCi), and serial images taken of the cranial abdomen. The tracer is usually not visible 60 minutes after administration in normal horses.[58]

Alternatively, if nuclear scintigraphy is not available, one can use acetaminophen absorption testing as an indirect determination of gastric emptying.[58,65] One performs this test by administering 20 mg/kg of acetaminophen orally and measuring subsequent blood values and calculating the time to reach maximum serum concentrations and the absorption constant. In human beings, the proximal small intestine absorbs almost all of the acetaminophen.[66] The median time to reach peak plasma levels using acetaminophen absorption in horses was 47.7 minutes.[58]

13.1.9 Histopathologic Examination

One often requires histopathologic examination of tissue s from the intestine to diagnose chronic inflammatory, infiltrative, or neoplastic conditions, and such examinatio n can be useful in evaluating the extent of injury after obstruction or ischemia. Rectal mucosal biopsies are easy to collect with few complications. However, to collect a full-thickness biopsy of the intestine requires a surgical approach (flank or ventral midline approach). Laparoscopy offers a safer technique to observe the large intestine and other abdominal structures.[67] One can obtain biopsies of masses, lymph nodes, mesentery, or intestinal serosa via laparoscopy and mucosal biopsies of the upper gastrointestinal tract via endoscopy.

13.1.10 Advanced Diagnostics

Other diagnostics, specifically laparoscopy and computed tomography, are available but require specialized equipment and personnel with specific training. Flexible or rigid endoscopes used for laparoscopic evaluation of the abdomen allow for visualization of visceral organs and potentially for collection of biopsy material from

masses or organs. Full-thickness biopsies of the intestines are not routinely possible through the laparoscope and usually require flank or ventral midline laparotomy. The laparoscopic procedure can be done in the standing or recumbent horse. Advantages of this technique over a flank or ventral midline celiotomy include smaller incisions, less healing time, and less procedure time. Disadvantages include the large amount of equipment needed, skill involved, and the limitation as a diagnostic modality, rathe r than a treatment.[68] Clinical applications of diagnostic laparoscopy include rectal tears, percutaneous abscess drainage, assessment of adhesions, displacements, and integrity of the serosa of various bowel segments, and biopsy of abdominal masses.[67]

Computed tomography scans are available at several universities across the country. They have been used frequently to evaluate dental disease and may be useful in evaluating tumors and masses of the head, larynx, pharynx, and proximal esophagus.[69] Computed tomography also has promise for evaluating abdominal disorders in foals. Most equipment can accommodate up to 400 lb. Restrictions to computed tomography as a diagnostic aid include expense, availability, expertise, and weight and size limitation.

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13.2

13.2—Pathophysiology of Gastrointestinal Inflammation

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The inflammatory response of the gastrointestinal tract is a mechanism ultimately aimed at eliminating pathogens, initiating tissue repair, and restoring the gastrointestinal barrier. Inflammation alters blood flow, endothelial permeability increases, cells are recruited rapidly into the tissue, plasma protein cascades are activated, and a myriad of soluble products are released that coordinate the response, trigger innate and adaptive immunity, and mobilize reparative elements. Although the cellular and vascular response and the secreted mediators of

inflammation are important for killing pathogens and limiting invasion of injured tissues by commensal organisms, they can be damaging to host cells and proteins if not tightly regulated. Thus if the inciting stimulus is not eliminated quickly, the inflammatory response itself causes significant tissue injury. The mechanism regulating inflammation has been the focus of much research to identify therapeutic targets to modulate the damage to host tissues during many gastrointestinal diseases. Recent work has provided some of the molecular and cellular details of this complex physiology and has led to novel therapeutic strategies for treating inflammation.

13.2.1 Initiation of the Inflammatory Response

13.2.1.1 EPITHELIUM

The gastrointestinal epithelium interfaces with a luminal environment inhabited by potentially hostile microbial organisms. The epithelium presents a physical barrier to invasion by the flora of the gastrointestinal tract, consisting of the apical cellular membrane, intercellular tight junctions the permeability of which is highly regulated, and a secreted layer of mucus. Breaching of the mucosal barrier by invading pathogens generates potent soluble and neural signals that initiate an inflammatory response.[1] Conceptually, the epithelium can be thought of as a sensory organ detecting pathogen invasion to trigger an appropriate host defense and reparative response.

Noninfectious mucosal injury or invasion of epithelial cells by pathogenic organisms such as *Salmonella* activates synthesis of proinflammatory chemokines (chemoattractants) by epithelial cells and triggers a robust influx of neutrophils into the tissue within hours of the damage.[1] Of the chemoattractants produced by epithelium, interleukin-8 (IL-8) has a particularly important role in initiating inflammation by recruiting neutrophils from blood[2–4] and regulating neutrophil migration through tissue matrix adjacent to epithelium.[5,6] Bacteria-derived formylated chemotactic peptides also act as potent chemoattractants that are fully capable of stimulating a robust inflammatory response in the intestine if the epithelial barrier permits the diffusion of the peptides across the mucosa.

Epithelial cells activated during infection produce cytokines such as tumor necrosis factor α (TNF-α), 780
arachidonic acid metabolites, and other proinflammatory mediators that activate recruited leukocytes.[7] 781
Bacterial products, particularly lipopolysaccharide and other cell wall components, are potent activators of leukocytes recruited into the tissue. Once the inflammatory response has been initiated, TNF-α, IL-1β, and other proinflammatory products of neutrophils, monocytes, mast cells, and epithelial cells amplify the inflammatory response.

The enteric nervous system has a key role in sensing and regulating inflammatory responses in the intestine. For example, *Clostridium difficile* toxin A activates a neural pathway that triggers mast cell degranulation and neutrophil influx into the tissue.[8,9] Blockade of this neural pathway is sufficient to abolish the profound inflammatory response induced by toxin A and many of the effects of toxin A on enterocyte secretion. Other pathogens and immune-mediated hypersensitivity reactions similarly stimulate inflammation by mechanisms that involve the enteric nervous system. Thus the epithelium interacts in a highly complex manner with the intestinal milieu, the enteric nervous system, and inflammatory cells to regulate the tissue response to injury and infection.

13.2.1.2 MACROPHAGES

Resident macrophages located in the lamina propria, submucosa, and intestinal lymphoid organs are among the first cells beyond the epithelium to respond to infection or injury. Macrophages are activated by bacterial products via pattern recognition receptors and begin to produce proinflammatory molecules important for recruiting and activating neutrophils and monocytes. Pattern recognition receptors recognize microbial products ranging from lipopolysaccharide to peptidoglycan and even CpG-containing bacterial DNA and signal the invasion by pathogens. Of the pattern recognition receptors, the lipopolysaccharide receptor complex is perhaps the best defined. Lipopolysaccharide activates macrophages via the CD14–Toll-like receptor complex to initiate transcription of the inflammatory cytokines TNF-α and IL-1β, which synergize with lipopolysaccharide to amplify the macrophage response.[10] Lipopolysaccharide, particularly in concert with inflammatory cytokines, stimulates macrophages to produce copious amounts of nitric oxide, which is microbicidal and vasoactive. Nitric oxide and other nitrogen radicals react with reactive oxygen intermediates (ROIs) to produce some of the most toxic molecules of the host defense system: the peroxynitrites.[11] IL-8 is produced as well to recruit neutrophils. As the response progresses, other inflammatory mediators, particularly the arachidonic acid–derived lipids, are produced. These lipids have potent vasoactive effects and are important stimuli of endothelial cells, neutrophils, and platelets.

13.2.2 Vascular Response During Inflammation

Four important changes occur in the intestinal vasculature during inflammation:

1. Alteration of blood flow
2. Increased vascular permeability
3. Increased adhesiveness of endothelial cells, leukocytes, and platelets
4. Exposure of the basement membrane and activation of the complement, contact, and coagulation cascades

A wide range of mediators alters blood flow during intestinal tract inflammation, from gasses such as nitric oxide (a major vasodilator of the intestinal vasculature) to lipids (prostaglandins, leukotrienes, thromboxanes, and platelet-activating factor), cytokines, bradykinin, histamine, and others. The major sources for these mediators include activated leukocytes, endothelial cells, epithelial cells, and fibroblasts. The primary determinant of blood flow early in inflammation is vascular caliber, which initially decreases in arterioles, but then quickly changes to vasodilation coincident with opening of new capillary beds, increasing net blood flow. The increase in blood flow is short lived, for the viscosity of the blood increases because of fluid loss and tissue edema through leaky capillaries. Leukocyte margination, platelet adhesion to endothelial cells and exposed matrix, and areas of coagulation protein accumulation further decrease local circulation.

Inflammatory mediator actions on the endothelial cells initially increase vascular permeability. Histamine, leukotrienes, platelet-activating factor, prostaglandins, bradykinin, and other mediators stimulate endothelial cell contraction, and interendothelial gaps form.[12,13] This stage of increased vascular permeability is readily reversible. Concurrently, mediators such as the cytokines TNF-α and IL-1β induce a structural reorganization of the interendothelial junctions, resulting in frank discontinuities in the endothelial monolayer.[14] Cytokines also stimulate endothelial cells to express adhesion molecules that support adhesion of leukocytes and platelets,[15]

leading to the next and perhaps most devastating event. Leukocytes (primarily neutrophils) and platelets adhere to exposed basement membranes and activated endothelial cells. Adherent neutrophils and platelets then are exposed to the mediators of inflammation present in the surrounding milieu, which activates the cells to release oxidants and proteases (particularly elastase) that injure the endothelium and have the potential to cause irreparable harm to the microvasculature.[16–18] Marginated neutrophils begin to transmigrate between endothelial 781
cells (as described in later sections), and if their numbers are large enough, they disrupt the integrity of the 782
interendothelial junctions, worsening the vascular leakage.[17]

Conceptually, these stages of enhanced vascular permeability can be thought of as a mechanism to allow plasma proteins to enter the tissues and to potentiate the critical influx of leukocytes into tissues. However, if not regulated precisely, alterations in hydrostatic and oncotic forces and irreversible damage to the vascular bed may have devastating consequences. Moreover, inappropriate activation of plasma protein cascades and leukocytes by activated endothelium and exposed matrix proteins can contribute to systemic inflammation (systemic inflammatory response syndrome; see Chapter 13.7 for more information) characterized by hypotension, generalized vascular leak syndrome, and multiorgan dysfunction, which may be fatal. Phosphodiesterase inhibitors reduce endothelial permeability in ischemia-reperfusion injury and other models of inflammation-induced vascular leakage[19,20] by increasing endothelial tight junction integrity and thus may be a viable therapeutic strategy to prevent or reduce the permeability alterations associated with inflammation.

13.2.3 Cellular Effectors of Inflammation

13.2.3.1 ENDOTHELIAL CELLS

Endothelial cells respond to products of activated epithelial cells and macrophages in the intestinal tissue to recruit cells and humoral mediators of inflammation into the tissue. Activated endothelial cells display a range of molecules critical for neutrophil and platelet adhesion. Intercellular permeability increases to expose basement membrane proteins that trigger humoral defense systems (complement, coagulation, and contact system cascades) and to provide access for these macromolecules to the tissue. Endothelial cells are an important source of inflammatory mediators that amplify the response and vasoactive substances (particularly nitric oxide) that alter blood flow.

13.2.3.2 NEUTROPHILS

13.2.3.2.1 Recruitment

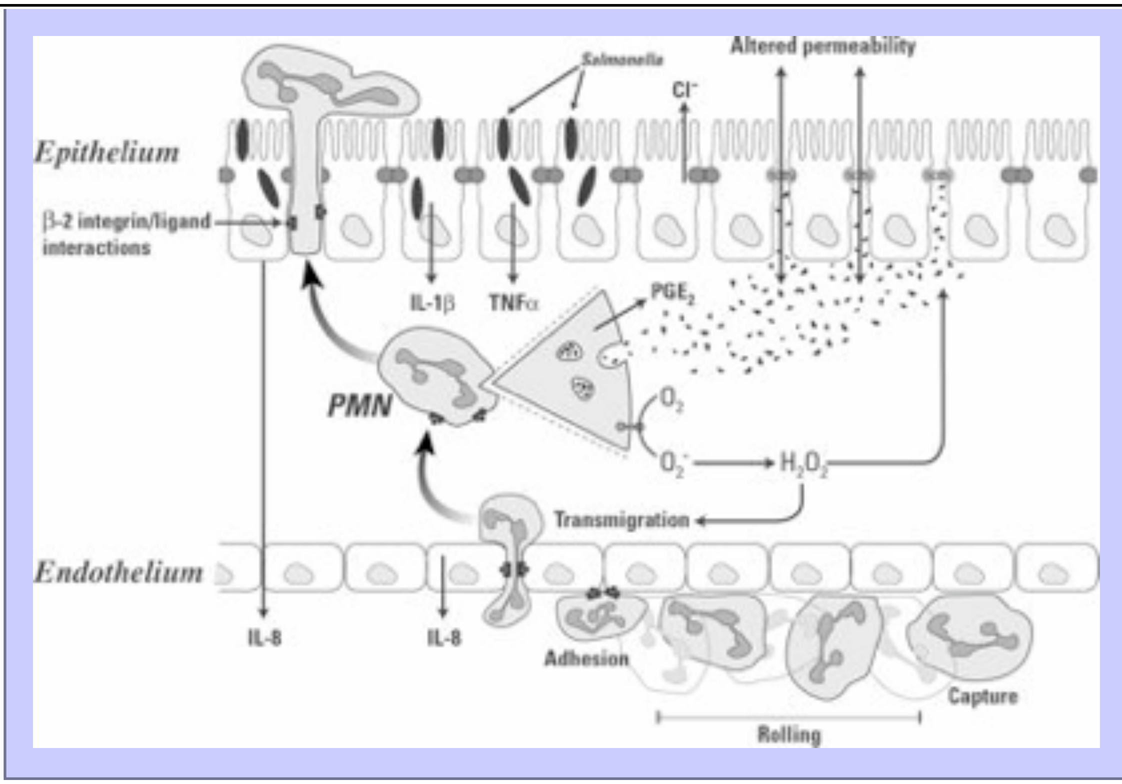
Infection or injury to the gastrointestinal mucosa causes an influx of leukocytes from the blood that lay the foundation of the inflammatory response. Neutrophils, being the first to arrive during inflammation, have a dominant role in the acute response. Within minutes, neutrophils are recruited into the tissue where they are activated to release products that not only are proinflammatory and lethal to pathogens but also may damage host cells and tissues. Not surprisingly, much attention has been paid to the role of neutrophils in the pathophysiology of many inflammatory conditions.[21] Neutrophil depletion is protective in many models of gastrointestinal inflammatory disease. Of interest to clinicians, blockade of neutrophil migration into inflamed tissues prevents many of the pathophysiologic events associated with infectious enteritis, ischemia-reperfusion injury, and other gastrointestinal diseases.[16,22–26]

Neutrophil transendothelial migration is a multistep process that is temporally and spatially regulated and has a degree of cell type specificity (Figure 13.2-1). The predominant sites of neutrophil transendothelial migration are in the postcapillary venules and, in some tissues, the capillaries. Endothelial cells in these vessels respond to cytokines and other soluble signals by expressing molecules that promote neutrophil adhesion and transmigration, including selectins and counterreceptors for integrins. As neutrophils flow through these vessels, they are first tethered to activated endothelium. Tethering is mediated by selectin molecules expressed on neutrophils (L-selectin) and on activated endothelial cells (P- and E-selectins) that bind to P-selectin glycoprotein ligand-1 (PSGL-1), E-selectin ligand-1 (ESL-1), and other mucin counterreceptors.[27,28] Tethering functions to increase the exposure of the neutrophil to activating chemokines presented on the surface of the endothelial cells.

Stimulation of neutrophils by IL-8 and other chemokines activates the second step of transendothelial migration. Chemokine binding to their receptors on the neutrophil generates signals that activate the binding of integrin adhesion receptors to their ligands, called intracellular adhesion molecules or vascular cell adhesion molecules expressed on endothelial cells in inflamed mucosa. Integrin ligation to cellular adhesion molecules arrests the tethered neutrophils, resulting in firm adhesion to the endothelium. Of the integrins expressed on neutrophils, the β_2 integrins have a particularly important role in transendothelial migration. Calves and human beings with a disorder known as leukocyte adhesion deficiency illustrate the requirement for β_2 integrin–mediated adhesion in neutrophil function. Leukocyte adhesion deficiency results from an autosomal recessive trait causing the lack of the β_2 integrin expression. The neutrophils from these individuals cannot migrate into most tissues and do not function normally, resulting in poor tissue healing and profound susceptibility to infection, especially at epithelial barriers.[29,30] Other integrins also have a role in transendothelial migration. β_1 Integrins mediate transendothelial migration in some cells and seem to be particularly important for mediating emigration of monocytes into many tissues.[31]

Following this firm adhesion step, neutrophils migrate through the endothelium along a chemotactic
gradient of IL-8 and other chemoattractants such as leukotriene B_4.[3,17,32] Neutrophils migrate across the 782
endothelial monolayer at intercellular junctions via a mechanism involving a series of integrin-ligand 783
interactions mediated by β_2 and β_1 integrins and other adhesion molecules[28] that is generally capable of maintaining the integrity of the endothelial barrier.[33] However, massive flux of neutrophils through the endothelium alters endothelial tight junctions and injures the basement membrane, resulting in increased endothelial permeability to molecules as large as plasma proteins and even endothelial cell detachment from the basement membrane.[17,18] Nonintegrin molecules such as platelet/endothelial cell adhesion molecules (PECAMs) also are involved in transendothelial migration of neutrophils.[28] Homotypic binding of PECAMs on adjacent endothelial cells form part of the intercellular junction. Neutrophils express an
integrin of the β_3 family that can bind PECAM, and via sequential binding of β_3 integrins to PECAM, the 783
neutrophil can "unzip" the intercellular junction and migrate through, closing it behind itself. 784

Figure 13.2-1 Depiction of neutrophil responses during intestinal inflammation in response to salmonella infection. Salmonellae infect epithelial cells, stimulating the production of chemokines (interleukin-8 [IL-8]), cytokines (IL-1β and tumor necrosis factor-α [TNF-α]), and other proinflammatory mediators. Endothelial cells stimulated by inflammatory mediators produce chemoattractants (such as IL-8) and display adhesion molecules that promote neutrophil emigration. The three steps of neutrophil (polymorphonuclear [PMN]) emigration— capture/rolling (mediated by selectins), adhesion (mediated by β_2 integrins), and transendothelial migration (mediated by integrins and platelet/endothelial cellular adhesion molecule [PECAM])— occur on activated endothelium. Chemoattractant molecules, such as IL-8 trigger neutrophil emigration. In inflamed tissues, cytokines (IL-1β and TNF-α) and a variety of other proinflammatory mediators stimulate the neutrophil oxidase complex to produce reactive oxygen intermediates (ROIs; O_2^- and H_2O_2 and their derivatives). Activated neutrophils degranulate to release proteases and other hydrolases, cationic peptides (defensins), myeloperoxidase, and other products into the tissue. Activated neutrophils synthesize a variety of inflammatory mediators, including prostaglandins (PGE_2) that modulate the inflammatory response. The products of activated neutrophils (ROIs, proteases, and mediators) stimulate epithelial secretion and alter tight junction permeability, promoting diarrhea. Neutrophils eventually migrate across the infected epithelium by a mechanism that involves integrins, disrupting tight junction integrity and increasing permeability to bacterial products, thus exacerbating the inflammatory response.



13.2.3.2.2

Activation

A key feature of neutrophils and other leukocytes is the requirement for integrin-mediated adhesion to extracellular matrix proteins (ECMs) or other cells to achieve an optimal effector phenotype.[34] Critical components of the ECMs in inflamed tissues include fibronectin, fibrinogen, and vitronectin, deposited in tissues as a result of plasma leakage and by synthesis of new proteins by stromal cells and resident macrophages in response to inflammatory mediator activation. The changing composition of the matrix proteins deposited in tissues during inflammation serves as a clue as to the nature of the tissue environment for recruited inflammatory cells as they become activated. Individual gene expression studies have demonstrated that adhesion to matrix proteins induces the expression of cytokines and chemokines and their receptors, arachidonic acid–derived lipid mediator synthases, metalloproteinases, growth factors, transcription factors, and other genes that influence the differentiation and activation of inflammatory cells.[35] ROI production, phagocytosis, degranulation, and other effector functions stimulated by inflammatory mediators and bacterial products are optimal only when neutrophils adhere to the ECMs.[34] Adhesion to distinct ECM proteins selectively activates signaling pathways and gene expression of neutrophils, monocytes, and other leukocytes with differing abilities to promote certain functions such that the composition of ECMs in many ways controls the development of the ultimate effector phenotype. Thus integrin-mediated adhesion provides a mechanism by which neutrophils and other leukocytes can sense the complex tissue environment and respond appropriately.

Of the activators of neutrophils at sites of inflammation, complement (C3-opsonized particles), cytokines (TNF-α and IL-1β), platelet-activating factor, immune complexes, and bacterial products are among the most potent stimuli. Other mediators produced during inflammation may modify neutrophil activity, particularly formylated bacterial peptides, chemokines, complement fragments (C5a), leukotriene B_4, and

prostaglandins. Activated neutrophils are highly phagocytic; produce large amounts of ROIs; degranulate to release myeloperoxidase, cationic antimicrobial peptides (defensins), serine proteases (mainly elastase), and metalloproteinases; and secrete inflammatory mediators (TNF-α, IL-1β, prostaglandins, leukotrienes, and others) (see Figure 13.2-1).

13.2.3.3 MAST CELLS

Mast cells strategically reside in mucosal tissues, including the submucosa and lamina propria of the gastrointestinal tract, and constitute a crucial first line of defense at epithelial barriers. However, they are also important effector cells of the pathophysiology of inflammatory gastrointestinal diseases.[36] Experimental depletion of mast cells, genetic deficiency in the development of mast cells, or pharmacologic stabilization of mast cells to prevent degranulation have a protective effect in a variety of models of gastrointestinal inflammatory disease, including dextran- or trinitrobenzenesulfonic acid–induced colitis,[37,38] ischemia-reperfusion injury,[39,40] and immediate hypersensitivity responses.[41]

Mast cells are activated by a wide variety of microbial products and host-derived mediators.[42] Among the activators of mast cells the so-called anaphylatoxins (complement fragments C3a, C5a, and C4a), are potent stimuli causing release of mediators of inflammation. In addition, mast cells are the primary effector cells of immunoglobulin E–mediated anaphylaxis (type I hypersensitivity reactions) by virtue of their high affinity receptors for immunoglobulin E. Cross-linking of receptor-bound immunoglobulin E on mast cell surface by antigens (i.e., food antigens) causes rapid degranulation, resulting in the explosive release of granule contents.[43] Neural pathways in the intestine also regulate mast cells. Mast cells respond to enteric pathogen invasion via neural reflexes that stimulate the release of inflammatory mediators.

Activated mast cells release preformed histamine, 5-hydroxytryptamine, proteases, heparin, and cytokines from granules. Activation also stimulates de novo synthesis of a range of inflammatory mediators, including prostaglandins, platelet-activating factor, and leukotrienes. Transcription of a number of peptide mediators, such as the cytokines TNF-α and IL-1β among many others, also increases on stimulation of mast cells. Mast cell products have profound effects on the vasculature, increasing endothelial permeability and causing vasodilation.[44] Moreover, mast cell–derived mediators greatly enhance epithelial secretion by a mechanism that activates neural pathways of epithelial secretion and directly stimulates epithelial cells.[43] Mast cell products significantly alter intestinal motility, generally increasing transit and expulsion of intestinal contents. Mast cell–derived leukotrienes and TNF-α also have a crucial role in host defense against bacterial pathogens, acting to recruit and activate neutrophils.[45,46]

Mast cells have a newly identified role in host defense and inflammatory responses to bacterial pathogens, which in part is caused by the release of proinflammatory mediators during bacterial infection, which is critical for recruiting and activating other innate host defense cells such as neutrophils. However, mast cells are also phagocytic, have microbicidal properties, and can act as antigen-presenting cells to the adaptive immune system.[47] Although accumulating evidence was establishing the role of mast cells in innate immunity, a seminal study that unconditionally identified the importance of mast cells in host defense demonstrated that mast cell–deficient W/W^v mice have impaired responses to gram-negative bacterial peritonitis, resulting in a significant increase in mortality. The role for mast cells in host protective responses appears to be as a sensor of bacterial invasion. Unlike immunoglobulin E–mediated responses, bacterial products seem to elicit a highly regulated and selective response from mast cells.

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13.2.4 Humoral Mediators of Inflammation

13.2.4.1 COMPLEMENT

The complement cascade is a fundamental part of the inflammatory response. Activation of the complement cascade by immune complexes (classical pathway) or by bacteria or bacterial products, polysaccharides, viruses, fungi, or host cells (alternative pathway) results in the deposition of complement proteins on the activating surface and the release of soluble proteolytic fragments of several complement components. In particular, activation of either pathway results in the deposition of various fragments of the complement protein C3, which are potent activators of neutrophils and monocytes.[48] Opsonization of particles with C3 fragments constitutes a major mechanism of target recognition and phagocyte activation.[49] During the activation of the complement cascade culminating in deposition of C3, soluble fragments of C3 (C3a), C5 (C5a), and C4 (C4a) are liberated. These fragments, termed *anaphylatoxins,* have potent effects on tissues and cells during inflammation. Perhaps most notably, anaphylatoxins are chemotactic for neutrophils (particularly C5a), activate neutrophil and mast cell degranulation, and stimulate ROI release from neutrophils.[48] The termination of the complement cascade results in the formation of a membrane attack complex in membranes at the site of complement activation, and if this occurs on host cells such as endothelium, it may cause irreversible cell injury. Although the primary source of complement is plasma, epithelial cells of the gastrointestinal tract also produce C3, suggesting that local production and activation of the complement cascade during inflammation occurs in intestinal tissues.

Clearly, if the regulatory mechanisms of the complement cascade fail, then the inflammatory response may be inappropriate and tissue injury can occur. The role of complement in gastrointestinal inflammation has been most studied extensively in models of ischemia-reperfusion injury. Activation of the complement cascades has a major role in altered endothelial and epithelial permeability in these models. Several lines of evidence support the importance of complement in intestinal injury. Mice deficient in C3 or C4 are protected against ischemia-reperfusion injury.[50] Moreover, administration of monoclonal antibodies against C5 reduced local and remote injury and inflammation during intestinal reperfusion injury in a rat model.[51] Administration of a soluble form of complement receptor 1, a regulatory protein that halts the complement cascade by dissociating C3 and C5 on host cell membranes, reduced mucosal permeability, neutrophil influx, and leukotriene B_4 production during ischemia-reperfusion injury in rats and mice.[50,52] Although neutrophils and mast cells mediate many of the pathophysiologic effects of the complement cascade, the membrane attack complex may have a primary role in altered vascular permeability during ischemia-reperfusion injury.[53]

13.2.4.2 CONTACT SYSTEM

Four components initiate the contact system of coagulation: Hageman factor (HF), prekallikrein, factor XI, and high-molecular-weight kininogen. HF is a large plasma glycoprotein that binds avidly to negatively charged surfaces.[54] Bacterial cell walls, vascular basement membranes, heparin, glycosaminoglycans, and other negatively charged surfaces in the intestine capture HF and the other three important initiators of the contact system in a large multimolecular complex. Of the surfaces that bind HF, the extracellular matrix is a potent activator of the contact system. Once bound, HF is converted to HF-α, which cleaves prekallikrein to kallikrein and factor XI to factor XIa. The ultimate result is further cleavage of HF by kallikrein and triggering of the contact system cascade, activation of intrinsic coagulation by factor XIa, activation of the alternative

pathway by HF, and proteolytic cleavage of high-molecular-weight kininogen by kallikrein, releasing biologically active kinins.

The products of the contact system, particularly bradykinin, have several important biologic properties that drive many of the vascular and leukocytic responses during inflammation.[54] Bradykinin induces endothelial cell contracture and intracellular tight junction alterations that increase vascular permeability to fluid and macromolecules. Bradykinin also affects vascular smooth muscle contracture, resulting in vasoconstriction or vasodilation depending on the location. Bradykinin also increases intestinal motility, enhances chloride secretion by the intestinal mucosa, and intensifies gastrointestinal pain. In neutrophils, kinins stimulate the release of many inflammatory mediators, including cytokines, prostaglandins, leukotrienes, and ROIs.[55] Kallikrein cleaves C5 to release C5a, a potent chemotactic factor for neutrophils, and thus has a role in recruiting and activating inflammatory leukocytes.

The plasma kallikrein-bradykinin system is activated in a variety of acute and chronic inflammatory diseases 785
of the gastrointestinal tract.[56,57] Recent evidence has demonstrated that blockade of the pathophysiologic 786
effects of bradykinin has clinical applications. Oral or intravenous administration of the bradykinin receptor antagonist icatibant reduces the clinical signs, onset of diarrhea, and many of the histopathologic changes in experimental models of colitis in mice.[58] Inhibition of kallikrein by oral administration of P8720 attenuated the intestinal inflammation, clinical score, and systemic manifestations in a model of chronic granulomatous enterocolitis.[57] Thus the contact system is a viable therapeutic target for inflammatory diseases of the intestine.

13.2.5 Tissue Injury During Inflammation

Changes in blood flow to the mucosa and other regions of the intestine that reduce perfusion of the tissues can potentiate the initial damage caused by infection or injury. For example, reperfusion of ischemic tissues is associated with platelet and neutrophil clumping in the small vessels of the mucosa, which can impede blood flow.[59] Platelets are activated and adhere to exposed basement membrane and activated endothelial cells and provide a surface for leukocyte adhesion. The accumulation of platelets and leukocytes can reduce vessel diameter and blood flow significantly while potentiating local coagulation and thrombus formation.

Soluble mediators released by activated leukocytes and endothelial cells also affect blood flow. Histamine and the vasoactive lipids derived from arachidonic acid (leukotrienes, prostaglandins, thromboxane, prostacycline, and platelet-activating factor) have a prominent role in regulating local perfusion during inflammation and also may have systemic effects on blood flow. Procoagulant mediators released by inflammatory cells in response to the inflammatory process (i.e., tissue factor produced by macrophages or endothelial cells), exposed basement membrane proteins, and bacterial components can trigger the contact system and the coagulation and complement cascades, the products of which affect blood flow. Nitric oxide, whether produced by endothelial cells or leukocytes (macrophages), is a potent regulator of blood flow and has a significant role in the control of perfusion during inflammation.[60] Many of the mediators that affect perfusion also affect endothelial permeability, altering osmotic and hydrostatic balance and tissue edema. In extreme cases, local and systemic coagulopathies initiated by vascular injury and absorption of microbial products and inflammatory mediators induce a hypercoagulable state, leading to microthrombus formation, which can reduce blood flow, or macrothrombus formation, which causes tissue infarction.

The cellular mediators of inflammation have the potential to inflict severe injury to intestinal tissues. Neutrophils have an important role in the pathophysiology of many intestinal diseases, including ischemia-reperfusion injury,

[16,22] infectious enterocolitis,[23–25] nonsteroidal antiinflammatory drug–induced mucosal ulceration,[26] and others. Depletion of neutrophils, blockade of their emigration into tissues, or inhibition of neutrophil activation reduce the severity of these and other inflammatory diseases.[61] Thus many antiinflammatory therapies are emerging that specifically target neutrophil adhesion, migration, and activation.

Migration of neutrophils through endothelium during emigration into inflamed tissues is remarkable in that the permeability of the endothelial monolayer is preserved under most circumstances. However, a limit exists above which neutrophil migration alters the permeability characteristics of the endothelium. The effect is in part physical in that mere movement of large numbers of neutrophils through the endothelium is sufficient to disrupt the tight junctions mechanically and is caused in part by toxic products of neutrophils that damage endothelial cells and basement membranes.[59,62] Serine proteases (particularly elastase) and metalloproteinases released by degranulating neutrophils destroy tissue matrix proteins and cell-surface proteins that make up endothelial intercellular junctions. These degradative enzymes are particularly damaging to basement membranes and the cellular barriers of the endothelium, thus contributing to vascular permeability (and local tissue edema) and thrombosis. The permeability may be affected to the extent that not only water but macromolecules (albumin, matrix proteins, complement, etc.) leak into the interstitium. Blockade of neutrophil adhesion to endothelium with anti–β_2 integrin antibodies has a sparing effect on the microvasculature in experimental intestinal ischemia-reperfusion injury, reducing the alterations in vascular permeability and histopathologic evidence of microvascular damage.[59]

Similar to the endothelium of inflamed tissues, massive neutrophil transmigration occurs across the epithelium in response to infection or injury. Neutrophil transepithelial migration increases epithelial permeability by disrupting tight junctions.[62] Like the endothelium, neutrophils disrupt the epithelial barrier mechanically as they migrate through (see Figure 13.2-1). Proteases, particularly elastase, degrade basement membrane components and tight junction proteins. Products of activated neutrophils (TNF-α and interferon-γ) increase tight junction permeability by direct effects on the enterocytes. Prostaglandins released by activated neutrophils stimulate epithelial secretion, thus contributing to diarrhea. Subepithelial accumulation of neutrophils can lead to deadhesion of the epithelial cells from the basement membrane and mild to severe ulceration. The physiologic 786
results of the effects of neutrophils and their products on the epithelial barrier include protein-losing enteropathy 787
and absorption of bacterial cell wall constituents, which potentiates the local and systemic inflammatory responses.

Neutrophils in inflamed tissues stimulated by potent host-derived activators (such as IL-1β and TNF-α) and bacterial products (lipopolysaccharide) release copious amounts of ROIs (see Figure 13.2-1). Although these oxygen and oxyhalide radicals are important for killing pathogens, they are also potentially toxic to epithelial and endothelial cells and matrix proteins. Reactive nitrogen intermediates produced primarily by macrophages during inflammation combine with ROIs to form peroxy nitrites, which are particularly toxic.[11] In addition to injury to mucosal tissues, ROIs also have an as yet ill-defined role in recruiting and activating neutrophils, thereby potentiating the inflammatory response.[63] In support of the role of ROIs in inflammatory diseases of the gastrointestinal tract, administration of inhibitors of ROI production or pharmacologic ROI scavengers can be protective in many models of reperfusion injury or enterocolitis. Many therapies are aimed at inhibiting neutrophil activation and effector functions in tissues have been evaluated for use in intestinal diseases. Phosphodiesterase inhibitors, by causing cyclic adenosine monophosphate accumulation in neutrophils, are antiinflammatory by virtue of their ability to suppress neutrophil activation and ROI production. New phosphodiesterase inhibitors selective for the predominant neutrophil isoform of phosphodiesterase hold promise for use in many inflammatory diseases.

Subepithelial mast cells also have an important role in altering epithelial permeability in inflamed intestine. During the intestinal hypersensitivity response, subepithelial mast cell release of mast cell protease II by degranulation increases epithelial permeability via an effect on tight junctions.[41,64,65] This alteration in tight junction permeability results in enhanced transepithelial flux of macromolecules, including proteins and bacterial products. Cytokines released by mast cells and phagocytes also regulate tight junction permeability. Interleukin-4, a product of mast cells and macrophages, increases epithelial permeability.[66] Moreover, TNF-α and interferon-γ, products of many inflammatory cells, synergistically increase tight junction permeability.[67]

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13.3

13.3—Pathophysiology of Diarrhea

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Acute equine colitis causes rapid, severe debilitation and death in horses (more than 90% of untreated horses die or require euthanasia).[1] Since 1919, several reports have described a number of different acute diarrheal conditions in the horse that appear to share a common characteristic clinical presentation.[2–10] Diarrhea associated with acute equine colitis occurs sporadically, is highly fatal, and is characterized by intraluminal sequestration of fluid, moderate to severe colic (abdominal pain), and profuse watery diarrhea with resultant endotoxemia, leukopenia, and hypovolemia.[7,10,11] The condition can affect adult horses of all ages but usually occurs in horses between 2 and 10 years of age. Disease onset is sudden with a rapid progression and often is preceded by a stressful event. A definitive diagnosis is made in only about 20% to 30% of cases.[7,11] Most ante- and postmortem diagnostic tests remain speculative.

Treatment of the condition in horses is costly because of the massive fluid therapy required. Currently, no curative treatment is available for acute colitis in horses, human beings, or other mammals. Treatment regimens provide rehydration, electrolyte and plasma protein replacement, mitigation of the effects of circulating endotoxin, and antimicrobial therapy when indicated. Attempts during the past 40 years to develop appropriate treatments (i.e., vaccines or pharmacologic agents) have been hampered by the unavailability of acceptable equine models and have been unsuccessful because of the complex pathophysiology of the intestinal epithelium.

Although the mechanisms responsible for the fluid losses are not known, inflammatory cells may play an integral role because this condition is characterized by large numbers of granulocytes infiltrating the large intestinal mucosa.[12–16] Equine cecal and colonic tissues collected during the acute stages of experimentally induced acute equine colitis (equine ehrlichial colitis, lincomycin with and without *Clostridium* spp. inoculation, nonsteroidal antiinflammatory drug administration) reveal the presence of numerous neutrophils and eosinophils in the lamina propria and submucosa.[12,15,17,18] Granulocyte-derived reactive oxygen intermediates are crucial to antimicrobial defenses in the gut and stimulate chloride and water secretion by interactions with enterocytes.[19,20] Normal equine intestinal tissue is unique compared with that in most other mammalian species for a preponderance of eosinophils located in the intestinal mucosa and submucosa.[6,21] Production of reactive oxygen intermediates by stimulated phagocytic granulocytes following mucosal barrier disruption may be responsible for the massive fluid secretory response that occurs during the early stages of acute equine colitis.

Colitis refers to inflammation and mucosal injury of the colon and cecum (typhlocolitis) that may occur in response to a number of causes.[22,23] The cause of the colonic injury may be well-defined such as in naturally occurring infectious or experimentally induced colitis. However, many cases of human and animal diarrhea have a speculative or unknown diagnosis or no diagnosis.[11,24,25] Irrespective of the underlying or initiating cause of colonic injury, the colon apparently has a limited repertoire of responses to damage because most forms of colitis demonstrate similarities in histopathologic appearance and clinical presentation. Various degrees of mucosal erosion and ulceration, submucosal/mucosal edema, goblet cell depletion, and presence of an inflammatory cellular infiltrate within the mucosa and submucosa are common to many types of human and animal colitis.[21,23–25] Characteristic clinical manifestations include intraluminal fluid sequestration, abdominal discomfort, hypovolemia, and most often profuse, watery diarrhea.

13.3.1 Pathophysiology of Colitis

Large bowel diarrhea results from abnormal fluid and ion transport by cecal and colonic mucosa. Loss of fluid by the large intestine can result from malabsorptive or hypersecretory processes and is often a combination of the two.[26] Colonic secretory processes are a function of the crypt epithelium, whereas absorptive processes are limited to surface epithelial cells.[27] Under normal baseline conditions, an underlying secretion by crypt epithelium is masked by a greater rate of surface epithelial cell absorption. Abnormal forces influencing the rates 789
of secretion and absorption can result in massive, uncontrolled secretion and malabsorption by large intestinal 790
mucosal epithelial cells, leading to rapid dehydration and death.[26,27]

Two intracellular processes control colonic secretion: the cyclic nucleotide (cyclic adenosine monophosphate [cAMP] and cyclic guanosine monophosphate [cGMP]) and the calcium system.[28,29] Agents may activate adenyl cyclase (vasoactive intestinal peptide, prostaglandin E_2 [PGE_2]) or guanyl cyclase (bacterial enterotoxins) and induce increases in cAMP or cGMP, respectively. This reaction causes phosphorylation of specific protein kinases that induce the actual apical and basolateral membrane transport events. Increases in intracellular free

calcium may arise from cyclic nucleotide–dependent release of stored calcium within the cell or from increased calcium entry across the cell membrane.[26,27] Calcium may act through calmodulin, which then can activate membrane-phosphorylating protein kinases.

At least four central systems control intestinal secretion: (1) the hormonal system, (2) the enteric nervous system, (3) bacterial enterotoxins, and (4) the immune system.[29,30] Hormonal control of colonic electrolyte transport is exerted primarily through the renin-angiotensin-aldosterone axis.[31,32] The enteric nervous system controls transport through three separate components: (1) extrinsic nerves of the parasympathetic and sympathetic pathways; (2) intrinsic ganglia and nerves, secreting a variety of neurotransmitters including peptides; and (3) neuroendocrine cells (intraepithelial lymphocytes) that reside in the epithelium and release messengers onto the epithelial cells in a paracrine manner.[26,29–32] Many bacterial enterotoxins can induce intestinal secretion by cAMP or cGMP signal transduction.[33] Bacterial enterotoxins can stimulate enteric neurons, providing evidence for interaction between two controlling systems.[34]

Preformed inflammatory mediators such as histamine, serotonin, or adenosine and newly synthesized mediators such as prostaglandins, leukotrienes, platelet-activating factor, various cytokines, the inducible form of nitric oxide, and reactive oxygen metabolites can initiate intestinal secretion by directly stimulating the enterocyte and by acting on enteric nerves indirectly to induce neurotransmitter-mediator intestinal secretion.[30] For instance, when added to the T84 colonic cell line, the known mast cell mediators histamine, adenosine, and PGD_2 induce chloride secretion.[35–37] Prostaglandins of the E and F series can cause an increase in chloride secretion in intact tissue and isolated colonic cells.[38–40] Leukotrienes, platelet-activating factor, and a number of cytokines have been shown to have no effect on T84 cell secretion but have a significant effect on electrolyte transport in intact tissue, suggesting that intermediate cell types may be involved in these secretory responses.[41–43]

The epithelial cell chloride secretory response occurs via prostaglandin- and adenosine-mediated increases in cellular cAMP, whereas histamine acts by H_1 receptor induction of phosphatidylinositol turnover, production of inositol triphosphate, and mobilization of intracellular calcium stores.[30,33] Lipoxygenase products (leukotrienes) are capable of activating a colonic secretory response and do not appear to involve the cyclic nucleotides or calcium ions.[41] Phagocyte-derived reactive oxygen mediators (ROMs) can induce colonic electrolyte secretion in vitro, suggesting that oxidants may contribute directly to the diarrhea associated with colitis.[44–48] Reactive oxygen species initiate the secretory response by increasing cellular cAMP or stimulating mesenchymal release of PGE_2 or prostacyclin, which in turn stimulates the epithelial cell or enteric neuron, respectively.[44–49] Sodium nitroprusside, an exogenous source of nitric oxide, stimulated an increase in chloride secretion in rat colon that was mediated by cyclooxygenase products and enteric neurons.[50] Table 13.3-1 summarizes inflammatory mediator– induced epithelial cell chloride secretion.

13.3.2 Role of Inflammatory Cells

Acute colitis rarely develops by a simple cause or effect phenomenon but is influenced by many extrinsic and intrinsic host and microorganism factors. Inflammatory mediators released from mast cells and monocytic or granulocytic phagocytes cause intestinal chloride and water secretion and inhibit neutral sodium and chloride absorption.[29,30,67] Inflammatory cells, particularly the phagocytic granulocytes, play an important role in mucosal pathophysiology in cases of colitis.[20,68] Large numbers of these cells are observed on histopathologic examination of tissues from human and animal cases of colitis. Products of cell activation stimulate direct and

indirect secretory responses in intestinal cells and tissues.[28–30,45–49] Products of phagocyte secretion may amplify the inflammatory signal or have effects on other target cells in intestine such as enterocytes and smooth muscle cells (Table 13.3-2).

13.3.3 Role of Phagocyte-Derived Reactive Oxygen Metabolites

The NADPH-oxidase system of phagocytes (neutrophils, eosinophils, monocytes/macrophages) is a potent inducer of superoxide radicals used as a host defense mechanism to kill invading microorganisms.[20,69] During inappropriate stimulation such as inflammation, trauma, or ischemia followed by reperfusion, increased levels of toxic oxygen species are produced, causing damage to host tissues. Engagement of any of several receptor and 790
nonreceptor types including phagocytosis mediators, chemotactic agents, various cytokines, and microbial 791
products can stimulate phagocytes.[20] Resident phagocytes or those recruited to colonic mucosa early in the disease process are considered to augment mechanisms causing fluid and electrolyte secretory processes, a so-called amplification process.[70,71]

TABLE 13.3-1 Inflammatory Mediators That Stimulate Epithelial Cell Chloride Secretion

MEDIATOR	ACTION	REFERENCE
Prostaglandin E_2	Increases Cl secretion.	51
	Decreases neutral NaCl absorption.	
Vasoactive intestinal peptide	Increases cAMP-mediated NaCl secretion.	52
	Activates cholinergic nerves.	53
Endotoxin	Increases Na absorption.	54
	Increases cell membrane permeability.	
Serotonin	Increases fluid and electrolyte secretion.	55
Interferon-γ	Decreases tight junctions and causes increase in cell membrane permeability.	56
Interleukin-1α and interleukin-1β	Increase prostaglandins E_2 and $F_{1\alpha}$ and thromboxane B_2.	57
Histamine (H_1)	Increases Cl secretion via Ca-mediated pathways.	[58] and [59]
Bradykinin	Increases Cl secretion through prostaglandin-mediated pathways.	60 and 61
Reactive oxygen mediators	Increase Cl secretion.	44 and 62
Thromboxanes	Increase Cl secretion.	63
	Decrease neutral NaCl absorption.	
Lipoxygenase products	Increase Cl secretion via prostaglandin-mediated pathways.	64
Platelet-activating factor	Increases Isc (Cl secretion).	65
Adenosine	Increases Cl secretion.	66

cAMP, Cyclic adenosine monophosphate.

Activation of the respiratory burst results in the production and release of large amounts of superoxide anion (O_2^-) and H_2O_2.[69,72] In addition to these ROMs, activated phagocytes secrete peroxidase enzyme (myeloperoxidase from neutrophils and eosinophil peroxidase from eosinophils) into the extracellular space. The peroxidases catalyze the oxidation of Cl^- by H_2O_2 to yield HOCl, the active ingredient in household bleach products. The peroxidase-H_2O_2-halide system is the most cytotoxic system of the phagocytes; HOCl is 100 to 1000 times more toxic than O_2^- or H_2O_2.[69] HOCl is a nonspecific oxidizing and chlorinating agent that reacts rapidly with a variety of biologic compounds including DNA, sulfhydryls, nucleotides, amino acids, and other nitrogen-containing compounds. HOCl reacts rapidly with primary amines (RNH_2) to produce the cytotoxic N-chloramines (RNHCl). The mechanisms by which HOCl and RNHCl damage cells and tissue remain speculative, but possibilities include direct sulfhydryl oxidation, hemoprotein inactivation, protein and amino acid

degradation, and inactivation of metabolic cofactors of DNA.[73] Peroxidase-derived oxidants have been shown to degrade hyaluronic acid and collagen.[74] In addition, luminal perfusion of specific ROMs increased mucosal permeability and serosal application caused increases in Cl^- secretion in vitro.[75]

TABLE 13.3-2 Phagocyte-Derived Inflammatory Mediators

ENZYME	MEDIATOR	MONOKINE	REACTIVE OXYGEN MEDIATOR
Protease	Platelet-activating factor	Interleukin-1	O_2^-
Kallikrein	Prostaglandin	Tumor necrosis factor	H_2O_2
Phospholipase	HPETE	Interferon-γ	OH^-
	Leukotriene		HOCl

HPETE, Hydroperoxyeicosatetraenoic acid.

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Tissue myeloperoxidase activity, an index of tissue granulocyte infiltration, is used clinically and experimentally to assess degree of intestinal inflammation.[76,77] Myeloperoxidase activity is elevated in acute flare-ups of human inflammatory bowel disease and various animal models of acute colitis.[76–80]

The acute inflammatory response in these conditions is characterized predominantly by neutrophils, the predominant source of myeloperoxidase activity. However, this assay measures total hemoprotein peroxidase, which includes monocyte and eosinophil peroxidase in addition to neutrophils.[77] Moreover, levels of peroxidase activity in equine circulating eosinophils are greater than in circulating neutrophils,[81] and this may apply to resident tissue eosinophils as well.

Arachidonic acid metabolites are thought to play a role in intestinal inflammation in diarrheal disease.[30,45,82] Elevated levels of these intermediate metabolites have been demonstrated in natural disease and experimental models of colitis and appear to parallel increases in ROMs in inflamed intestine.[82] Addition of H_2O_2 or HOCl to rat colonic tissue in Ussing chambers has been shown to induce PGE_2 release and active Cl^- secretion.[47,48] Prostaglandins can stimulate increases in Cl^- secretion in intact intestinal tissue[45,46,48] and in isolated colonic T84 cells.[47,49] Interactions between ROMs and mesenchymal release of PGE_2/PGI_2 may be relevant to the mechanisms producing the diarrheic condition. Fibroblasts co-cultured or juxtaposed to colonic T84 cells greatly increased the Cl^- secretory response to H_2O_2 in vitro through the release of PGE_2.[49] In addition, equine colonic mucosa has an increased sensitivity to endogenously released prostaglandin by exhibiting a significant secretory response under in vitro conditions.[83]

13.3.4 Role of Endotoxin, Malnutrition, Immunodeficiency, and Intestinal Microflora

13.3.4.1 ENDOTOXIN

Endotoxin, the lipopolysaccharide component of the outer cell wall of gram-negative bacteria, is present in large quantities in the large intestine of healthy horses.[81,83] Endotoxins are released into the immediate

surroundings when gram-negative bacteria undergo rapid proliferation or die.[84,85] The intact bowel forms an effective barrier to the transport of significant amounts of these highly antigenic toxins, but the diseased gut absorbs these macromolecules in large amounts, causing the subsequent adverse systemic effects that are often life threatening.[86]

Disruption of the intestinal barrier (i.e., ischemia, trauma, inflammatory conditions) overwhelms the capacity of the liver to clear endotoxins, and systemic endotoxemia ensues. Endotoxins have been shown to be potent activators of the inflammatory process, stimulating the production and release of numerous cytokines by activated macrophages and other immunocytes.[87] In vitro studies suggest that endotoxin activates phagocytic granulocytes to secrete ROMs, increase release of lysozymes, and enhance the migratory response to chemotactic stimuli.[88] Prostacyclin and thromboxane A_2 mediate hemodynamic dysfunction, and lipoxygenase products may induce tissue ischemia.[89] The cytokine interleukin-1 causes a febrile response and initiates the acute phase response.[90] Tumor necrosis factor contributes to many of the abnormal physiologic responses, particularly hemostatic functions that potentiate coagulopathy.[91] Additional mediators include interleukin-6, platelet-activating factor, procoagulant mediators, and various other speculated substances.[84]

Endotoxins trigger mucosal immune cells and subsequent release of inflammatory mediators in cases of colitis. The first report of experimentally induced endotoxemia described clinical signs and hematologic findings that closely paralleled those reported for severe colitis in horses.[92] Studies in which endotoxin was administered intravenously in human beings and laboratory animals caused significant dose-related gastrointestinal changes, ranging from mild diarrhea to bloody, watery diarrhea.[93,94] In vitro studies on the effects of endotoxin on intestinal water and electrolyte transport in adult male rats showed a significant decrease in net colonic sodium absorption and increased colonic permeability.[55]

In animal models of protein energy deficiency, endotoxin-induced mortality increased compared with that of well-nourished control animals. Endotoxin depresses lymphocyte responses to specific mitogens.[95] Thus the adverse effects of malnutrition and endotoxin are mutually aggravating.

13.3.4.2 IMMUNODEFICIENCY

The importance of a normal immune system to the defense of the mucosal surface of the gastrointestinal tract is evident in the immunosuppressed state. Primary immunodeficiencies affecting the gastrointestinal tract are well documented.[96–98] Common agammaglobulinemia is the most frequently reported gastrointestinal disease and causes B cell deficiency–associated giardiasis.[99] Interestingly, selective immunoglobulin A (IgA) deficiency rarely results in intestinal disease because of a speculated increase in mucosal IgM response. However, combined IgA and IgM deficiencies with a higher incidence of intestinal disease occur. A selective deficiency of secretory IgA has been associated with intestinal candidiasis. Certain mucosal pathogens may enhance their pathogenicity by producing IgA proteases.[99] Defects in cell-mediated immunity are associated 792
most commonly with intractable diarrhea, and organisms frequently involved include *Salmonella* spp., 793
Escherichia coli, and *Shigella* spp.[100] Acquired immunodeficiency or immunosuppression in adults can result from infectious diseases (particularly viral), nutritional deficiencies, aging phenomenon, and drugs (corticosteroids, azathioprine, cyclophosphamide).

TABLE 13.3-3 Vitamin and Mineral Deficiency–Related Immunodeficiencies

DEFICIENCY	FUNCTION
Pyridoxine, folic acid, vitamin C, vitamin E	Impairs cell-mediated immunity and reduces antibody response.
Vitamin B_6	Decreases lymphocyte stimulation response to specific mitogens.
Zinc	Deficiency affects humoral and cell-mediated immunity.
Iron	Inhibits bacterial multiplication.
Copper	Depresses antibody response.

13.3.4.3 NUTRITIONAL DEFICIENCIES

Nutrition is a critical determinant of immunocompetence and risk of illness.[101,102] Impaired systemic and mucosal immunity contribute to an increased frequency and severity of intestinal infections observed in cases of undernourishment. Abnormalities occur in cell-mediated immunity, complement system, phagocytic function, mucosal secretory antibody response, and antibody affinity. Morbidity caused by diarrheal disease is increased particularly among individuals with stunted growth rate because of malnourishment.[102]

The critical role of several vitamins and minerals in immunocompetence has been substantiated in animals deprived of one dietary element and findings in human patients with single-nutrient deficiency. Tables 13.3-3 and 13.3-4 summarize nutritional-related immune system abnormalities.

TABLE 13.3-4 Vitamin and Mineral Excess–Related Immunodeficiencies

EXCESSES	FUNCTION
Vitamin A	Increases the immune response.
β-Carotene	Increases the number of $CD4^+$ cells.
Vitamin E and selenium	Enhance immunocompetence and increase resistance to microorganisms.
Zinc	Depresses neutrophil function and lymphocyte responses.
Iron	Needed by neutrophils and lymphocytes for optimal function, which may be related to myeloperoxidase and ribonucleotidyl reductase deficiencies.

In summary, nutritional deficiency can cause increased colonization of the intestine with microorganisms, alter the symbiotic characteristics of resident intestinal bacterial populations, and impair defenses of the gastrointestinal tract, allowing increased risk of systemic spread of infection and absorption of macromolecules (in particular, endotoxin).

13.3.4.4 INTESTINAL MICROFLORA

Indigenous microflora greatly impede colonization of the gastrointestinal mucosa by pathogenic organisms. The ability of a potential pathogen to initiate an infection depends on its ability to breach the mucosal epithelial barrier. One mechanism by which the normal flora inhibit establishment of pathogens is by preventing adherence of the pathogen to mucosal cells by occupying the site or by stearic hindrance.[102–105] Resident microbes also produce byproducts such as antibacterial factors that allow symbiosis rather than competition between them. Another hindrance mechanism is production of volatile fatty acids by normal microbial digestive processes to create an environment that is toxic to many bacterial populations, particularly the Enterobacteriaceae.[106]

13.3.5 Factors Affecting Motility

Disturbances in motility patterns occur during inflammatory diseases of the colon, but the role of motility alterations in the pathogenesis of diarrhea remains unclear. Invasive bacteria cause characteristic motor patterns in the colon consisting of rapid bursts of motor activity that appear to decrease transit time through the large intestine. The result is reduced clearance of bacteria from the large intestine, which may contribute to the virulence of the organism.[107] Absorption of endotoxin and the release of inflammatory mediators such as prostaglandins disrupts the motility patterns of the large intestine, resulting in less coordinated contractions, and may contribute to the alterations in motility seen with invasive bacteria. Although the effect of endotoxin and prostaglandins on transit time is not profound, the disruption of coordinated activity may play a role in causing diarrhea.[108] Thorough mixing and prolonged retention time of ingesta are important not only in microbial digestion of nutrients but also in absorption of microbial byproducts and fluid.[32,109] The ingesta is viscous and therefore must be mixed to bring luminal ingesta in contact with the mucosa for absorption.[109] In addition, poor mixing increases the thickness of the unstirred layer, decreasing contact of ingesta with the mucosa and decreasing absorption.[32,109]

Progressive motility must be present, however, if a diarrheal state is to occur.[32,109] Ileus may be accompanied by 793
increased fluid in the lumen of the large intestine, but without progressive motility the fluid is not passed. 794
Frequently, acute colitis causes a period of ileus characterized by scant stool. Diarrhea is apparent only when motility returns and the ingesta is passed. Increased progressive motility has been suggested to cause diarrhea by decreasing transit time and is thought to play a role in irritant catharsis and in the mechanism of action of some laxatives.[110] Irritation and distention increase motility and may well decrease transit time, but increased secretion also is thought to contribute to diarrhea caused by these substances.[111]

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13.4

13.4—Malabsorption Syndromes and Maldigestion: Pathophysiology, Assessment, Management, and Outcome

Malcolm C. Roberts

13.4.1

Pathophysiology

Malabsorption and maldigestion are recognized clinical problems in human medicine. In the horse the clinician often reaches the diagnosis of malabsorption for a condition by exclusion in the workup of a horse with chronic weight loss and wasting. The term *malabsorption* implies impairment of digestive and absorptive processes arising from functional or structural disorders of the small intestine and related organs, the pancreas, liver, and biliary tract. The condition can affect absorption of carbohydrates, proteins, fats, vitamins, minerals, and to a lesser extent, water and electrolytes. In the horse the resulting pathophysiologic changes may influence large intestinal function adversely through alterations in the substrate presented for fermentation. Malabsorption syndromes are encountered more frequently in human beings and small animals than they are in horses. 796

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One should direct the clinical investigation of malabsorption at ascertaining and trying to localize the source of the abnormality. In medical practice, impairment of one or more phases of the digestion and absorption of dietary constituents may precipitate clinical signs that are associated primarily with carbohydrate, protein, or fat malabsorption. This level of differentiation is not possible in the horse because of the herbivorous diet and the contribution of large intestinal functions.

In human and small animal medicine, disturbances in digestive processes especially from exocrine pancreatic insufficiency or reduced intestinal bile salt concentration are principal determinants of many clinical

malabsorption syndromes. The rarity of pancreatic problems in horses and the herbivorous diet makes maldigestion less of a concern and difficult to pursue diagnostically. Nevertheless, maldigestion undoubtedly contributes to chronic weight loss conditions in horses, which may be significant with severe infiltrative disease of the small intestine with partial to total villous atrophy and flattened mucosa. Impairment of digestive processes may exacerbate diarrhea in the suckling foal through reduced intestinal bile salt concentrations from hepatic or ileal dysfunction.

Malabsorption is not synonymous with diarrhea, although diarrhea may be a feature. Adult horses rarely exhibit diarrhea with small intestinal problems unless large intestinal involvement is concomitant. Chronic diarrhea is predominantly a large intestinal disorder that reflects an overload of water and electrolytes and thus may be considered a state of impaired absorption. Primary small intestinal disease is more likely to occur in neonates and young foals. For example, acquired small intestinal brush border lactase deficiency may result in increased lactose fermentation in the large intestine and induction of osmotic diarrhea.[1] Box 13.4-1 lists conditions that have been or potentially could be associated with malabsorption syndromes and maldigestion in the horse.

13.4.1.1

BOX 13.4-1 CONDITIONS THAT HAVE BEEN OR COULD BE ASSOCIATED WITH MALABSORPTION SYNDROMES AND MALDIGESTION IN THE HORSE

13.4.1.1.1

Malabsorption

13.4.1.1.1.1

Inadequate absorptive surface area

- Small intestinal resection (short bowel syndrome), villous atrophy (idiopathic), and mucosal atrophy

13.4.1.1.1.2

Small intestinal bacterial overgrowth (ileocecal valve bypass; physiologic)

13.4.1.1.1.3

Inflammatory or infiltrative disorders: chronic inflammatory bowel disease

- Granulomatous enteritis
- Multisystemic eosinophilic epitheliotropic disease (includes all eosinophilic conditions, such as eosinophilic granulomatosis, chronic eosinophilic gastroenteritis, and chronic eosinophilic dermatitis, except eosinophilic enterocolitis)
- Basophilic enterocolitis
- Lymphocytic-plasmacytic enterocolitis
- Lymphosarcoma, alimentary form; other forms with alimentary involvement
- Amyloidosis

13.4.1.1.1.4 Parasitic causes (larval cyathostomes)

13.4.1.1.1.5 Infectious causes (response to infectious agents)

- *Mycobacterium* spp., *Rhodococcus equi, Salmonella* spp., *Lawsonia intracellulare* (proliferative enterocolitis), *Histoplasma* spp., and others

13.4.1.1.1.6 Immunologic causes

- Immediate hypersensitivity to antigens presented to or absorbed from intestinal tract; food allergy

13.4.1.1.1.7 Biochemical defects (with or without microscopic cellular damage)

- Disaccharidase deficiency: acquired lactase deficiency in foals and monosaccharide transport defects

13.4.1.1.1.8 Lymphatic obstruction

- Lymphadenopathy; lymphangiectasia abscesses

13.4.1.1.1.9 Miscellaneous

- Partial intestinal obstruction, adhesions, mural thickening, and toxin-induced

13.4.1.1.2 Maldigestion

13.4.1.1.2.1 Gastric disorders

- Deficiency or inactivation of pancreatic lipase
- Exocrine pancreatic insufficiency: chronic pancreatitis and pancreatic carcinoma

13.4.1.1.2.2 Reduced intestinal bile salt concentration (with impaired lipid micelle formation)

- Hepatic dysfunction: parenchymal liver disease and cholestasis
- Interrupted enterohepatic bile circulation; ileal inflammatory disease or resection
- Abnormal bacterial proliferation in the small intestine: stagnant (blind) loops, incompetent ileocecocolic valve, bypass and resection, and hypomotility
- Drug-induced sequestration of bile salts: neomycin and calcium carbonate
- Small intestinal brush border enzyme deficiency

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Malabsorption in horses has no pathognomonic clinical syndrome. Case recognition derives from the robust investigation of horses with chronic wasting. Prevalence is unknown. No strict case definition exists, even for

chronic wasting. Interest generated by unusual clinical test results and their related pathologic findings has stimulated publication of reports of cases considered as malabsorption in horses. Pathologic description of the predominant cellular infiltrate and the pattern of intestinal distribution have resulted in the classification of many conditions as representing examples of chronic inflammatory bowel disease (CIBD) (see Box 13.4-1), drawing analogies from human medicine. In the affected animal, coexistent enteric protein loss reflecting changes in mucosal integrity from extensive infiltration and inflammation in the intestinal tract is likely to be more debilitating than the malabsorption.

13.4.2 Clinical Assessment

The principal concern of the owner is the weight loss and poor condition of the horse. Many clinical examination findings, except for body condition, may appear within normal limits. Investigation of the weight loss, together with the clinical pathologic findings, helps to eliminate other more commonly encountered causes of wasting. Box 13.4-2 lists clinical signs that may be associated with malabsorption syndromes.

13.4.2.1 BOX 13.4-2 CLINICAL SIGNS THAT MAY BE ASSOCIATED WITH MALABSORPTION SYNDROMES

Vital signs usually within normal limits: occasional slight fever, diurnal or cyclic (inflammation)

Mucosa: pallor; chronic anemia

Appetite: anorexia; poor, normal, or ravenous

Body condition: weight loss, chronic wasting, or more rapid and dramatic decline

Demeanor: normal, dull, or depressed

Energy and activity level: normal, decreased, or lethargic

Fecal consistency: formed, scant, increased, or diarrhea

Large intestinal involvement: diarrhea, unstructured feces, or blood

Pain: persistent low grade or intermittent abdominal discomfort

Edema: reduced albumin absorption, enteric protein loss, lymphatic obstruction, or liver disease

Palpable rectal abnormalities: thickened bowel wall or enlarged mesenteric lymph nodes

Extraintestinal signs: generalized skin lesions, exudative dermatitis, ulcerative coronitis, or arthritis

No characteristic clinical pathologic profile of malabsorption exists. Findings relate to the stage of the underlying disease process and intercurrent problems. The syndrome tends to cause anemia (normocytic, normochromic) and neutrophilia. Hemolytic or macrocytic anemia and thrombocytopenia have been observed in alimentary lymphosarcoma. Lymphocytosis (leukemia) rarely is encountered. Eosinophilia is uncommon even with suspected immune-mediated conditions and widespread tissue eosinophilia. Many animals are hypoalbuminemic and hypoproteinemic; horses with alimentary lymphosarcoma may exhibit hyperproteinemia and hypergammaglobulinemia. Serum or plasma may be lipemic. The clinician may find elevated hepatic and biliary

tract enzymes (γ-glutamyltransferase and alkaline phosphatase) in multisystemic conditions; for example, eosinophilic granulomatosis (multisystemic eosinophilic epitheliotropic disease; EG [MEED]). Abdominocentesis has been of diagnostic value in several alimentary lymphosarcoma cases, but rarely in the granulomatous conditions.

Ultrasonographic examination of the abdomen can yield infomation on intestinal distention, wall thickness, and unexplained masses detected on rectal palpation. Rectal biopsy is easy to perform and may provide an indication of cellular infiltration that could be present at more proximal locations. However, pathologists examine few equine rectal samples, and the interpretation is frequently equivocal. Adoption of standardized grading or classification would improve the diagnostic value. A proposed classification system was based on a retrospective study of 130 rectal biopsies from 116 horses ages 1 to 18 years with clinical signs of intestinal disease. Necropsy results were studied from 40 horses. Biopsy specimens (21 horses) and necropsy rectal tissue (9 horses) from 30 horses ages 4 to 22 years served as controls. Simple proctitis, the presence of neutrophils in the crypt or surface epithelium, was an abnormal finding compared with mild scattered neutrophil infiltration in controls. Simple proctitis was found in association with malignant lymphoma and other inflammatory disorders. Inflammatory bowel disease was diagnosed from rectal biopsy specimens in 6 of 12 EG (MEED) cases and 4 of 9 granulomatous enteritis cases confirmed at necropsy.[2] Rectal biopsy aided diagnosis for 3 of 7 horses in a series of lymphocytic-plasmacytic enterocolitis cases.[3] Eosinophils were demonstrated on impression smears of rectal mucosal biopsies from 1 of 2 horses with eosinophilic enterocolitis.[4]

Skin biopsies or ultrasound-guided biopsies of liver, lymph node, or lung may reveal evidence of multisystemic disease. One can obtain intestinal and lymph node biopsies via a standing laparotomy. Exploratory laparotomy 798
facilitates rigorous inspection of the gastrointestinal tract and associated organs to obtain multiple biopsies from 799
intestinal sites and lymph nodes. The procedure may provide a diagnosis, enabling one to make decisions on potential treatment and management options. Cost and potential postoperative complications may limit surgical procedures for diagnosis. Laparoscopy may provide an alternative means to facilitate biopsy of certain tissues. However, one should consider surgical exploration as an option early in the process rather than as a last resort.

The noninvasive breath hydrogen test used to assess carbohydrate malabsorption in human beings has not proved reliable in equine studies.[5]

13.4.2.2 CARBOHYDRATE ABSORPTION TESTS AND INTESTINAL FUNCTION TESTS

Intestinal function tests can provide a practical and inexpensive means to assess the absorptive capability of the small intestine. For clinical practice purposes this is limited to carbohydrate absorption. Abnormality of carbohydrate absorption has become an important precept on which to base a diagnosis of malabsorption in the horse. However, results of the oral glucose tolerance test (OGTT) or d-xylose absorption test require cautious interpretation. Pathologic changes in the mucosa and submucosa must be extensive and widely distributed to greatly affect the peak plasma concentration and shape of the curve. The tests are easy to perform in practice and require a baseline blood sample predosing and further samples for up to 6 hours after administration of the solution. Many commercial laboratories conduct glucose and xylose assays.

13.4.2.2.1 Oral Glucose Tolerance Test

The immediate dietary history, gastric emptying rate, intestinal transit, age, and hormonal effects of the horse influence glucose peak and curve shape. Higher glucose peaks are recorded from healthy animals eating grass or hay than from those eating concentrates. Recent appetite or the level of cachexia may affect

test results. Maximum plasma glucose level (>85% baseline) is reached by 120 minutes in healthy animals given 1 g glucose per kilogram body mass as a 20% solution.[6,7] Break points below which the probability increases of carbohydrate malabsorption associated with intestinal morphology changes have been proposed.[7]

A referral population of 42 mature horses with chronic weight loss was divided into three groups based on OGTT results to determine if any concurrence with the morphologic diagnoses existed. Group 1 ($n = 5$) had a normal OGTT (peak glucose concentration at 120 minutes >85% baseline) and contained animals that had normal small intestinal morphology, and a few with large intestinal lesions. Group 2 ($n = 25$) had partial malabsorption and included 18 horses with small intestinal infiltrative disease that allowed some glucose uptake. Diagnoses included lymphosarcoma, villous atrophy, granulomatous enteritis and EG (MEED). Seven horses had normal small intestinal histologic findings. Peak glucose concentrations were less than 85% and greater than 15% of baseline at 120 minutes. Seventeen horses in the group had large intestinal pathologic conditions. Group 3 horses ($n = 12$) had total malabsorption; the peak concentration at 120 minutes was less than 15% above baseline. These horses had severe infiltration throughout most of the small intestine that was attributed predominantly to lymphosarcoma or granulomatous enteritis.

However, the test is far from definitive; one cannot assume a flat curve indicates malabsorption and a poor prognosis. Two horses with chronic weight loss initially diagnosed with malabsorption based on flat OGTT curve s subsequently showed more normal OGTT responses.[8] Full-thickness intestinal biopsies were unremarkable. One horse had an elevated serum immunoglobulin E to oat allergen. Oats and oat straw were removed from access. Dexamethasone was given on a tapered protocol, and a repeat OGTT was normal at 18 months. The other horse received oral probiotics to counter suspected small intestinal bacterial overgrowth, was clinically normal in 2 months, and had an improved OGTT with a 60 minute peak. Therefore malabsorption, as defined by an absorption test and weight loss, may occur in the horse without significant morphologic changes in the intestine, and the condition may be transient. Demonstration of carbohydrate malabsorption in 16 of 24 horses with chronic diarrhea showed poor diagnostic sensitivity for small intestinal involvement. Impaired glucose absorption was recorded in horses with predominantly large intestinal problems, cyathostomiasis, chronic colitis, alimentary lymphosarcoma, and MEED.[9]

13.4.2.2.2 D-Xylose Absorption Test

Although prior dietary history influences peak plasma xylose concentration, xylose is not confounded by hormonal effects or mucosal metabolism. Gastric emptying rate, intestinal motility, intraluminal bacterial overgrowth, and renal clearance do affect curve shape. Healthy mares not fed for up to 96 hours had flatter curves and a slower decrease in plasma xylose than when deprived for 12 to 36 hours.[10] Hence recent appetite or the level of cachexia may influence test results.

Abnormal d-xylose absorption represented by a flat curve or delayed absorption is considered indicative of significant jejunal disease and has been observed with most examples of CIBD, parasitism, and idiopathic villous atrophy.[11,12] Ponies may have lower peak d-xylose concentrations at 60 and 90 minutes than horses, although the range of peak values at the test dosage (0.5 g D-xylose per kilogram body mass in a 10% solution) is wide. 799

800 Potentially diagnostic discriminatory cut off points for peak plasma xylose concentrations have not been determined.

Abnormal absorption curves have been detected in the absence of small intestinal histologic changes,[13] and interpretation is clouded further by findings from small intestinal resection studies in healthy ponies. Nine

ponies with 70% distal small intestinal resection and four sham-operated controls were placed on interval feeding for 5 weeks and then turned out to pasture until 6 months after surgery. Grazing was supplemented by twice daily (meal feeding) concentrate rations. All ponies gained weight and were clinically normal, and none developed diarrhea. However, the mean peak xylose concentration at 60 minutes declined progressively (at monthly intervals) over the study period in the resection group to 15% of that of controls. Lack of clinical malabsorption was attributed to adaptation of the residual 30% of healthy small intestine and of large intestinal function.[14] Bacterial overgrowth in the small bowel remnant from refluxed cecal contents (resected ponies had ileocecal valve bypass) may have contributed to the abnormal xylose assimilation. By contrast, xylose absorption decreased over 6 months, associated with substantial weight loss, lethargy, and diarrhea, in an earlier study of extensive (≥60%) small intestinal resection in ponies.[15] An important difference was the feeding pattern; those ponies received pelleted feed twice daily for the entire 6 month follow-up period.

Consequently, horses with suspected malabsorption may adapt to an interval feeding regimen. The critical factor could be the availability of sufficient unaffected or minimally affected small intestine and large intestinal functional capacity. The outcome for animals with small intestinal disease and some unknown degree of large intestinal pathologic dysfunction may be less successful than shown in the experimental study.

Abnormal xylose absorption reverted to normal following 35 days of corticosteroid therapy in an adult Thoroughbred gelding with a 6-week history of weight loss and diarrhea for 3 weeks; peak xylose concentration at 60 minutes was within normal limits and the horse had gained weight.[4] d-xylose absorption was abnormal in an adult Standardbred gelding with a 2-month history of poor performance, weight loss, intermittent fever, mesenteric lymphadenopathy, elevated fibrinogen, and decreased albumin and globulin levels. Multiple full-thickness small intestinal biopsies revealed evidence of granulomatous enteritis. The horse received antibiotics postoperatively and then corticosteroids parenterally for 4 to 5 months. After 3 weeks, peak plasma xylose had increased, although absorption was delayed. Five months after cessation of corticosteroid therapy, the horse had regained weight and was bright and alert, and d-xylose absorption was normal.[16]

Diagnostic predictions were made retrospectively by examining d-xylose absorption in horses with granulomatous enteritis compared with those with EG.[17] Peak xylose concentrations were much lower in horses with granulomatous enteritis than those with EG, whereas in EG the absorption curve shifted to the right with the peak occurring at 240 minutes. The small intestine is affected predominantly in granulomatous enteritis with extensive villous atrophy and more diffuse lesions in the large intestine, whereas in EG (MEED) the large intestine is more involved. Hence, the extent and distribution of pathologic changes in the small and large intestines may influence xylose absorption test results.

13.4.3 Management, Therapy, and Outcome

The chronic wasting horse with suspected malabsorption and probable enteric protein loss has at best a guarded to poor prognosis. Prognosis may be improved through early and aggressive investigation to achieve a diagnosis, and perhaps assess the stage in the natural progression of the disorder. The owner may elect euthanasia of the animal or may be willing to determine whether the condition can be improved. In the short term, intravenous infusion of plasma or colloids, with or without fluids and electrolytes, may be necessary to stabilize the condition. Prognosis is much worse for the horse that is inappetent. Prolonged intensive total parenteral nutrition and/or oral alimentation may not be a realistic course of action. The overall therapeutic and management plan

can prove to be expensive. The owner must be cognizant from the start that the outcome may not be altered, even after protracted therapy. One cannot make predictions for outcome of therapy without meaningful data because only a few case reports of successful responses with long-term follow up exist.

13.4.3.1 NUTRITION

Some level of digestive and absorptive capability remains in the diseased small intestine. Interval feeding of small quantities of food may be beneficial if the horse continues to eat, and particularly for animals with ravenous appetites that seem able to maintain their reduced state of body condition without further losses. Diet should include feeds with a high fiber content to favor large intestinal fermentation, including grass hay and access to pasture complemented by commercial high-fiber rations based on beet pulp and soybean hulls. Energy intake can be increased through feeding high-energy dense fats that provide 2.25 times more calories than carbohydrates. Most affected horses should tolerate high fat (5% to 10%) processed feeds containing vegetable oils or rice bran (up to 20% of the concentrate mix, equivalent to 8% vegetable oil) to achieve the 800
higher-fat composition. Changeover to a higher-fat concentrate should be gradual. Even in healthy animals 801
that can eat up to 20% added fat, appetite may decrease as the percentage increases, and fecal consistency may change. Clearly, the objective for the horse with suspected malabsorption is to sustain, and preferably increase, dietary intake, value, and efficiency.

The owner of an affected horse must be prepared to experiment with feeds, must be patient, and must keep records. No standard procedure exists. Exposure to a feed component may contribute to the problem as an allergen eliciting a hypersensitivity reaction. Identifying the potential allergen through immunologic testing or by stepwise removal and outcome assessment over a longer period may be difficult. The clinician should give immunosuppressive drugs early in the process.

13.4.3.2 DRUG THERAPY

Immunosuppressive agents have produced the most promising responses to ameliorate the effects of conditions associated with malabsorption, particularly CIBD. Short-duration, and in some cases more prolonged and sustained, improvements in body condition, weight gain, demeanor, energy and activity levels have occurred following corticosteroid administration. One should start treatment as early as possible. One should follow initial parenteral (intramuscular or intravenous) loading doses of dexamethasone (sodium phosphate) with a series of depot injections, or orally administered prednisolone or prednisone, on a tapered dose protocol over a period of months. Interval low-dose therapy may be necessary if clinical signs return after treatment ends. One uses the lowest dose to control the clinical signs for alternate-day therapy. Clinical benefits far outweigh concerns over potential adverse effects. Chemotherapeutic agents such as vincristine, cytosine, cyclophosphamide, and hydroxyurea have been tried in a few cases of CIBD or lymphosarcoma with no apparent success, probably related to the advanced stage of the disease when treatment was initiated and the dose selected.

13.4.3.3 SURGERY

Resection of a segment of intestine that is edematous, hemorrhagic, or constricted is an option in localized forms of CIBD,[18,19] particularly if gross changes are not discernible in adjacent or distant parts of the intestinal tract, that is, malabsorption is not a feature. Long-term outcome has been favorable. Removal of a substantial proportion of the diseased small intestine may be indicated in a horse with malabsorption, considering that resection of 70% distal small intestine was performed in healthy animals without inducing

adverse effects. However, because pathologic changes may exist in normal-appearing small or large intestine that is not resected or biopsied, the prognosis remains guarded. Two young horses with granulomatous enteritis had the thickened terminal small intestine resected with positive outcomes; one survived 4 months, the other has a follow up extending more than 10 years.[20]

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13.5

13.5—Pathophysiology of Mucosal Injury and Repair

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13.5.1

Mucosal Barrier Function

To gain an appreciation of the mechanisms whereby the mucosa is injured and subsequently repaired, one must understand how the integrity of the mucosa is regulated physiologically. Regulation of mucosal integrity is referred to as mucosal barrier function, which is vital because it prevents bacteria and associated toxins from gaining access to subepithelial tissues and the circulation. However, the mucosa has two conflicting functions: it must serve as a protective barrier and continue to absorb solutes necessary to maintain well-being of the host. This conflict is most notable at the intercellular (paracellular) space, which allows passage of select solutes and water,[1–4] but which does not admit large molecules, including bacterial toxins.[5] The paracellular space is regulated almost exclusively by the tight junction,[6] which is the interepithelial junction at the apical-most aspect of the paracellular space. Although these tight junctions originally were viewed as inert cellular adhesion sites, what has become clear in recent years is that tight junction permeability depends on tissue-specific molecular structure and is regulated by a complex array of intracellular proteins and the cytoskeleton. Tight junctions consist of a group of transmembrane proteins that interdigitate from adjacent cells. Although occludin originally was thought to be the predominant tight junction transmembrane protein, a group of proteins termed *claudins* appear to be more critical.[7] These transmembrane proteins interact with the cytoskeleton via a series of intracellular proteins, including zonula occludens 1, 2, and 3; cingulin; and others.[8] In addition, local regulatory proteins such as the small guanosine triphosphatase-Rho are also critical to tight junction function. In general the relative contractile state of the actin cytoskeleton determines the degree to which tight junctions are open or closed, but the complexities of regulation of this process are understood poorly.[9,10]

The most sensitive measure of mucosal barrier function is transepithelial electric resistance, which is measured by mounting mucosa in an ex vivo system called an Ussing chamber, because this measurement is largely a reflection of the permeability of mucosa to ions.[11,12] Ions may follow one of two routes when traversing epithelium: transcellular and paracellular.[5] Because cell membranes have a resistance to passive flow of ions 1.5 to 3 log units greater than that of the epithelium as a whole, measurements of transepithelial resistance largely reflect the resistance of the paracellular space, and in particular the tight junctions that regulate paracellular flow of ions.[12] Because tight junctions differ in structure from different portions of the mucosa,[13] measurements of transepithelial resistance reflect the net resistance of epithelium of variable permeability within a given tissue. For example, tight junctions in the intestinal glandular structures called crypts are leakier than those in the surface epithelium because of fewer and less organized tight junction strands.[11,14] Conversely, surface epithelium has a greater number of well-organized tight junction strands that result in epithelium with a high resistance.[11] This correlates well with the absorptive function of epithelium located on the mucosal surface and the secretory function of crypt epithelium. Structure of tight junctions also varies with the segment of intestine. For example, tight junctions have more strands in the ileum than in the jejunum, which is reflected by a higher transepithelial resistance in the ileum.[15]

13.5.1.1

GASTRIC MUCOSAL BARRIER FUNCTION

The stomach has four regions based on the type of mucosal lining (in an orad to aborad order): nonglandular stratified squamous epithelium, cardiac epithelium, proper gastric mucosa, and pyloric mucosa.[16] Stratified squamous epithelium has distinct differences in terms of barrier function compared with the remainder of the gastrointestinal tract. This epithelium has baseline transepithelial resistance measurements of approximately 2000 to 3000 Ω/cm[2], which is an order of magnitude higher than the adjacent cardiac mucosa.[17,18] Thus the stratified squamous mucosa is exceptionally impermeable. This in effect is the only mechanism this mucosa has to defend itself against injury. The stratified squamous epithelium consists of four layers: the outer stratum corneum, stratum transitionale, stratum spinosum, and the basal stratum germinativum. However, not all layers contribute equally to barrier function, the barrier being composed mostly of interepithelial tight junctions in the stratum corneum and mucosubstances secreted by the stratum spinosum.[17,19] The relative impermeability of stratified squamous mucosa can be demonstrated by the effects of HCl on this type of epithelium in vitro, which has little effect until it reaches a pH of 2.5 or lower.[18] Thus although most of the literature on equine ulceration pertains to the effects of HCl and inhibitors of HCl secretion,[20–23] other factors may be critical to the development of gastric ulcer disease.

The site of HCl secretion (proper gastric mucosa) also is protected from so-called back-diffusion of H^+ by a high transepithelial electric resistance (compared with cardiac mucosa), but a number of other critical mechanisms also exist to prevent acid injury. The gastric mucosa secretes mucus and bicarbonate, which together form a HCO_3^--containing gel that titrates acid before it reaches the lumen.[24,25] The mucus layer is formed principally by glycoproteins (mucins) secreted by goblet cells but also includes other gastric secretions and sloughed epithelial cells. Mucins consist of core peptides with a series of densely packed O-linked polysaccharide side chains that, once secreted, become hydrated and form a viscoelastic gel. However, the mucus layer does not form an absolute barrier to back-diffusion of acid. Thus for acid that does back-diffuse into the gastric mucosa, epithelial Na^+/H^+ exchangers are capable of expelling H^+ once the cell reaches a critical pH.[25]

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Recent studies have renewed interest in the protective mechanisms of mucus because of the discovery of a group of compounds secreted by goblet cells called trefoil peptides. The name of these peptides is derived from a highly conserved cloverleaf structural motif, which confers substantial resistance to degradation by proteases including pepsin. Three members of this group are known, pS2, SP, and intestinal trefoil factor, the latter of which is secreted solely by goblet cells in the small and large intestine. pS2 and SP are secreted by goblet cells within the stomach and are believed to intercalate with mucus glycoproteins, possibly contributing to the barrier properties of mucus.[26] These peptides also play a critical role in repair of injured mucosa.

An additional mucosal function that serves to reduce the level of injury is adaptive cytoprotection, wherein application of topical irritants to gastric mucosa results in subsequent protection of mucosa in response to repeated exposure to damaging agents. For example, pretreatmen t with 10% ethanol protected against mucosal damage in response to subsequent application of absolute ethanol, and this effect was abolished by treatment with the cyclooxygenase inhibitor indomethacin.[27] The cytoprotective effect of prostaglandins has been demonstrated directly in studies in which preadministration of prostaglandins protected gastric mucosa from damage by agents such as concentrated HCl and hypertonic saline.[28] Prostaglandins appear to be cytoprotective in the stomach at doses less than those used to inhibit gastric acid secretion, ruling out a simple

antacid mechanism.[29] Although not fully characterized, cytoprotection has been attributed in part to prostaglandin-stimulated mucus production.[30] An associated beneficial effect of prostaglandins is the increased production of bicarbonate, which is trapped within mucus on the surface of the mucosa.[31,32] Interestingly, prostaglandin E_2 (PGE_2) appears to lose its cytoprotective activity in the presence of the mucolytic agent *N*-acetylcysteine. Attention also has been directed at enhanced mucosal blood flow as a potential mechanism for prostaglandin-mediated cytoprotection. For example, pretreatment with PGI_2 protected against ethanol-induced mucosal damage as a result of increased mucosal blood flow.[33] Although PGE_2, which is also cytoprotective, does not increase blood flow,[34] it may prevent vascular stasis associated with irritant-induced vascular damage resulting from inhibition of neutrophil adherence to damaged endothelium.[35]

Sensory nerves also have been implicated in cytoprotective mechanisms. These nerves are distributed throughout gastrointestinal mucosa. As an example of their importance in mucosal cytoprotection, pretreatment of newborn rats with capsaicin (to which sensory nerves are sensitive) renders the mature rats more susceptible to gastric injury.[36] Alternatively, use of a low dose of capsaicin, which stimulates rather than destroys sensory nerves, protects gastric mucosa against injurious agents.[37,38] Sensory nerves contain neuropeptides such as calcitonin–gene-related peptide (CGRP) and substance P, which may play a protective role via vascular mechanisms. For instance, CGRP stimulates increased gastric blood flow, which is theorized to reduce injury in much the same way as prostaglandins do. In fact, recent studies suggest that the roles of prostaglandins and CGRP in gastric cytoprotection are intertwined intimately. In particular, PGI_2 is believed to sensitize sensory nerves following treatment with a mild irritant, with resultant increases in CGRP release and mucosal flow. Similar studies have shown that antagonists of CGRP inhibit the cytoprotective action of PGE_2.[39] Another neural mediator, nitric oxide, also has been implicated in adaptive cytoprotection. Interestingly, nitric oxide has a number of actions that are similar to those of prostaglandins, including maintenance of mucosal blood flow.[40]

13.5.2 Intestinal Barrier Function

Regulation of barrier function in the intestine is not as well characterized as that of the stomach, although mechanisms of barrier function, including secretion of mucus and regulation of mucosal blood flow, are presumed to be similar. The proximal duodenum also has to protect itself from acid damage as it receives gastric contents, and this involves secretion of mucus and bicarbonate in much the same way as the stomach. One other mechanism that helps the stomach and the intestine to maintain mucosal barrier function is the speed with which the mucosa repairs. Thus for a defect to develop in the mucosal barrier, injurious factors have to outpace mucosal recovery. Such recovery initially involves epithelial migration across denuded regions of basement membrane 803
(restitution),[26] a process so rapid that epithelial defects may be resurfaced within minutes. For example, in bile 804
salt–injured colon, denuded surface mucosa was covered completely by restitution.[41] In the small intestine, villi greatly amplify the surface area of the mucosal luminal surface, which in turn takes far longer to resurface with restituting epithelium once it has become denuded.[42] However, intestinal villi are able to reduce the denuded surface area considerably by extensively contracting.[43] These mechanisms are described in detail under Mechanisms of Gastrointestinal Mucosal Repair.

13.5.3 Mechanisms of Gastric Injury

13.5.3.1 ULCERATION OF STRATIFIED SQUAMOUS MUCOSA

Although the stratified squamous epithelium is relatively impermeable to HCl, a number of factors can enhance the damaging effects of HCl in this epithelium. In particular, bile salts and short-chain fatty acids are capable of breaking down the squamous epithelial barrier at an acid pH, thereby exposing deep layers to HCl, with subsequent development of ulceration.[18,44] High concentrations of short-chain fatty acids normally exist within the equine stomach because of microbial fermentation.[17] These weak acids penetrate squamous mucosa and appear to damage Na^+ transport activity principally located in the stratum germinativum. Bile salts also may be present in the proximal stomach because of reflux from the duodenum. Although such reflux has a high pH, bile salts appear to adhere to stratified squamous epithelium, becoming lipid soluble and triggering damage once the pH falls below 4.[45] Diet and management (e.g., periods of fasting) also play crucial roles in the development of conditions conducive to gastric ulceration. Typically, a pH gradation in horses exists from proximal to distal compartments of the stomach, with the lowest pH values in the distal stomach.[46] However, fasting disrupts this stratification such that low pH values may be recorded in the proximal stomach.[47] Fasting conditions also increase the concentration of duodenal contents within the proximal stomach, particularly bile.[45]

13.5.3.2 ULCERATION OF PROPER GASTRIC MUCOSA

Proper gastric mucosa is exposed to injurious agents, including pepsin, bile, and acid. Parietal cells in the horse secrete acid constantly as an adaptation to near-continuous intake of roughage,[16] but the enterochromaffin-like cells within the proper gastric mucosa and G and D cells within the pyloric mucosa tightly regulate acid secretion. Histamine released by enterochromaffin-like cells amplifies acid secretion and interacts with H_2 receptors on parietal cells and G cells, which release the prosecretory hormone gastrin. A combination of histamine and gastrin can have a synergistic effect on parietal cell gastric secretion, because these mediators have distinct receptors and second messengers. However, D cells are sensitive to an acidic environment and release somatostatin, which inhibits acid secretion.[48] Nonetheless, gastric mucosa may be exposed to acid for prolonged periods of time, particularly in horses that are extensively meal fed and that do not have the benefit of roughage, which tends to buffer stomach contents.[45,48]

Aside from peptic ulceration induced by combinations of acid and pepsin, research in human medicine has revealed the tremendous importance of *Helicobacter pylori* in inducing ulceration. Infection with this organism has the effect of raising gastric pH because of disruption of gastric glands and also induces an inflammatory reaction that causes damage.[49] However, little evidence to date indicates that this organism is involved in gastric ulcers in horses. In the absence of a known role for infectious agents in gastric ulceration in animals, ulceration likely develops from injurious factors similar to those found in the proximal stomach, including gastric acid and bile. However, some factors that are important to induction of squamous epithelial ulceration may not be important in development of proper gastric mucosal ulceration. For example, feed deprivation and intensive training reproducibly induce squamous epithelial ulceration in horses but have little effect on proper gastric mucosa in horses.[50] Gastric acid likely plays a key role, whereas other factors such as nonsteroidal antiinflammatory drugs (NSAIDs) serve to reduce gastric defense mechanisms. In particular, inhibition of prostaglandin production reduces mucus and bicarbonate secretion while also reducing gastric

mucosal blood flow.[51] Some of the NSAIDs also have a topical irritant effect, although this appears to be of minor significance because the route of administration (oral or parenteral) seems to have little influence on development of ulceration.[52]

The source of prostaglandins responsible for gastric protection originally was assumed to be cyclooxygenase 1 (COX-1), because this isoform is expressed constitutively in gastric mucosa, whereas COX-2 is not expressed in the stomach unless induced by inflammatory mediators. However, mice in which the COX-1 gene has been knocked out fail to develop spontaneous gastric lesions,[53] possibly because of compensatory increases in prostaglandin production by COX-2.[54] This concept agrees with recent data indicating that inhibition of both COX isoforms is required to induce gastric ulceration.[55] From a clinical perspective this data indicate that drugs selective for COX-1 or COX-2 may be less ulcerogenic in the horse. Because COX-2 elaborates prostaglandins induced by inflammatory stimuli, selective inhibitors of COX-2 may be particularly useful because of their ability to serve as antiinflammatory agents that are less ulcerogenic.[56] 804

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13.5.4 Intestinal Ischemia-Reperfusion Injury

The most notable cause of intestinal mucosal injury in horses, particularly those suffering from colic, is ischemia. Initially, that a reduction in gastrointestinal blood supply leads to mucosal injury seems intuitive. However, the anatomy of the gastrointestinal tract and the differing structure of the intestinal mucosa at various anatomic locations have a significant influence on the extent of mucosal injury. Furthermore, ischemic injury may be induced by several different mechanisms, including occlusion of arterial supply by a thrombus, strangulation of intestinal vasculature, and generalized reduction in blood flow associated with various shock states. In addition, a number of seemingly distinct mechanisms of intestinal injury, such as intestinal distention, also trigger mucosal injury via an ischemic mechanism. Finally, reperfusion injury also may influence the extent of mucosal injury following an ischemic episode and has been pro posed as a potential site of therapeutic intervention.[57,58] Thus understanding the mechanisms of ischemia-reperfusion injury is critical to developing an understanding of the severity of various clinical conditions and beginning to formulate a therapeutic approach to diseases characterized by this devastating form of injury.

13.5.4.1 REGULATION OF INTESTINAL BLOOD FLOW

The intestinal circulation is capable of closely regulating blood flow during periods of low systemic perfusion pressure.[59,60] In particular, local regulation of resistance vessels within the microvasculature is particularly prominent, whereby metabolic end products of adenosine triphosphate (ATP) result in continued dilation of resistance vessels despite reductions in systemic arterial pressure. Dilation results in continued perfusion of gastrointestinal tissues during the early stages of shock, while other organs such as skeletal muscle undergo massive shunting of blood resulting from increased constriction of resistance vessels. The reasons for these differences in regulation are not entirely clear but may relate to the high level of energy required to fuel the intestinal mucosa and the serious systemic effects of breaches in the mucosal barrier. However, as blood flow falls below a critical level, regulatory systems are no longer effective and oxygen uptake by the gastrointestinal tissue decreases, culminating in tissue damage.[59]

The tip of the villus is the most susceptible region affected by hypoxia in the equine small intestine, largely because of the countercurrent exchange mechanism of blood flow in the small intestinal villus.[59] This countercurrent exchange mechanism is attributable to the vascular architecture, which consists of a central arteriole that courses up the core of the villus, arborizes at the tip, and is drained by venules coursing down the

periphery of the villus.[61] As oxygenated blood flows into the central arteriole, oxygen tends to diffuse across to the adjacent venules, which flow in the opposite direction. This series of events takes place along the length of the villus, resulting in a tip of the villus that is hypoxic even under normal conditions. Furthermore, reduced blood flow as occurs in shock exacerbates the countercurrent exchange of oxygen, and the tip becomes absolutely hypoxic.[59] This mechanism might explain why the small intestinal mucosa is more susceptible to ischemic injury, compared with the colon, which has no villi. For example, the duration required to produce severe morphologic damage to the equine colon is approximately 25% longer than in the small intestine.[62]

13.5.4.2 ISCHEMIC EPITHELIAL INJURY

Intestinal mucosal epithelium is susceptible to hypoxia because of the high level of energy required to fuel the Na^+/K^+-ATPase that directly or indirectly regulates ion and nutrient flux. The first biochemical event to occur during hypoxia is a loss of oxidative phosphorylation. The resulting diminished ATP concentration causes failure of the energy-dependent Na^+/K^+-ATPase resulting in accumulation of sodium, and subsequently intracellular water. The pH of the cytosol drops as lactic acid and inorganic phosphates accumulate from anaerobic glycolysis. The falling pH damages cell membranes, including lysosomal membranes, resulting in the release and activation of lysosomal enzymes into the cytosol, further damaging cellular membranes. Damage to the cell membrane allows the accumulation of high concentrations of calcium in the cytosol, which activates calcium-dependent degradative enzymes.[63] These events result in cytoplasmic blebbing of the basal membrane with subsequent detachment of cells from the underlying basement membrane.

Recent studies on epithelial injury during ischemia suggest that most epithelial cells undergo programmed cell death (apoptosis) during ischemia and reperfusion rather than necrosis, allowing retention of reusable components of irreversibly injured cells.[64] In one study, 80% of detached epithelium during small intestinal ischemia and reperfusion underwent apoptosis.[65] Although the most obvious result of apoptosis is loss of surface epithelium, a number of cells on the lower portion of the villus (in the small intestine) and cells within the crypts also may undergo apoptosis that only may become evident up to 24 hours following reperfusion of ischemic tissue.[66]

Morphologic changes observed in ischemic-injured small intestinal mucosa follow a similar sequence regardless of whether ischemia alone or ischemia and reperfusion induce injury (Table 13.5-1).[67] Initially, 805
epithelium separates from the underlying basement membrane, forming a fluid-filled space termed 806
Grüenhagen's space (Figure 13.5-1). The mechanism of fluid accumulation in this space is not understood entirely but may result from continued epithelial absorption of NaCl and water before it has detached fully from neighboring epithelial cells. This fluid accumulation likely exacerbates epithelial separation from the basement membrane. Subsequently, epithelium progressively sloughs from the tip of the villus toward the crypts, which are the last component of the intestinal mucosa to become injured.[68–70] Injury of crypts likely relates to the vascular architecture, because crypts receive a blood supply separate from the vasculature involved in the villous countercurrent exchange mechanism. The early morphologic changes observed in the equine large colon during ischemia are different from those described in the equine small intestine because of the lack of intestinal villi. However, as might be expected, the more superficially located surface cells are sloughed before those in crypts.[62,71] The orderly progression of tissue injury has been used by one group of investigators to predict accurately the survival of horses with large colon volvulus. The researchers took biopsies from the pelvic flexure, which has been shown previously to reflect mucosal changes along the length of the colon accurately,[72] and examined them histologically for the width of the crypts and intercrypt

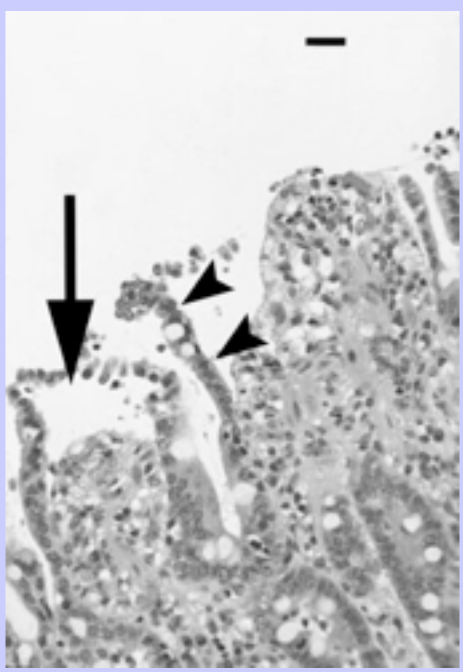
interstitial space. They expressed the latter measurements as a ratio of interstitium to crypt width (I:C) and defined nonviable colon as that which has greater than 60% loss of crypt and an I:C ratio greater than 3. Using this methodology, researchers correctly predicted survival in 94% of horses.[73]

TABLE 13.5-1 Grading System for Ischemia-Reperfusion Injury in Small Intestinal Mucosa

GRADE	DESCRIPTION
1	Separation of epithelium at the tip of the villus, creating a small space between epithelium and basement membrane called Grüenhagen's space
2	Loss of epithelium from the tip of the villus
3	Loss of epithelium from the upper third of the villus
4	Complete loss of villus epithelium
5	Injury or loss of epithelium within the crypt in addition to complete loss of villus epithelium

Modified from Chiu CJ, McArdle AH, Brown R et al: Intestinal mucosal lesion in low-flow states. 1. A morphological, hemodynamic, and metabolic reappraisal, *Arch Surg* 101:478–483, 1970.

Figure 13.5-1 Histologic appearance of Grüenhagen's space in ischemic injured ileal mucosa. Separation of epithelium at the tip of the villous from its basement membrane creates a space *(arrows)*. Epithelium subsequently sloughs into the lumen *(arrowheads)*. 1-cm bar = 100 μm.



13.5.4.3
STRANGULATING OBSTRUCTION

Because of the dramatic decline in *Strongylus vulgaris*–induced colic, which was associated frequently with infarction of intestinal arterial blood supply,[74] most ischemic lesions are associated with strangulating obstruction. Therefore considering mechanisms of ischemic injury in horses with naturally occurring strangulating lesions is important. The majority of experimental work has assessed complete ischemia (complete occlusion of the arterial blood supply)[62] or low-flow ischemia (during which arterial blood flow is reduced).[75,76] However, during intestinal strangulation, a disparity between the degree of occlusion of the veins and arteries occurs whereby veins are occluded before arteries because of differences in compliance of vascular walls. Thus strangulating lesions are typically hemorrhagic (hemorrhagic strangulating obstruction) as the arteries continue to supply blood to tissues that have little or no venous drainage. The result is ischemic injury, as previously outlined, but also a tremendous congestion of the tissues. Such hemorrhagic congestion has two opposing effects: it disrupts tissue architecture, including the mucosa and its epithelium, and continues to provide oxygenated blood to the tissues during much of the ischemic episode. In contrast, when strangulation results in sudden cessation of arterial blood flow (ischemic strangulating obstruction), tissues appear pale, and the mucosa rapidly degenerates because of a complete lack of oxygenated blood.[70] From a clinical standpoint, this makes assessing the degree of mucosal injury in horses with strangulating injuries 806
difficult because intestine that may look nonviable (dark red) may in fact have less mucosal injury than that of 807
ischemic strangulated intestine.[77]

An additional consideration in clinical strangulating obstruction is the degree of ischemia that intestinal distention may induce. For example, experimental distention (18 cm of H_2O for 2 hours) and decompression (2 hours) of jejunum resulted in a significant increase in microvascular permeability and a significant decrease in tissue oxygenation similar to that which would be expected with low-flow ischemia.[78,79] In particular, microscopic evaluation of vasculature revealed capillary endothelial cell damage and local edema formation.[80] This data suggest that distended intestine proximal to an obstruction may undergo mucosal injury despite its normal appearance. Indeed, in one study, intraluminal pressures greater than 15 cm H_2O in naturally occurring cases of colic correlated with a poor prognosis for survival.[81]

13.5.4.4
REPERFUSION INJURY

Although that reperfusion of ischemic tissues results in exacerbation of mucosal injury recently has been taken for granted, one should remember that mechanisms underlying intestinal reperfusion injury have been defined largely in laboratory animals under specific conditions.[82–86] However, studies on reperfusion injury in horses have had some conflicting results.[68,76,87] The conflict may be attributable to the way in which the studies have been performed. In particular, the type of ischemia used in most laboratory animal studies has been low-flow ischemia (in which the blood flow typically is reduced to 20% of baseline flow), whereas studies in horses have used a number of different ischemic models, including various types of strangulating obstruction. Although strangulating obstruction is of great clinical relevance, this type of ischemic insult is less likely to develop reperfusion injury.[68,88,89] Conversely, low-flow ischemia appears to prime tissues for subsequent injury once the tissue is reperfused, and considerable evidence supports the presence of reperfusion injury in horses following low-flow ischemia.[75,76,80,90] Nonetheless, low-flow ischemia may not be a common clinical entity.

TABLE 13.5-2 Comparison of Mean Levels of Xanthine Oxidase/Xanthine Dehydrogenase and Myeloperoxidase (as an Indication of Granulocyte Numbers) in the Small Intestine of Various Species

SPECIES	INTESTINAL SEGMENT	TOTAL XO/XDH (mU/gTISSUE)	MYELOPEROXIDASE (U/gTISSUE)	REFERENCE
Cat (adult)	Jejunum	80	12	86, 143
	Ileum	NR	NR	
Rat (adult)	Jejunum	405–523	1.9	96, 144
	Ileum	150	NR	96, 145
Pig (6–8 weeks)	Jejunum	3.4 (0)*	NR	96
	Ileum	0.4 (0.9)	2.2	
Horse (adult)	Jejunum	100–131 (60)†	0.02	92, 96
	Ileum	30–48 (0)	0.1	96

XO/XHD, Xanthine oxidase/xanthine dehydrogenase; *NR,* not reported.

* Value in parentheses for the neonatal piglet.

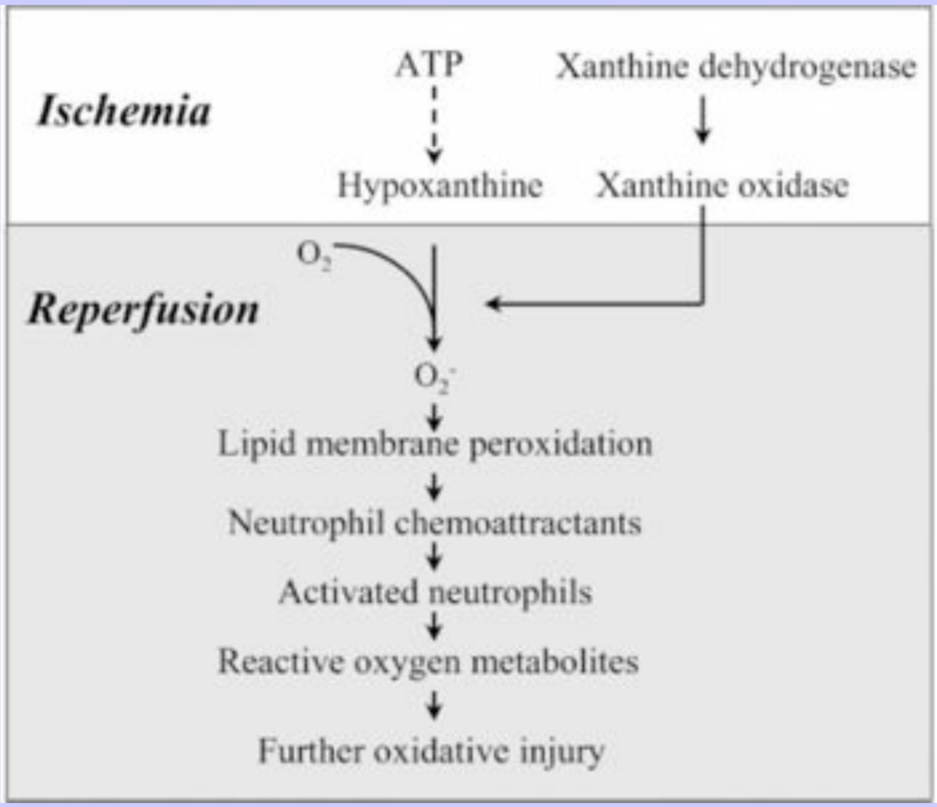
† Value in parentheses for the foal.

In addition to the type of ischemia, other factors are involved in priming tissues for reperfusion injury, including species and anatomic-specific variation in oxidant enzyme and neutrophil levels (Table 13.5-2). For example, the foal appears to have low levels of small intestinal xanthine oxidase, an enzyme that has been shown to play a critical role in triggering reperfusion injury in laboratory animals,[84,85,91] whereas adult levels are much greater, particularly in the proximal small intestine.[92] In addition, horses appear to have low numbers of resident neutrophils in the intestinal mucosa,[93] and this population of neutrophils (rather than those recruited from the circulation) appears to be most critical for induction of reperfusion injury.[86] However, studies demonstrating reperfusion injury in the equine colon following low-flow ischemia have shown significant accumulation of neutrophils within the mucosa.[75] Therefore a complete understanding of mechanisms of neutrophilic infiltration and the mechanisms whereby they damage tissue requires further study.

Reperfusion injury is initiated during ischemia when the enzyme xanthine dehydrogenase is converted to xanthine oxidase and when its substrate, hypoxanthine, accumulates simultaneously because of ATP use (Figure 13.5-2).[57,94] However, little xanthine oxidase activity occurs during ischemia, because oxygen is 807
required as an electron acceptor. During reperfusion, xanthine oxidase rapidly degrades hypoxanthine in the 808
presence of oxygen, producing the superoxide radical as a by-product.[57] The superoxide radical contributes to oxidative tissue damage and, most importantly, activates neutrophil chemoattractants.[84,85] Thus inhibition of xanthine oxidase in feline studies of intestinal ischemia-reperfusion injury prevents infiltration of neutrophils and subsequent mucosal injury.[83,84] However, inhibition of xanthine oxidase has had no effect on ischemia-

reperfusion injury in equine small intestine[87] and colon,[95] suggesting that reperfusion injury is simply a continuation of injury initiated during ischemia, as suggested in some equine studies,[63] or that the classic reperfusion injury pathway is activated by alternate sources of reactive oxygen metabolites. The latter has been suggested by studies in feline models of ischemia-reperfusion injury in which the source of a significant proportion of reactive oxygen metabolites is unknown and is independent of xanthine oxidase and neutrophils.[83]

Figure 13.5-2 Intestinal reperfusion injury cascade. Reperfusion injury is initiated by elaboration of superoxide by metabolism of hypoxanthine by xanthine oxidase and subsequent infiltration of neutrophils.



A veterinary review of the pathogenesis of intestinal reperfusion injury in the horse suggested the concept of a therapeutic window wherein treatment of reperfusion injury would be beneficial.[57] The basis of this concept is that certain conditions exist under which ischemic injury is minimal and that tissues are damaged severely during reperfusion.[88] Thus under conditions of low-flow ischemia, little injury is demonstrated during 3 hours of ischemia, but remarkable injury occurs during 1 hour of reperfusion.[83–85] However, a therapeutic window may not exist under conditions of strangulating obstruction in which severe injury occurs during ischemia and minimal injury occurs during reperfusion.[96] This in turn greatly reduces clinicians' ability to ameliorate ischemia-reperfusion injury with treatments such as antioxidants at the time of reperfusion.

13.5.5 Mechanisms of Gastrointestinal Mucosal Repair

13.5.5.1 GASTRIC REPARATIVE MECHANISMS

Mechanisms of gastric repair depend greatly on the extent of injury. For instance, superficial erosions can be covered rapidly by migration of epithelium adjacent to the wound; a process termed *epithelial restitution*. However, ulceration (full-thickness disruption of mucosa and penetration of the muscularis mucosa) requires repair of submucosal vasculature and extracellular matrix. The formation of granulation tissue initiates repair and supplies connective tissue elements and microvasculature necessary for mucosal reconstruction. Connective tissue elements include proliferating fibroblasts that accompany newly produced capillaries that form from proliferating endothelium. Recent studies indicate that nitric oxide is critical to both processes,[40,97] which likely explains the reparative properties of nitric oxide in the stomach.[98]

Once an adequate granulation bed has formed, newly proliferated epithelium at the edge of the wound begins to migrate across the wound. In addition, gastric glands at the base of the ulcer begin to bud and migrate across the granulation bed in a tubular fashion.[99] Repairing epithelium expresses epidermal growth factor, which appears to facilitate these processes.[100] In addition, a mucoid cap facilitates these events and retains reparative factors and serum adjacent to the wound bed.[51] Once the ulcer crater has been filled with granulation tissue and the wound has been reepithelialized, the subepithelial tissue remodels by altering the type and amount of collagen. Despite the remodeling process, ulcers tend to recur at sites of previous ulceration, and the concern is that this remodeling can result in excessive deposition of collagen and fibrosis.[26]

13.5.5.2 INTESTINAL REPARATIVE MECHANISMS

Reparative mechanisms are similar in the intestine, except that in the small intestine, mucosal villi contribute to mucosal repair. Once intestinal epithelium is disrupted, two events occur almost immediately to reduce the size of the denuded portion of the villus: contraction of the villus and epithelial restitution (Figure 13.5-3). For example, in porcine ileum subjected to 2 hours of ischemia, villi were 60% of their former height and 50% of the denuded villous surface area was covered in flattened epithelium within 6 hours.[42] Enteric nerves appear to regulate villous contraction, because inhibition of enteric nerve conduction prevents villous shortening 808
following injury. The contractile component of the villus is a network of myofibroblasts distributed 809
throughout the lamina propria of the villus and along the central lacteal. Inhibition of villous contraction results in retarded epithelial repair because of the larger denuded surface that remains to be covered by migrating epithelium compared with similarly injured villi that have contracted.[43] PGE_2 also has been implicated in regulating villous contraction, because application of PGE_2 resulted in villous contraction when perfused through normal rat ileum.[101] As villi contract, assuming the basement membrane is intact, epithelium from the margins of the wound migrates centripetally to resurface toward the tip of the villus.[43] The process of restitution is similar in denuded colonic mucosa, except that it may proceed more rapidly because of the lack of villi.[41] Epithelial restitution is solely a migratory event that does not depend on provision of new enterocytes by proliferation. Cellular migration is initiated by extension of cellular lamellipodia that receive signals from the basement membrane via integrins. Intracellular signaling converges on the actin cytoskeleton, which is responsible for movement of lamellipodia. Specific components of the basement membrane appear to be critical to the migratory process. For example, application of antibodies to collagen types III and IV, which

are important components of intestinal mucosal basement membrane, impeded epithelial restitution.[102,103] Other elements of the basement membrane, including proteoglycans, hyaluronic acid and noncollagenous proteins such as fibronectin and laminin also may provide important signals.[104] These subepithelial matrix components that facilitate restitution may form the basis for clinical treatments designed to speed up the repair process, analogous to administration of matrix components to horses with articular cartilage damage.

Although epithelial restitution results in gross closure of previously denuded regions of gastrointestinal mucosa, closure of interepithelial spaces ultimately is required to restore normal epithelial barrier resistance. Because the tight junction is principally responsible for regulating the permeability of the interepithelial space, repair and closure of this structure likely is critical to restore intestinal barrier function. Recent research indicates that prostaglandins play a vital role in recovery of tight junction resistance,[105] indicating that administration of nonselective COX inhibitors to horses with colic, particularly those recovering from strangulating obstruction, may be deleterious. Therefore judicious use of NSAIDs is appropriate until more selective drugs that allow continued production of reparative prostaglandins are available for use in horse.[56]

After restoration of the epithelial barrier, the epithelium must reestablish normal mucosal architecture to allow normal gut absorptive and digestive function. In porcine ileum subjected to 2 hours of ischemia, the epithelial barrier was restored within 18 hours, but villi were contracted and covered in epithelium with a squamous appearance. Restoration of normal villous architecture required another 4 days.[42] Newly proliferated crypt epithelium replaces the flattened villous epithelium that characterizes restitution. Under normal circumstances the dividing stem cells, of which the base of each mucosal crypt has approximately four, form new enterocytes. Newly divided enterocytes migrate from the crypt onto the villus.[106] During migration, enterocytes differentiate and acquire specific absorptive and digestive functions. Fully differentiated enterocytes reside on the upper third of the villus for 2 to 3 days and then are sloughed into the intestinal lumen.[107] This process accelerates during mucosal repair and requires increased proliferative rates. A variety of locally available gut-derived factors, including luminal nutrients, polyamines, and growth factors, may stimulate increased proliferation within 12 to 18 hours.[42] The return of the normal leaflike shape of the villus occurs following the appearance of normal columnar epithelium.

13.5.6 Mediators of Repair

13.5.6.1 PROSTAGLANDINS

Although prostaglandins have been implicated in mucosal cytoprotective function, few studies have assessed their importance in mucosal repair. One study implicated prostaglandins in growth factor-stimulated restitution,[108] but a more prominent role of prostaglandins in mucosal repair is their ability to close interepithelial tight junctions.[105,109,110] For instance, ischemic-injured small intestine rapidly recovers barrier function (as measured in vitro as transepithelial resistance) in the presence of PGI_2 and PGE_2, despite the fact that these prostanoids had little effect on villous contraction and epithelial restitution. However, electron microscopic examination of tissues reveals dilation of tight junctions in tissues treated with NSAIDs,[110] whereas those additionally treated with prostaglandins have closely apposed tight junctions (Figures 13.5-3 and 13.5-4). Prostaglandins stimulate closure of tight junctions via the second messengers cyclic adenosine monophosphate and Ca^{2+},[105] which interestingly were among the first mediators found to modulate tight junction permeability.[111,112] Such tight junction closure is of importance to patients with intestinal injury that are treated with NSAIDs, because reduced prostaglandin levels may result in increased intestinal permeability.

For example, in a study on ischemic-injured porcine ileum, treatment with the NSAID indomethacin resulted in a significant increase in intestinal permeability to inulin and lipopolysaccharide compared with tissues that were treated additionally with PGI_2 and PGE_2.[105]

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Figure 13.5-3 Histologic appearance of repairing intestinal mucosa 6 hours following a 2-hour ischemic episode. Blunting of the villi, attributable to villous contraction, and evidence of epithelial restitution *(arrows)* are notable. 1-cm bar = 100 μm.

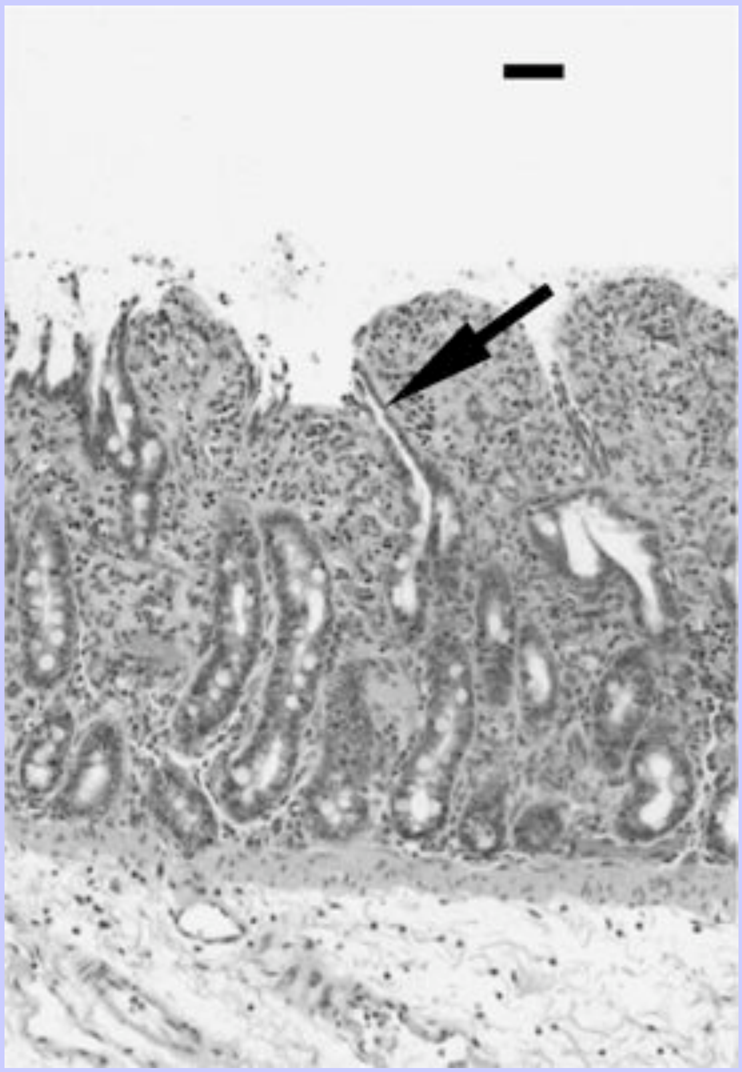
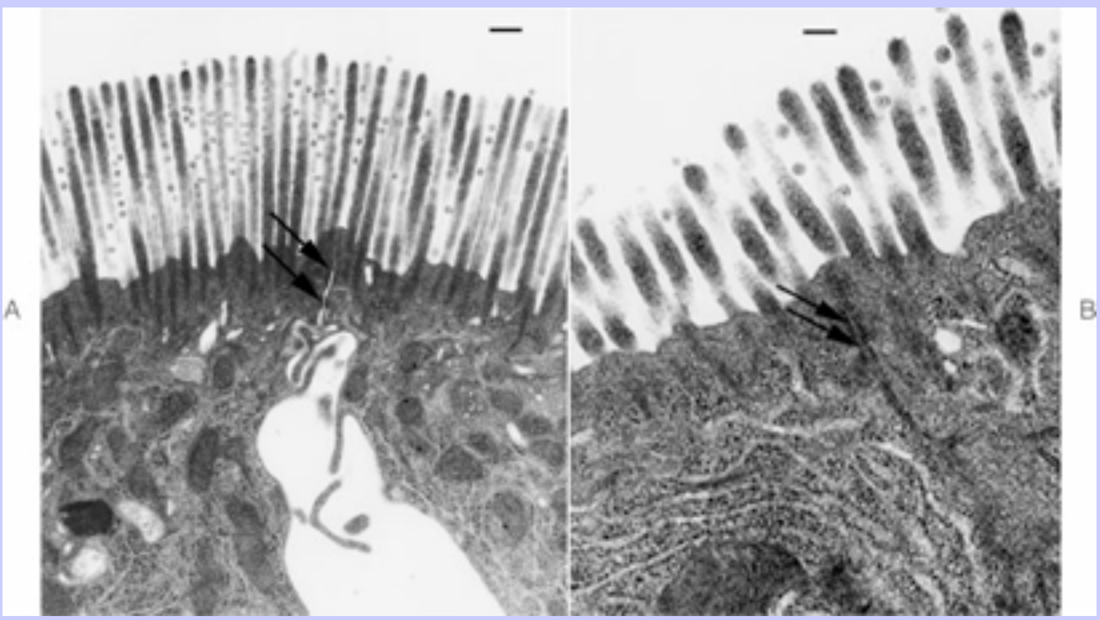


Figure 13.5-4 Ultrastructural appearance of repairing ischemic-injured mucosa. **A,** Restituting epithelium 2 hours following a 1-hour ischemic episode in the presence of the nonselective cyclooxygenase inhibitor indomethacin. Dilation of the interepithelial space and the apical tight junction *(arrows)* correlates with a leaky intestinal barrier, **B,** Similar restituting epithelium had been treated additionally with prostaglandins E_2 and I_2. The close apposition of the tight junction *(arrows)* and the interepithelial space correlate with normalization of intestinal barrier function. 1-cm bar = 6 μm.



13.5.6.2 POLYAMINES

The process of restitution absolutely depends on a group of compounds called polyamines.[113,114] The rate-limiting enzyme in the formation of the polyamines spermine, spermidine, and putrescine is ornithine decarboxylase. In rats with stress-induced duodenal ulcers, systemic administration of the ornithine decarboxylase inhibitor α-difluoromethylornithine significantly reduced polyamine levels and greatly reduced epithelial restitution. Furthermore, intragastric treatment of these same rats with putrescine, spermidine, and spermine prevented the delayed mucosal repair induced by α-difluoromethylornithine.[113] Interestingly, gastric tissue levels of ornithine decarboxylase increased in rats with stress-induced gastric ulcers, suggesting that tissue injury enhances polyamine production, which may contribute to the normal rapid rate of epithelial restitution.[115]

The mechanisms whereby polyamines stimulate epithelial restitution are not clear. McCormack, Wang, Viar, et al. hypothesized that polyamines increase transglutaminase activity, an enzyme that catalyzes the cross-linking of cytoskeletal and basement membrane proteins.[116] Further investigation of the role of polyamines in IEC-6 cell migration showed that depletion of polyamines resulted in disruption of the cytoskeleton and 810
reduced the physical extension of lamellipodia.[117] More recent studies have clarified this pathway. In 811
particular, polyamines have been shown to regulate cytoskeletal cellular migration via activation of the small guanosine triphosphatase-Rho-A by elevating intracellular Ca^{2+} levels. These elevations in Ca^{2+} result from polyamine regulation of expression of voltage-gated K^+ channels and altered membrane electric potential.[118]

Polyamines also play a role in the normal physiologic regulation of crypt cell proliferation and differentiation.[119,120] Polyamines are produced by fully differentiated enterocytes at the tip of the villus and may reach the crypt within sloughed luminal epithelium or via local villous circulation.[121] Following intestinal injury, polyamines appear to stimulate enhanced proliferation by increasing the expression of protooncogenes, which control the cell cycle.[122] The mechanism whereby polyamines influence gene expression likely relates to the cationic nature of these compounds, which may influence the tertiary structure of negatively charged DNA and RNA.[113]

13.5.7 GROWTH FACTORS

Locally produced growth factors—including epidermal growth factor (EGF), transforming growth factor α (TGF-α), TGF-β, and hepatocyte growth factor—have the ability to modulate mucosal recovery. The most important of these growth factors in early mucosal repair events is TGF-β, which is a potent stimulus of epithelial restitution and modulator of the extracellular matrix.[26] Neutralization of TGF-β retards epithelial migration in vitro, and TGF-β apparently may serve as a point of convergence for mediators of restitution, because neutralizing TGF-β also inhibits the effects of other peptides. However, TGF-β paradoxically inhibits epithelial proliferation, thereby reducing the supply of new enterocytes for mucosal repair. Conversely, EGF, produced by the salivary glands and duodenal Brunner's glands, and the related TGF-α, produced by small intestinal enterocytes, are potent stimulants of enterocyte proliferation. These growth factors share approximately 30% of their amino acid structure, bind to the same receptor on the basolateral surface of enterocytes, and are not related to TGF-β.[123] The physiologic role of EGF is difficult to discern because it is present in the intestinal lumen, with no apparent access to its basally located receptor. However, EGF has been proposed to act as a "surveillance agent" that gains access to its receptor during epithelial injury (when the EGF receptor likely would be exposed) to stimulate proliferation.[124] TGF-α presumably has a similar role, but it is present in greater concentrations in the small intestine because it is produced by differentiated villous enterocytes. The mature peptide is cleaved from the extracellular component of the transmembrane TGF-α precursor and released into the lumen.[123]

13.5.7.1 TREFOIL PEPTIDES

Another group of proreparative peptides produced within the gastrointestinal tract are the trefoil peptides. Under physiologic conditions, trefoil peptides are secreted by mucus-producing cells at distinct anatomic sites. For example, gastric epithelium produces the trefoil peptide pS2, whereas the small and large intestine mucosa produce intestinal trefoil peptide.[125] However, any of the trefoil peptides may be upregulated within repairing epithelium regardless of anatomic site.[26,126] In addition, trefoil peptides have the ability to induce their own

expression, amplifying the level of these reparative factors at sites of mucosal repair.[127] Trefoil peptides are the most potent stimulants of epithelial migration in vitro, and their effects are independent of growth factors, including TGF-β.[128] However, recent evidence suggests that EGF receptor activation is required for induction of pS2 and another of the trefoil peptides, termed *spasmolytic peptide,* in gastric epithelium in vitro. The importance of trefoil peptides to the mucosal repair response in vivo is illustrated by gene knockout studies, in which mice deficient in intestinal trefoil factor have greatly reduced ability to repair intestinal injury.[129] In fact, detergent-induced mucosal injury was lethal because of a lack of restitution compared with wild-type mice that fully recovered from similar mucosal injury. The fact that administration of intestinal trefoil factor restored restitution has important therapeutic implications. The mechanism whereby trefoil peptides stimulate epithelial migration is yet to be characterized fully but appears to involve translocation of the adherens junction protein E-cadherin, thereby allowing cells to become untethered from neighboring cells.[26]

13.5.7.2

INTESTINAL NUTRIENTS

The principal metabolic fuel of enterocytes is glutamine and of colonocytes, butyrate. However, recent studies suggest that glutamine and butyrate have more specific proliferative actions aside from their role as nutrients. For example, in the piglet IPEC-J2 enterocyte cell line, glutamine enhanced gene transcription by increasing mitogen-activated protein kinase activity.[130,131] Similarly, butyrate stimulated mucosal growth following colonic infusion in the rat.[132] Because of such growth-promoting actions, glutamine was shown to prevent intestinal mucosal atrophy and dysfunction that accompanies starvation[133,134] and long-term total parental nutrition.[135,136] Additionally, glutamine improves function of transplanted small intestine[137,138] and protects intestinal mucosa from injury if administered before chemotherapy[139] and radiation therapy.[140,141] Intestinal 811
nutrients also may synergize with other proliferative agents. For example, administration of glutamine and 812
TGF-α to porcine ileum that had been subjected to 2 hours of ischemia resulted in a synergistic increase in mitogen-activated protein kinase activity, enterocyte proliferation, and villous surface area.[42] Although concern has arisen that such early return to normal surface area may result in dysfunctional mucosal digestive and absorptive function because of resurfacing denuded mucosa with immature epithelium, nutrients and growth factors also appear to promote early differentiation. In the case of glutamine and TGF-α restoration of postischemic small intestine, rapid recovery of digestive enzymes also was documented.[142]

13.5.8

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13.6

13.6—Gastrointestinal Ileus

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Effective gastrointestinal motility involves a complex interaction between the enteric nervous system, muscular wall, and luminal contents. Additional factors that influence the net transit of digesta include gravity, the volume and viscosity of the contents, and pressure gradients created by simultaneous contraction and relaxation of adjacent segments of bowel. Casual use of the term *intestinal motility* in veterinary medicine often underestimates the complexity of the processes involved in the transit of intestinal contents. This is particularly true when the term is used to describe the frequency and or intensity of intestinal sounds, or borborygmi. The existence of borborygmi does not always equate with progressive movement of intestinal contents.

Disruption to normal motility occurs commonly in horses for a variety of reasons. Examples of diseases in which altered motility may be present include gastroduodenal ulceration, intraluminal obstruction or impaction, excessive wall distention, strangulating obstructions, peritonitis, and inflammatory bowel diseases such as duodenitis proximal jejunits or colitis. Ineffective intestinal motility is also a feature of several neonatal diseases, including prematurity, systemic sepsis, and perinatal asphyxia. Certain parasitic infections, electrolyte derangements, and endotoxemia can modify digesta transit in horses of all ages. General anesthesia and specific sedatives, such as xylazine, romifidine, or detomidine, also disturb motility.

13.6.1

Postoperative Ileus

The inhibition of propulsive bowel activity usually is referred to as ileus. Ileus is ascribed most frequently to the condition that occurs after laparotomy and is termed *simple* or *uncomplicated postoperative ileus* (POI). The 815

816 term *complicated* or *paralytic ileus* describes intestinal motility disturbed for longer periods after surgery. POI in horses is associated most commonly with surgery of the small intestine, particularly after resection and anastomosis,[1,2] is a common complication of small intestinal surgery, and can have a negative effect on short-term postoperative survival.[3,4] Motility dysfunction likely is present in all horses after laparotomy, but many are affected subclinically and require minimal or no specific intervention. In symptomatic animals, clinical signs are apparent shortly after recovery and include colic, tachycardia, dehydration, decreased borborygmi and fecal output, and sequestration of fluid within the stomach. Rectal examination and ultrasound reveal small intestinal distention with rare or absent wall movement. The severity and duration of intestinal stasis varies, lasting from minutes to days.

13.6.2

Cecal Emptying Defect

A specific motility disorder involving the cecum or ileocecocolic region occurs sporadically in horses.[5–7] The condition most commonly occurs after general anesthesia and extraabdominal surgery, particularly orthopedic and upper airway procedures, and therefore often is categorized as a form of POI. Anecdotally, horses at greatest

risk are young male performance animals. Other cases occur spontaneously, often in animals with painful primary conditions such as uveitis or septic tenosynovitis. The syndrome is frustrating in that clinical signs are often subtle unless cecal perforation has occurred. In horses with a cecal emptying defect after anesthesia, overt signs are usually apparent 3 to 5 days after the procedure. The earliest detectable signs include depression and a reduction in feed intake and fecal output. Ineffective emptying results in overfilling of the cecum with moist contents, which is manifest by signs of mild to moderate colic. If the condition is recognized late or untreated, the cecum may rupture and result in fatal peritonitis.

13.6.3 Physiology

The inherent rhythmicity of electric activity in the intestine is controlled by the interstitial cells of Cajal, specialized cells that are electrically coupled to myocytes via gap junctions.[8] These cells are responsible for generating and propagating slow-wave activity and may be critically involved in a range of motility disorders. The enteric nervous system primarily controls and coordinates intestinal contraction. A combination of central and autonomic innervation influences events, but contraction does not require external neural input. The parasympathetic supply to the gastrointestinal tract is via the vagus and pelvic nerves, and the sympathetic supply is through postganglionic fibers of the cranial and caudal mesenteric plexuses. A complex network of interneurons within each plexus integrates and amplifies neural input; the intensity and frequency of resultant smooth muscle contractions are proportional to the amount of sympathetic and parasympathetic input. Additional binding sites for a number of other endogenous chemicals, including dopamine, motilin, and serotonin exist within the enteric nervous system and on smooth muscle cells.[9] Acetylcholine is the dominant excitatory neurotransmitter in the gastrointestinal tract and exerts its action through muscurinic type 2 receptors on smooth muscle cells. Sympathetic fibers innervating the gastrointestinal tract are adrenergic, postganglionic fibers with cell bodies located in the prevertebral ganglia. Activation of α_2-adrenergic receptors on cholinergic neurons within enteric ganglia inhibits the release of acetylcholine and therefore reduces intestinal contraction.

β_1-, β_2-, and β-atypical receptors are directly inhibitory to the intestinal smooth muscle.[10] Inhibitory nonadrenergic, noncholinergic neurotransmitters include adenosine triphosphate, vasoactive intestinal peptide, and nitric oxide.[11,12] These neurotransmitters are critical for mediating descending inhibition during peristalsis and receptive relaxation. Substance P is a nonadrenergic, noncholinergic neurotransmitter that may be involved in contraction of the large colon.[13,14]

The rate and force of intestinal contractions along the small intestine and large colon of the horse are important determinants of intestinal motility; of even greater importance to the net propulsion of digesta are the cyclical patterns of contractile activity. These patterns are known as the small intestinal and colonic migrating motility (or myoelectric) complexes (MMCs).[15,16] The colonic complex usually originates in the right ventral colon and variably traverses the ascending and descending colons. Many of these complexes are related temporally to a specialized motility event of the ileum, the migrating action potential complex.

13.6.4 Pathophysiology

13.6.4.1 INFLAMMATION

Local inflammation within the intestinal muscularis and inhibitory neural events are important initiators of intestinal ileus.[17,18] Intestinal inflammation not only is important in primary intestinal diseases in horses, such

as duodenitis-proximal jejunitis and colitis but also is induced after simple intestinal handling during laparotomy. Experimental data from other species suggests that handling of the small or large intestine at the time of surgery activates resident macrophages with resultant increased expression of P-selectin and intercellular adhesion molecule 1 on endothelial cells within the vasculature of the muscularis. The 816
upregulation of associated ligands on leukocytes leads to sequential “sticking and rolling,” followed by 817
neutrophil migration into the interstitium. The subsequent release of neutrophil products interferes with cell signaling and results in reduced intensity of smooth muscle contraction. Furthermore, the inflamed intestine fails to contract normally in response to putative prokinetic agents.

Another key factor in the development of intestinal stasis after inflammation is the local overproduction of nitric oxide caused by the upregulation of inducible nitric oxide synthase (iNOS) by resident macrophages. Nitric oxide is a key inhibitory neurotransmitter of the nonadrenergic, noncholinergic system.[12] Nitric oxide synthase inhibition has been a pharmacologic target in the treatment of experimental ileus.

13.6.4.2 DRUGS

The inhibitory effects of α_2-agonists such as xylazine and detomidine on cecal and large colon motility are well described.[19–24] Intravenously administered xylazine inhibits cecal and large colon motility for 20 to 30 minutes without seriously disrupting small intestinal myoelectric activity, and detomidine can reduce large intestinal myoelectric activity for up to 3 hours. The α_2-antagonist yohimbine has a weak but positive effect on cecal emptying in normal ponies, suggesting that normal motility is under constant α_2-adrenergic tone.[24]

Atropine is a postganglionic blocking agent that binds to muscarinic receptors. When administered at 0.04 mg/kg, atropine inhibits individual small intestinal, cecal, and colonic contractions for about 120 minutes but supresses small intestinal and colonic migrating complexes for up to 8 hours.[25]

13.6.4.3 NEURAL REFLEXES

Neural reflexes also may mediate inhibition of motility associated with peritoneal inflammation.[26,27] The afferent segment is composed partly of capsaicin-sensitive visceral afferent C fibers that terminate in the dorsal horn of the spinal cord, where they can activate inhibitory sympathetic fibers or synapse directly on the sympathetic ganglia. Consequently, the efferent limb of the reflex expresses increased sympathetic outflow, primarily mediated through stimulation of α_2-adrenoreceptors, and inhibition of acetylcholine release, which provides the rationale for α_2-blockade in treating ileus. Intraluminal infusion of capsaicin before abdominal surgery ameliorated the severity of POI in experimental rats. This finding highlights the importance of visceral afferent fibers in the development of POI.[28]

13.6.4.4 DISTENTION

Ileus also can occur in association with intestinal obstruction or displacement. Mild to moderate distention of the bowel, such as that occurring in the early stages of an intraluminal obstruction, evokes an increase in local contractile activity.[29,30] Excessive distention results in inhibition of motility within the distended segment of bowel. Intestinal stasis is not always detrimental and under certain conditions may be protective.

13.6.4.5

ENDOTOXINS

Endotoxemia is a clinical feature of many diseases of the equine gastrointestinal tract, and endotoxins independently can exert a negative effect on intestinal motility and transit.[31] A variety of mediators likely are involved, but activation of α_2-adrenoreceptors and production of prostanoids appear to be important, for pretreatment with yohimbine or nonsteroidal antiinflammatory drugs (NSAIDs; phenylbutazone or flunixin), respectively, ameliorates the inhibitory effects of experimental endotoxin infusion.[32,33] Endotoxin infusion induced an inflammatory response in the intestine of rats that mimicked the response induced by handling during laparotomy.[34] The similarity of the responses were highlighted in a recent study that demonstrated that prior exposure of the muscularis to endotoxin protected the intestine from the effects of manipulation.[35]

The pathophysiology of cecal emptying defect is not known. This syndrome may best mimic POI in human beings and generally is considered a large intestinal disorder. An important difference in horses is that laparotomy is a rare predisposing factor, and most cases occur in horses undergoing routine extraabdominal surgical procedures. General anesthesia itself is a potent inhibitor of gastrointestinal motility in horses, but these effects are short-lived and reversible within hours of anesthetic withdrawal.[15] The return of normal motility in horses after experimental ileus was most delayed in the cecum, suggesting that this may be a common site of ileus in horses.[36] A link between routine postoperative medications, such as phenylbutazone and aminoglycoside antibiotics, has been suspected but not established. An inhibitory effect of NSAIDs on large colon contractility has been demonstrated using in vitro techniques.[37] Primary sympathetic overstimulation could be involved, for many of the affected animals are young, male horses or animals with painful diseases.

The duration of surgery influences the development of small intestinal POI, but not cecal emptying dysfunction.[7,38] Technique may have a weak influence on small intestinal POI after jejunojejunostomy. The duration of intestinal ileus was shorter in animals that received a side-to-side stapled anastomosis than those that had a hand sewn end-to-end procedure.[3] The duration of ileus after stapled end-to-end anastomosis was not different from that after either procedure.

Reported risk factors for the development of POI in horses include age (>10 years), small intestinal resection 817
and anastomosis, breed (Arabians had a greatest risk than other breeds), and duration of surgery.[38] 818
Interestingly, performing a pelvic flexure enterotomy and emptying the colon had a protective effect against POI.

13.6.5

Diagnosis

The diagnosis of ileus is based on history and physical examination findings. Important tests include determination of pulse rate and rhythm, auscultation and percussion of the abdomen, rectal palpation, and passage of a nasogastric tube. A complete blood count with fibrinogen estimation and cytologic analysis of peritoneal fluid may improve the accuracy of diagnosis. Affected animals may be colicky because of accumulation of fluid in the upper gastrointestinal tract (classical POI) or cecal contents (cecal emptying defect). Decompression of the stomach is important diagnostically and therapeutically in horses with POI after small intestinal surgery. Failure to relieve pain with gastric decompression could point toward mechanical obstruction, severe inflammation of the intestine, or peritonitis. Most animals with ileus are depressed and have reduced fecal output and intestinal borborygmi. One should interpret intestinal sounds with caution, however, because the

presence of borborygmi does not always equate to progressive intestinal motility and merely may reflect local, nonpropagated contractions.

Rectal palpation findings in cases of persistent POI or duodenitis-proximal jejunitis are usually nonspecific but may reveal dilated, fluid-filled loops of small intestine. The clinician occasionally can palpate roughened peritoneal surfaces if peritonitis is present. One can palpate cecal distention with digesta in horses with advanced cecal dysfunction.

Distinguishing functional ileus from mechanical obstruction is important and can be difficult, but horses with mechanical obstruction typically have sustained high volumes of gastric reflux that vary little over time.

13.6.6 Treatment

The management of intestinal ileus depends on the segment of gastrointestinal tract involved. Therapy for ileus of the proximal gastrointestinal tract involves a combination of gastric decompression, fluid and electrolyte therapy, and antiinflammatory drug therapy. Electrolyte therapy is critical, particularly for maintaining adequate extracellular concentrations of potassium, calcium, and magnesium. Calculation of the volume of fluid to be administered should include maintenance requirements (40 to 60 ml/kg/day) plus an estimate of losses, especially those lost through gastric decompression. One should consider parenteral provision of calories when feed has been withheld for more than 96 hours, particularly after the horse has had surgery. A combination of amino acids and dextrose with or without lipids effectively provides these calories. Hand walking also may provide some benefit to these animals but is not likely to have a direct effect on intestinal motility.

One should avoid drugs that may impair normal intestinal motility, including the anticholinergics such as atropine and opiate receptor agonists such as morphine and meperidine. Butorphanol appears to have little or no adverse effect on small or large intestinal motility.[39,40] One should use α_2-agonists sparingly because of their inhibitory effects on large intestinal motility.

Fluid therapy is the key component in managing cecal emptying defect, usually in combination with lubricants or laxatives, such as mineral oil or magnesium sulfate, and with careful use of antiinflammatory drugs. Horses with primary cecal impaction or impaction caused by an emptying defect frequently require surgery to prevent fatal rupture. The surgical management of these cases is controversial and may include typhlotomy alone, typhlotomy with a bypass procedure such as ileocolic or jejunocolic anastomosis, or a bypass without typhlotomy.[41] Most horses that undergo simple typhlotomy have an uneventful recovery,[42] although a small number experience impaction again and require a second laparotomy.

Experimental and anecdotal evidence provides a strong rationale for using antiinflammatory drugs to prevent and treat gastrointestinal ileus, particularly in animals that may have endotoxemia.[43] Flunixin meglumine is used widely in equine practice as an analgesic and antiinflammatory agent, and it ameliorates many of the adverse systemic effects of endotoxin, particularly those on the cardiovascular system. A potential negative effect of NSAIDs on large intestinal contractility has been suggested.

Broad-spectrum antimicrobials are indicated when one suspects sepsis or for the compromised immune system, as in cases of moderate to severe endotoxemia. Theoretical concerns have been raised regarding the use of aminoglycoside antibiotics in animals with ileus. High concentrations of aminoglycoside antimicrobials inhibited intestinal contractions in exposed sections of intestine in vitro, but this inhibitory effect is unlikely to occur at clinically relevant doses.[44]

Motility-enhancing drugs have been advocated to treat gastrointestinal ileus. Unfortunately, information directly pertinent to horses is limited and must be extrapolated cautiously from that of other species because of the differences in intestinal anatomy and physiology. Prokinetic drugs potentially can shorten the length of hospitalization, thereby reducing the cost of treatment and the number of potential complications such as weight loss, thrombophlebitis, and laminitis. Experimental evidence indicates that prokinetic drugs can minimize the 818

819 development of postoperative abdominal adhesions.[45] Most prokinetic drugs require a healthy gut wall to enhance intestinal contraction. Therefore one should not assume that many of these drugs would be effective in the presence of an inflammatory injury such as that which can occur after intestinal manipulation at surgery or that associated with duodenitis-proximal jejunitis.

Bethanechol is a parasympathomimetic agent that acts at the level of the myenteric plexus and directly on intestinal smooth cells through muscarinic receptors. Bethanechol is a synthetic ester of acetylcholine and is not degraded by anticholinesterase. Bethanechol has cholinergic side effects, including abdominal discomfort, sweating, and salivation, although these are minimal when the drug is administered at 0.025 mg/kg body mass subcutaneously or orally. Bethanechol has efficacy in diseases that involve abnormal gastric emptying and delayed small intestinal transit and has been shown to increase gastric contractility and hasten the emptying of liquid and solid phase markers from the stomach of normal horses.[46–47] Bethanechol also increases the strength and duration of wall contractions in the cecum and right ventral colon and consequently speeds up cecal emptying. Neostigmine increases receptor levels of acetylcholine by inhibiting cholinesterase. The drug (0.022 to 0.025 mg/kg intravenously) promotes cecal and colonic contractile activity and hastens the emptying of radiolabeled markers from the cecum.[24] Neostigmine has been used to manage small intestinal ileus, but it significantly delayed the emptying of 6-mm beads from the stomach of normal adult horses.[48]

Metoclopramide acts principally as a 5-hydroxytryptamine 4-receptor (5HT-4) agonist and 5HT-3–receptor antagonist. In contrast to newer generation benzamides, metoclopramide is also an antagonist at dopamine 1 (DA_1) and 2 (DA_2) receptors. Antagonism of prejunctional DA_2 receptors facilitates acetylcholine release and smooth muscle contraction. Metoclopramide crosses the blood-brain barrier, where its antagonist properties on central DA_2 receptors can result in extrapyramidal signs, including seizure. These signs were responsible for poor acceptance of the drug in equine practice. Most investigators have failed to demonstrate significant effects of metoclopramide in experimental animals, but constant intravenous infusion (0.04 mg/kg/hr) in a population of postoperative horses significantly decreased the volume and duration of gastric reflux over control and intermittent drug infusion groups.[49] Infusion was well tolerated and appeared to be superior to intermittent infusion or no treatment at all.

Cisapride is a second-generation benzamide that acts as a 5HT-4 agonist and 5HT-3 receptor antagonist but is without antidopaminergic action. Stimulation of 5HT-4 receptors within the enteric nervous system enhances release of acetylcholine from the myenteric plexus. Several reports suggest the efficacy of cisapride in managing intestinal disease in horses, including the resolution of persistent large colon impaction, treatment of equine grass sickness, and as a preventative for POI in horses after small intestinal surgery (0.1 mg/kg body mass intramuscularly during the postoperative period).[50–53] The horse erratically absorbs tablets administered rectally, but a method for preparing a parenteral form of the drug from tablets has been described.[54] Cisapride has the potential to cause adverse cardiac side effects mediated through blockage of the rapid component of the delayed rectifier potassium current that include lengthening of the QT interval and development of torsades de pointes, a potentially fatal arrhythmia. These adverse effects have resulted in withdrawal of the drug in the United States.

Domperidone acts as a competitive antagonist at peripheral DA_2 receptors. The drug is a therapeutic agent (1.1 mg/kg/day) for mares grazing endophyte-infected tall fescue, principally because of drug-enhanced prolactin release. The potential prokinetic effects of domperidone have not been studied extensively in horses, but a modest efficacy of domperidone (0.2 mg/kg intravenously) has been demonstrated in experimental ileus in two ponies.

Erythromycin is a direct motilin receptor agonist on smooth muscle cells and also may act within the enteric nervous system to facilitate the release of acetylcholine and motilin. Erythromycin enhances gastric emptying in normal horses but has a more pronounced effect on the hindgut.[47,55] Erythromycin lactobionate (1.0 mg/kg intravenously) hastens cecal emptying in normal animals and induces colonic MMC-like activity across the colon. Administration often is associated with defecation and abdominal discomfort. The drug may help prevent cecal impaction in horses after anesthesia, although its effectiveness on cecal motility in the immediate postoperative period may be reduced.[36] High doses, constant infusion, or prolonged use of erythromycin induces receptor downregulation and inhibition of activity. Erythromycin can induce diarrhea in adults, therefore one should avoid dosing over many days.

Naloxone (0.05 mg/kg intravenously) induces contractile activity in the cecum and left colon.[56] Defecation commonly follows administration of naloxone within 15 to 20 minutes.

α_2-Adrenoreceptor antagonists such as yohimbine or tolazoline counteract increased sympathetic outflow in response to nociceptive stimulation. Yohimbine infusion (75 µg/kg) also may attenuate the negative effects of endotoxin on motility.[32]

Intravenous infusion of lidocaine may suppress primary afferent neurons, thereby limiting reflex efferent inhibition of motility. An infusion dose of 15 to 20 mg/min over 5 to 6 hours has been recommended for horses. 819

Lidocaine infusion is associated with reversible side effects that include muscle fasciculations, ataxia, and seizure. Consequently, the rate of infusion requires close monitoring. 820

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13.7

13.7—Endotoxemia

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13.7.1

Introduction and Definitions

Endotoxemia is defined as the presence of endotoxin in the bloodstream. Most often, however, the term is used to refer to the associated clinical manifestations caused by an overshooting inflammatory reaction. In its pathophysiologic consequences the innate immune response to endotoxin (lipopolysaccharide) is similar to the response to other stimuli; for example, overwhelming bacterial infection, viral infection, or severe trauma. The term *systemic inflammatory response syndrome* therefore was introduced to describe a general systemic inflammatory process independent of cause. *Sepsis* is defined as the “systemic inflammatory response to infection,” and *septic shock* as “sepsis-induced hypotension, persisting despite adequate fluid resuscitation, along with the presence of hypoperfusion abnormalities or organ dysfunction.”[1] According to these definitions the

diagnosis of sepsis requires documentation of infection by culture in addition to two or more of the following findings: hypo- or hyperthermia, tachycardia, tachypnea or hypocapnia, and leukocytosis, leukopenia, or an increased proportion of immature leukocyte forms. Organ failure is a common sequela of endotoxic or septic shock, and the term *multiple organ dysfunction syndrome* describes insufficiency of two or more organ systems, as evident by clinical or clinicopathologic changes. In horses one should include the laminae of the feet in the list of organs susceptible to failure.

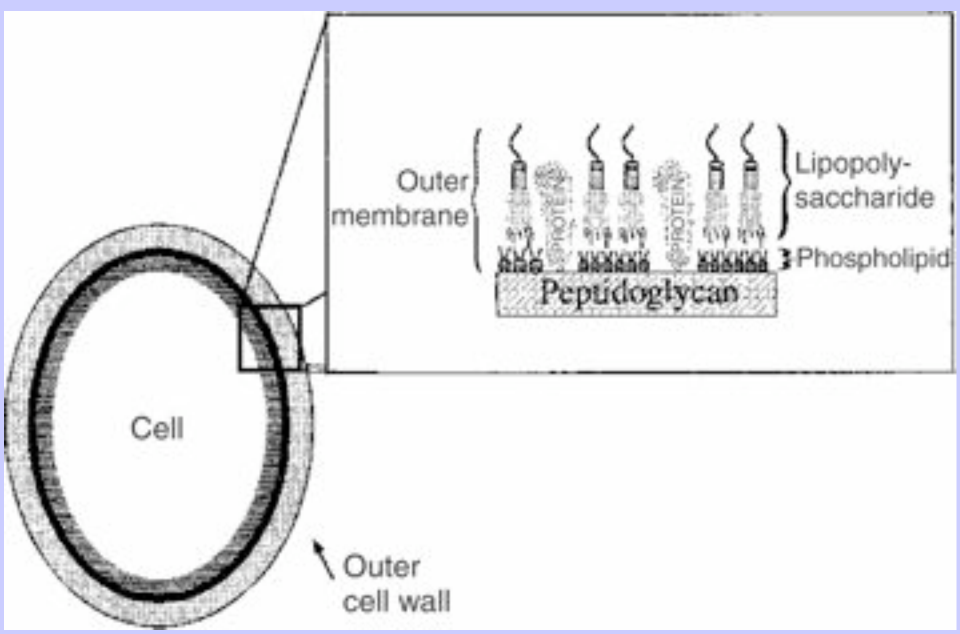
13.7.2 Endotoxin

German scientist Richard Pfeiffer (1858–1945), in working with *Vibrio cholerae,* first described endotoxin as a toxin "closely attached to, and probably integral of, the bacterial body."[2] He observed this toxin to be distinct from the actively secreted, heat-labile, and proteinaceous bacterial exotoxins. Endotoxin later was found to be a heat-stable lipopolysaccharide structure, and the terms *endotoxin* and *lipopolysaccharide* now are used interchangeably.

Lipopolysaccharide is a major structural cell wall component of all gram-negative bacteria, including noninfectious species (Figure 13.7-1). With 3 to 4 × 10[6] molecules per cell, lipopolysaccharide makes up about 75% of the outer layer of the outer cell membrane and is a key functional molecule for the bacterial outer membrane, serving as a permeability barrier against external noxious agents. The lipopolysaccharide molecule 821

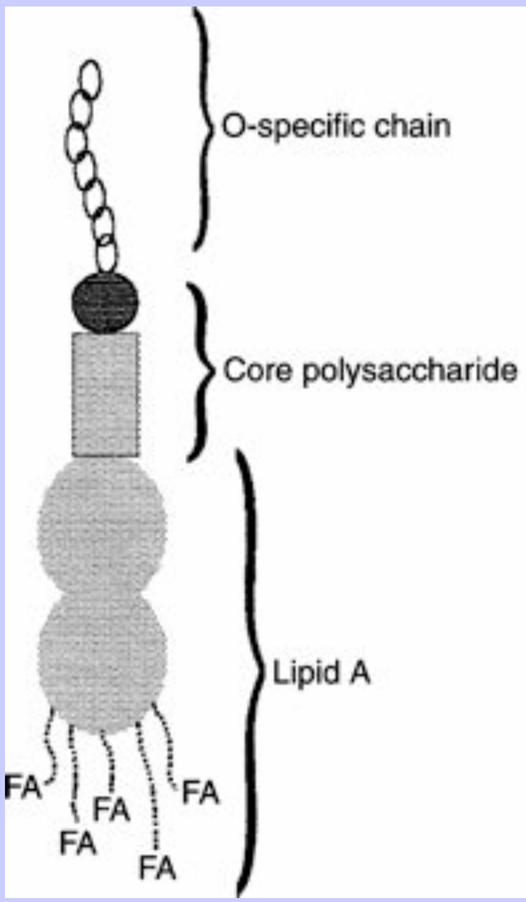
822 consists of four domains, which are essential for the virulence of gram-negative bacteria.[3] Three of the domains (inner core, outer core and O-specific chain) represent the hydrophilic polysaccharide portion of the molecule, whereas the lipid A portion represents the hydrophobic lipid portion (Figure 13.7-2). Combined, these domains confer the overall amphiphilic properties of the molecule that lead to the formation of micellar aggregates in aqueous solutions.

Figure 13.7-1



O-specific chains (also called O-antigen polysaccharides or O-chains) are characteristic of any given type of lipopolysaccharide and show enormous structural variability between bacterial serotypes.[4] O-chains are synthesized by addition of preformed oligosaccharide blocks to a growing polymer chain and therefore have a repetitive structure. O-specific chains determine part of the immunospecificity of bacterial cells[5] and, on interaction with the host immune system, serve as antigens for the production of species-specific antibodies.[6] O-specific chains are further responsible for the smooth appearance of gram-negative bacterial colonies on culture plates,[3] and lipopolysaccharide molecules containing an O-chain are termed *smooth lipopolysaccharide*.

Figure 13.7-2



The inner (lipid A-proximal) and outer (O-chain-proximal) core oligosaccharide portion is more conserved between different strains of gram-negative bacteria than the O-specific chain.[4] The core of all lipopolysaccharide molecules contains the unusual sugar KDO (3-deoxy-D-manno-oct-2-ulopyranosonic acid), which links the core region to the lipid A molecule. Synthesis of a minimal core is essential for the survival of bacteria,[7] and the smallest naturally occurring lipopolysaccharide structure consists of lipid A and KDO.[8] In contrast to the S-form colonies, colonies of gram-negative bacteria with lipopolysaccharide molecules that lack the O-specific chai n but contain a core region show a rough appearance on culture plates. Rough lipopolysaccharide molecules are denoted further as Ra, Rb, etc. to indicate the length of the core region. In Re-lipopolysaccharide (also called

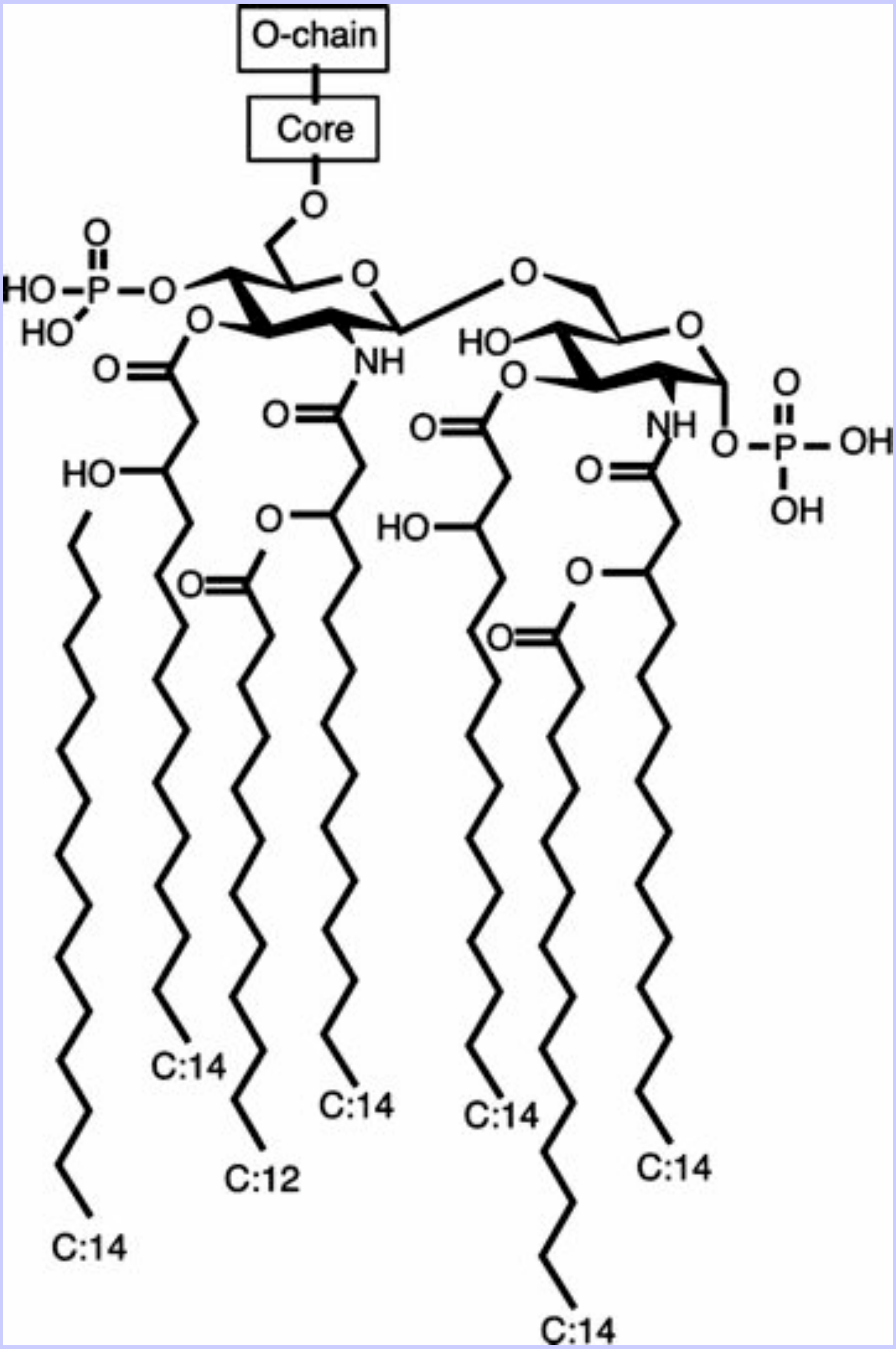
deep rough lipopolysaccharide), the core region is reduced to a KDO residue. Remutants often are used to raise antibodies against the core region in an attempt to provide cross-protection against a variety of bacterial species.

The lipid A portion serves to anchor the lipopolysaccharide molecule in the bacterial outer membrane and has been identified as the toxic principle of lipopolysaccharide,[9] and its structure is highly conserved among gram-negative bacteria. The common structure shared by lipid A molecules is a 1,4′-bisphosphorylated β1,6-linked D-glucosamine disaccharide backbone (lipid A backbone), which is acylated by up to six fatty acids.[4] Figure 13.7-3 shows the acylation pattern for *Escherichia coli* lipopolysaccharide. Variation in the lipid A structure between gram-negative bacteria affects the number, length, and position of fatty acids and the backbone structure and the substitution of phosphate by other polar groups.[6]

13.7.3 Causes of Endotoxemia in Horses

According to its nature as a structural cell wall component, the presence of endotoxin implies the presence of gram-negative bacteria as a source. Depending on the nature of the underlying disease, these bacteria may circulate in the bloodstream in their intact form (i.e., bacteremia), may be confined to a localized infectious process, or may be part of the endogenous bacterial flora colonizing the gastrointestinal tract. In any of these scenarios, endotoxin molecules are released as a by-product of bacterial growth and in large numbers on bacterial
cell death.[10] Common infectious conditions associated with endotoxemia in horses include neonatal gram- 822
negative sepsis, bacterial pneumonia and pleuropneumonia, endometritis, peritonitis, and infectious colitis with 823
bacteria such as *Salmonella* spp., that are not part of the normal intestinal flora. In one study, for example, endotoxin was detectable in plasma of 50% of foals evaluated for presumed sepsis.[11]

Figure 13.7-3 Chemical structure of the lipid A backbone. *C:14,* Myristic acid, *C:12,* lauric acid.



The term *translocation* describes entry of endogenous bacteria and bacterial products from the gastrointestinal tract into tissues and the systemic circulation.[12] The natural intestinal flora of horses consists mainly of gram-negative, anaerobic bacteria, and thus large amounts of endotoxin normally exist in the lumen of the equine intestinal tract.[13] Even in health, small amounts of endotoxin cross the intact mucosal barrier and reach the portal circulation and the liver. These molecules are cleared, however, by the mononuclear phagocytic system in the liver and only lead to a localized and restricted activation of the host immune system. For endotoxin translocation to become detrimental, excessive amounts have to cross the intestinal barrier and overwhelm the mononuclear phagocytic system or the capacity of the liver to detoxify lipopolysaccharide must be compromised. The latter may be a concern in conditions such as hepatitis, cholangiohepatitis, or portosystemic shunting of blood.

Permeability of the intestinal mucosal barrier frequently increases in cases of acute gastrointestinal disease. Colic patients are prime candidates to development endotoxemia, and plasma endotoxin was detectable in 10% to 40% of colic patients on admission.[14,15] A higher percentage of horses tested positive for endotoxin when only patients presented for surgical intervention were evaluated.[15] Aside from gastrointestinal rupture, increased permeability to intact bacteria or free endotoxin molecules is thought to be associated most commonly with ischemic insults such as strangulating obstruction and bowel infarction, severe inflammation as in proximal enteritis and colitis, bacterial overgrowth, and intraluminal acidosis, which occurs with grain overload.[16,17] One study, however, found no difference in plasma endotoxin detection between disease groups, therefore emphasizing the fact that any disease of the abdominal cavity can induce endotoxemia in horses. In the same study, endotoxin was approximately 3 times more likely to be detected in peritoneal fluid as opposed to plasma samples. Similarly, higher cytokine concentrations have been measured in peritoneal fluid than in plasma. The likely explanation for these findings is a local inflammatory response in the peritoneal cavity elicited by translocated bacteria and/or lipopolysaccharide molecules before their absorption into the systemic circulation.[14]

Although certainly the most important factor in horses, conditions other than gastrointestinal disease may result in translocation of endotoxin and bacteria. In experimental studies using laboratory animals, entry of gut-associated bacteria into the lymphatic system was demonstrated after hypovolemic shock, burn injuries, trauma, malnutrition, and starvation.[18–20] Furthermore, endotoxin itself caused bacterial translocation into mesenteric lymph nodes after intraperitoneal administration to mice.[21] These findings have received much attention in the literature concerning human patients because they serve to explain cases of endotoxic shock in the absence of demonstrable bacterial infection. One should keep in mind the possibility of translocation when evaluating cases of presumed systemic inflammatory response syndrome in horses, in which one cannot demonstrate bacterial infection or gastrointestinal disease. Endotoxin translocation also may be associated with strenuous exercise, which results in reduced splanchnic blood flow, hypoxemia, and a higher body temperature. In fit racehorses a significantly increased mean plasma lipopolysaccharide concentration was found after racing, whereas antilipopolysaccharide immunoglobulin G levels were decreased. Fit horses showed significantly higher 823
antilipopolysaccharide immunoglobulin G concentrations at rest than sedentary controls, suggesting leakage of 824
small amounts of endotoxin from the intestinal lumen during training and racing.[22] The clinical significance of these findings requires further investigation.

13.7.4 Mechanisms of Cellular Activation by Lipopolysaccharide

The initiating event in the pathophysiology of endotoxemia is the activation of lipopolysaccharide-responsive cells by endotoxin, resulting in altered cellular functions and increased expression of inflammatory mediators.

Immune cells such as macrophages, which are the first to encounter endotoxin, respond to minute amounts of lipopolysaccharide, which usually allows them to eliminate gram-negative bacteria and free lipopolysaccharide molecules efficiently. An important factor in the exquisite sensitivity to lipopolysaccharide is the presence of lipopolysaccharide-binding protein (LBP).[4] LBP is an approximately 60-kd plasma glycoprotein[23] synthesized by hepatocytes[24] and belongs to the group of acute phase proteins. Under the control of inflammatory agents and cytokines, LBP concentration in plasma increases approximately 100-fold within 24 hours of an inflammatory stimulus.[25] The main function of LBP is to transfer lipopolysaccharide to endotoxin-responsive cells, which include mononuclear phagocytes, neutrophils, lymphocytes, and endothelial cells. The importance of a highly sensitive response to lipopolysaccharide for protection against gram-negative bacterial infection is demonstrated in experiments using LBP “knock-out” mice (mice that lack the LBP gene and are therefore unable to synthesize LBP). Although these animals are resistant to the effects of isolated lipopolysaccharide, they are unable to control bacterial infection and rapidly succumb.[26] Despite its crucial importance for an effective host defense, LBP is not essential for lipopolysaccharide-receptor interaction per se, because high concentrations of lipopolysaccharide can activate cells in the absence of LBP.[27]

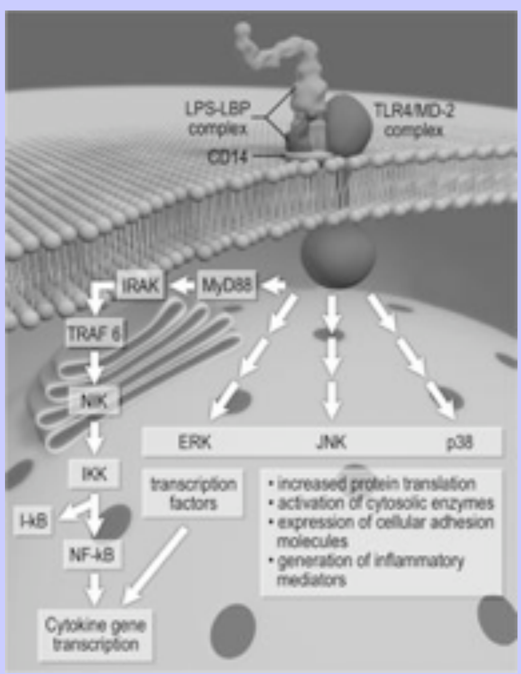
Aside from its role as a catalyst of cellular activation by lipopolysaccharide, LBP has opsonizing activity[28] and participates in the phagocytosis of lipopolysaccharide by macrophages and neutrophils.[29,30] Although phagocytosis of lipopolysaccharide is receptor dependent, it appears to be uncoupled from intracellular signaling events and occurs in the absence of cell activation.[31] LBP further catalyzes transfer of lipopolysaccharide to lipoproteins such as high-density lipoprotein, which neutralizes lipopolysaccharide activity.[32] This detoxifying effect may become important when large amounts of lipopolysaccharide are present. A protective effect of LBP against lipopolysaccharide challenge and infection has been demonstrated in a murine model.[33]

The most important lipopolysaccharide receptors known to date are cluster differentiation antigen 14 (CD14)[27] and Toll-like receptor 4 (TLR4).[34] Both are classified as pattern recognition receptors,[35] which means that they recognize lipopolysaccharide as a pattern common to all gram-negative bacteria. CD14 is a 53-kd protein that in its membrane-bound form (mCD14) is inserted into the cell membrane via a glycosyl-phosphatidyl-inositol anchor.[36] CD14 is expressed primarily on monocytes and tissue macrophages and to a lesser extent on neutrophils.[37] CD14 also is found in a free, or soluble, form (sCD14)[38] that can bind to cell types lacking CD14, such as endothelial cells, and make them lipopolysaccharide-responsive. In addition to this proinflammatory effect, high concentrations of sCD14 can sequester and neutralize lipopolysaccharide.[39] The amount of circulating sCD14 greatly increases during inflammation, which makes it a useful marker of acute and chronic inflammation.[37]

Although CD14 is known to be crucial for cellular activation, it cannot transmit signals to the inside of the cell because it lacks a transmembrane domain. The missing link between CD14 and the cytosolic environment is a Toll-like receptor in association with a molecule named MD-2.[40] The name *Toll-like receptor* stems from the homology of the mammalian receptor with a receptor type in *Drosophila* (Toll) that is important for dorsoventral orientation and immune responses in the fly. A number of Toll-like receptors have been identified in mammalian species so far, but TLR4 appears to be the receptor subtype most important for lipopolysaccharide signaling.[34] The importance of CD14 and TLR4 in the cellular response to lipopolysaccharide has been demonstrated in a number of experiments. Mice deficient in CD14 are incapable of mounting a normal inflammatory response to lipopolysaccharide,[39] whereas mutation or deletion of the gene encoding for TLR4 causes lipopolysaccharide hyporesponsiveness.[41–43]

After binding of lipopolysaccharide to cellular receptors, a multitude of signaling events takes place within the
cell and results in the alterations of cellular metabolism known as cell activation. Signaling pathways are
characterized by sequential phosphorylation and thereby activation of enzymatic activities. A typical end result
of intracellular signaling is the activation of transcription factors; for example, proteins that bind to DNA and
promote gene transcription. Translational mechanisms are activated in a similar manner. Among the best-
characterized pathways in endotoxin-induced cell signaling are the mitogen-activated protein kinase (MAPK)
pathways and the activation of transcription factor nuclear factor κB (NFκB) (Figure 13.7-4).[44,45] In the NFκB
pathway the intracellular domain of TLR4 associates with the adapter protein myeloid differentiation factor 88 824
and recruits interleukin-1 receptor-associated kinase to the complex. Activation of interleukin-1 receptor- 825
associated kinase, tumor necrosis factor receptor-associated factor, NFκB-inducing kinase, and IκB-kinase
follow, and lastly, IκB is phosphorylated. IκB is an inhibitor protein complex that sequesters and inactivates
NFκB in the cytoplasm. On phosphorylation, IκB is ubiquinated and degraded, and NFκB is translocated to the
nucleus where it unfolds its activity.[46] NFκB is a dimeric protein complex with several isoforms of which the
p65/p50 heterodimer is the most important inducible complex in mammals.[47] Proteins of importance for the 825
pathogenesis of septic shock, the genes of which contain promoter elements for NFκB, include cytokines, 826
inducible nitric oxide synthase, and cyclooxygenase 2 (COX-2).[44]

Figure 13.7-4 Mechanisms of cell activation by endotoxin. Lipopolysaccharide-binding protein *(LPB)* associates with lipopolysaccharide (*LPS*, endotoxin) and transfers it to the cellular surface, where lipopolysaccharide interacts with a receptor complex comprising CD14, Toll-like receptor 4 *(TLR4)*, and MD-2. Toll-like receptor 4, but not CD14, possesses a transmembrane portion and allows signal transduction to the cytosol. Signaling via the mitogen-activated protein kinases including extracellular signal-regulated kinase *(ERK)*, c-Jun-terminal kinase *(JNK)*, and p38 results in numerous alterations of cellular metabolism. Activation of IκB-kinase *(IKK)* via sequential phosphorylation of myeloid differentiation factor 88 *(MyD88)*, interleukin-1 receptor-associated kinase *(IRAK)*, tumor necrosis factor receptor–associated factor *(TRAF 6)*, and NFκB-inducing kinase *(NIK)* leads to phosphorylation, ubiquination, and degradation of IκB and release of NFκB. Transcription factors such as NFκB translocate to the nucleus and promote gene transcription. See the text for further details and alternative pathways of cellular activation.



Three groups of MAPKs known to be crucially important for lipopolysaccharide-induced signal transduction are extracellular signal-regulated kinase, c-Jun-terminal kinase, and p38. Final effects of signaling through MAPK pathways include the activation of several transcription factors, translation initiation factors, and cytosolic enzymes such as phospholipase A_2, as well as an increase in the expression of adhesion molecules on the cell surface.[44] Despite the characterization of seemingly separate pathways, one should recognize that interaction and synergy between pathways is likely to occur. For example, simultaneous activation of p38, c-Jun-terminal kinase, and extracellular signal-regulated kinase results in much higher levels of tumor necrosis factor (TNF) reporter expression than activation of a single pathway.[44,48] Aside from the mechanisms described here, pathways involving atypical protein kinase C[49,50] and receptor-independent integration of lipopolysaccharide into the cell membrane and ceramide-like second messenger activity of lipopolysaccharide[37] have been proposed. Additional pathways are likely to be uncovered in the ongoing investigation of intracellular signaling mechanisms and their in vivo significance.

13.7.5 Inflammatory Mediators

Although endotoxin can exert some direct effects, cytokines are a primary mediator of lipopolysaccharide effects. Cytokines are glycoprotein molecules that regulate inflammatory and immune responses by acting as a signal between cells.[51] Cytokines of major interest in the pathogenesis of endotoxemia include TNF, the interleukins, chemokines, and growth factors such as granulocyte-monocyte colony-stimulating factor. TNF is thought of as the most “proximal” cytokine released in response to lipopolysaccharide. Studies corroborate this by showing that administration of recombinant TNF mimics the effects of lipopolysaccharide,[52] and that antibodies directed against TNF protect against the lethal effects of endotoxin.[53] Increased plasma activity of TNF is associated with increased mortality in equine patients with acute gastrointestinal disease and in septic neonates.[14] Despite being a structurally diverse group of proteins, cytokines share several characteristics that allow them to execute their complex functions in the inflammatory response.[51] Any individual cytokine generally is produced by several different cell types, can act on different cell types, and has multiple effects on any given cell. Furthermore, cytokine effects are redundant, meaning that different cytokines can share the same effect. In endotoxemia, this is particularly true for the effects of interleukin-1 (IL-1) and TNF.[54] Many of the biologic activities of cytokines in vivo result from synergistic or antagonistic actions involving two or more cytokines.[55] Within itself the cytokine response is highly regulated: cytokines induce or suppress synthesis of other cytokines including their own (feedback regulation), regulate expression of cytokine receptors, and regulate cytokine activities. Additional regulatory mechanisms include the release of specific cytokine inhibitors such as soluble IL-1 and TNF-α receptors, cytokine receptor antagonists such as IL-1 receptor antagonist, and antiinflammatory cytokines including IL-10, IL-4, IL-13, and transforming growth factor β. Glucocorticoids also are produced increasingly in response to endotoxin and inhibit the production of cytokines.[56] During a controlled inflammatory response, therefore, cytokine secretion is a self-limited event, whereas excessive stimulation of cytokine release can lead to the perpetuation of the inflammatory response even after the initial stimulus has been removed. Conversely, the compensatory antiinflammatory reaction can become severe enough to cause anergy of the immune system and increased susceptibility to infection, which has been termed the *compensatory antiinflammatory response syndrome*. Overall, excessive and unbalanced stimulation of the immune system therefore may result in predominantly proinflammatory (systemic inflammatory response syndrome), antiinflammatory (compensatory antiinflammatory response syndrome), or combined (mixed antagonist response syndrome) responses.[57]

Interestingly, tolerance to endotoxin develops after repeated exposure to lipopolysaccharide.[58] Tolerance can be demonstrated in vitro and in vivo and encompasses decreased production of cytokines and a diminished clinical response.[58,59] Mechanisms that likely are responsible for the development of endotoxin tolerance include receptor downregulation and inhibition of intracellular signaling pathways.[60,61] Cytokines such as TNF are important mediators in the development of endotoxin tolerance.[62] The development of endotoxin tolerance in horses has been reported.[63,64]

Aside from cytokines, a number of other molecules function as inflammatory mediators in the pathogenesis of endotoxemia, the synthesis and release of which are stimulated by endotoxin and by cytokines. These mediators include the arachidonic acid metabolites or prostanoids, platelet-activating factor (PAF), oxygen-derived free radicals, nitric oxide (NO), histamine, kinins, and complement components. Table 13.7-1 summarizes the origins, targets, and effects of the most important inflammatory mediators involved in the pathogenesis of endotoxemia. Figure 13.7-5 shows the pathways of arachidonic acid metabolism by COX and lipoxygenase. COX products are the prostaglandins (PGs), prostacyclin (PGI_2) and thromboxanes, and the lipoxygenase produces the leukotrienes. 826

TABLE 13.7-1 Important Mediators of the Systemic Inflammatory Response to Endotoxin

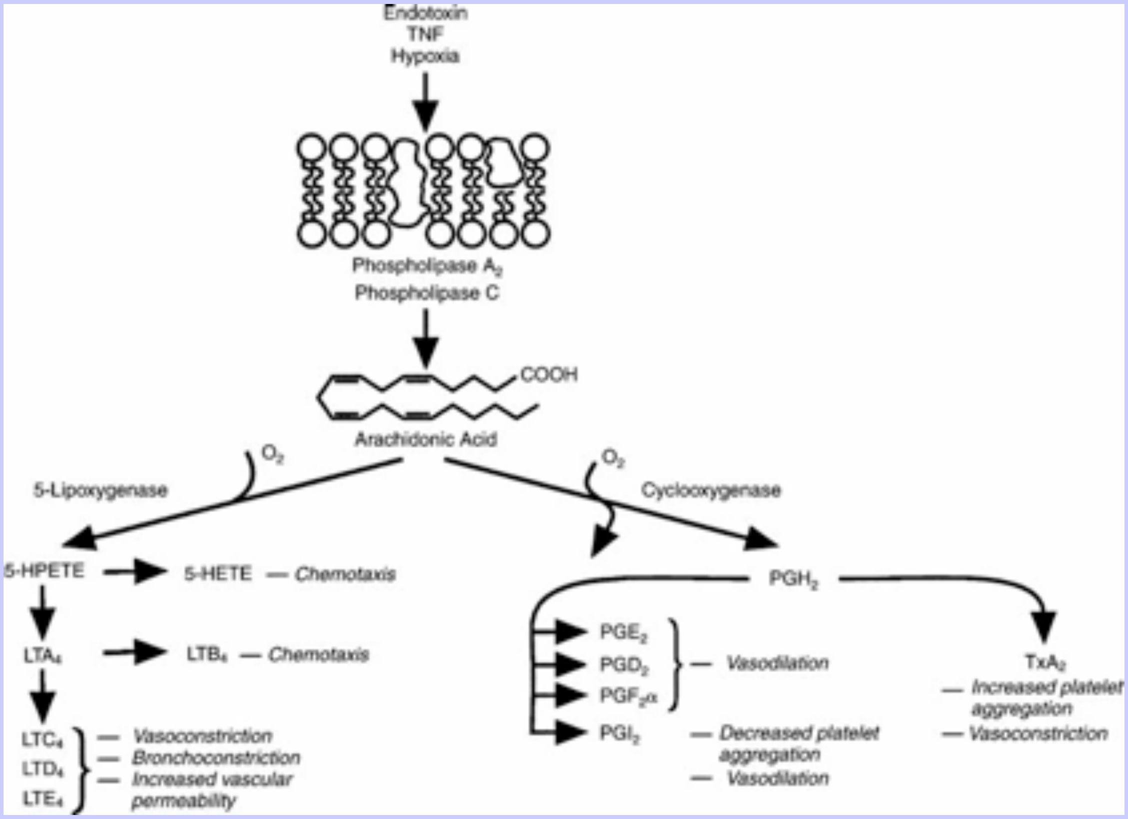
MEDIATOR	ORIGIN	EFFECTS
Tumor necrosis factor	Macrophages	Induces synthesis of TNF, IL-1, IL-6, and GM-CSF.*
	Monocytes	Activates neutrophils.
	Neutrophils	Activates fibrinolysis and coagulation.
	$CD4^+$ T cells	Activates contact and complement system.
	Natural killer cells	Induces a catabolic state.
		Induces insulin resistance.
		Is a pyrogen (direct action and via IL-1 induction).
		Induces synthesis of TNF, IL-1, IL-6, PGI_2, PAF, and GM-CSF.
Interleukin-1	Activated macrophages	Activates pyrogen.
	Endothelial cells	Induces malaise.
	Fibroblasts	Activates neutrophils and chemotaxis.
	Dendritic cells	Activates fibrinolysis and coagulation.
	Lymphocytes	Activates contact and complement system.
	Keratinocytes	Induces acute phase response.
		Increases activity of lipoprotein lipase.
		Mobilizes amino acids.
		Induces muscle proteolysis.
Interleukin-6	Activated macrophages	Induces acute phase response.
	Fibroblasts	Induces stress response.
	Keratinocytes	Is a weak pyrogen.
	T lymphocytes	
Interleukin-8	Macrophages	Activates neutrophils and chemotaxis.
	Endothelial cells	
Thromboxane A_2	Platelets	Induces vasoconstriction.
		Activates platelet aggregation.
Prostaglandin E_2	Most nucleated cells	Induces vasodilation.
		Activates platelet aggregation.
		Induces fever.

Prostaglandin I_2	Vascular endothelial cells	Induces vasodilation.
		Inhibits platelet aggregation.
Platelet-activating factor	Macrophages	Activates platelet aggregation.
	Platelets	Activates macrophages and neutrophils.
	Neutrophils	Induces hypotension.
	Mast cells	Increased vascular permeability.
	Eosinophils	Aids recruitment of leukocytes.
		Induces visceral smooth muscle contraction.
		Is a negative inotrope and arrhythmogenic.
		Induces ileus.
Prostaglandin $F_{2\alpha}$	Most nucleated cells	Induces vascoconstriction.
		Activates luteolysis.
Leukotriene B_4	Most nucleated cells	Is a chemoattractant.
		Promotes neutrophil interaction with endothelial cells.
Leukotrienes C_4, D_4, E_4	Most nucleated cells	Increase vascular permeability.
		Induce bronchoconstriction.
		Induce vasoconstriction.
Kinins	Produced from serum precursors	Increase vascular permeability.
		Induce smooth muscle contraction.
		Cause pain.
Complement components (C3a, C5a)	—	Activate neutrophils and chemotaxis.
		Induce smooth muscle constriction.
		Induce mast cell degranulation.
		Induce release of histamine and serotonin.
		Increase vascular permeability.
Oxygen-derived free radicals	Macrophages	Damage cell membranes.
	Neutrophils	Inactivate enzymes.
		Damage tissues.
Granulocyte-monocyte colony-stimulating factor	—	Induces rebound neutrophilia.

* *TNF,* Tumor necrosis factor; *IL,* interleukin; *GM-CSF,* granulocyte-monocyte colony-stimulating factor; *PG,* prostaglandin; *PAF,* platelet-activating factor.

827

Figure 13.7-5



13.7.6

Pathogenesis

The innate immune response to lipopolysaccharide is an efficient defense mechanism that provides maintenance of homeostasis and therefore health in the face of an almost continuous exposure to microorganisms and their products.[65] Detrimental consequences of this immune response only occur if excessive and uncontrolled mediator output results in endothelial damage, neutrophil-mediated tissue damage, and uncontrolled activation of the coagulation and fibrinolytic cascades and the complement system. Ultimately, the combination of these events culminates in cardiovascular instability, impaired hemostasis, organ failure, shock, and death. The following discussion addresses the various pathophysiologic events in the development of endotoxemia and shock and the role of inflammatory mediators.

13.7.6.1

ENDOTHELIAL DYSFUNCTION AND DAMAGE

Normal endothelium plays an important role in regulating blood pressure and regional tissue perfusion and provides an anticoagulant surface. Endothelial dysfunction and damage result in a decreased responsiveness to vasoactive agents (vasoplegia), increased vascular permeability, and a tendency for clot formation in the microvasculature. If the basement membrane and underlying matrix are compromised, further microvascular hemorrhage can occur. Endothelial cell damage is primarily neutrophil-mediated. More specifically, damage is

caused by oxygen-derived free radicals, which are produced within endothelial cells via reactions involving neutrophil-derived elastase and hydrogen peroxide molecules, endothelial cell enzymes such as xanthine oxidase, and endothelial cytosolic iron. The hypochloric anion radical (HO$^{\cdot}$) is thought to be responsible most directly for endothelial cell cytotoxicity. NO$^{\cdot}$ produced by constitutively expressed nitric oxide synthase in endothelial cells may afford protection from oxygen radical–induced endothelial cell damage. NO is able to scavenge superoxide radicals and react with them to form peroxynitrite. Variations in the ability to produce NO may explain why vascular beds in different organs vary in their susceptibility to neutrophil-mediated 828
damage.[66] Excessive production of NO by an inducible form of nitric oxide synthase (*i*NOS), however, 829
contributes to tissue damage, and increased peroxynitrite concentrations may be responsible in part for PAF-induced increases in vascular permeability.[67] In addition to oxygen-derived free radicals, activated neutrophils release matrix metalloproteinases, which contribute to tissue damage.[56] Vascular endothelial cells are further susceptible to direct effects of various cytokines, most prominently TNF-α and IL-1. These cytokines are thought to cause damage via the induction of COX activity and production of prostanoids and through generation of free radicals.

13.7.6.2 NEUTROPHIL ACTIVATION, MARGINATION, AND TRANSMIGRATION

Neutrophil activation by lipopolysaccharide and cytokines results in stimulation of phagocytosis and the respiratory burst, release of lysosomal enzymes and inflammatory mediators, and expression of adhesion molecules. Perhaps the single most specific clinicopathologic indicator of endotoxemia is pronounced neutropenia,[68] which temporally correlates with peak plasma concentrations of TNF.[69] Neutropenia is caused primarily by margination of neutrophils in the vasculature, whereas significant loss through active migration into peripheral tissues likely is limited to the presence of a localized source of infection. Margination is made possible by adhesion molecules on endothelial cells and leukocytes that interact and allow sticking of leukocytes to the endothelial lining of blood vessels. Endotoxin or cytokines such as TNF and IL-1 can initiate expression of adhesion molecules.[70] Subsequent transmigration of cells into tissue spaces is aided by the production of leukocyte collagenase, which allows enzymatic destruction of the vascular basement membrane. Margination and transmigration of neutrophils occurs in three phases.[70,71] In the first phase of tethering and rolling, endothelial cells are stimulated to express P-selectin and E-selectin, which bind to P-selectin glycoprotein ligand-1 and sialylated Lewis-X–like structures on leukocytes, respectively. Whereas P-selectin is stored preformed in Weibel-Palade bodies of endothelial cells, E-selectin is expressed only following stimulation by cytokines. Additionally, constitutively expressed L-selectin on neutrophils can bind to endothelial glycoproteins and glycolipids. During the second phase, firm adhesion is mediated by binding of neutrophil integrins (LFA-1 and Mac-1, also known as CD11a/CD18 and CD11b/CD18) to intercellular adhesion molecule 1 (an immunoglobulin structure) on endothelial cells. Although integrins are expressed constitutively on the leukocyte surface, activation signals are necessary to induce a high-affinity state and interaction with the endothelial surface. Transmigration finally requires the expression of yet another adhesion molecule, namely platelet/endothelial cell adhesion molecule 1, which is located at the intercellular junction of endothelial cells.[71] Chemotactic factors including activated complement factor C5a and the CXC chemokines control transmigration.[66] The latter group includes IL-8, which is expressed by endothelial cells in response to activation. Rebound neutrophilia, which is observed frequently following episodes of endotoxemia, is caused by neutrophil release from the bone marrow reserve pool and by stimulation of myeloid cell proliferation via granulocyte-macrophage colony-stimulating factor and is mediated primarily by TNF and IL-1.[54]

13.7.6.3

COAGULOPATHY AND DISSEMINATED INTRAVASCULAR COAGULATION

In health, coagulation and fibrinolysis underlie stringent control mechanisms that allow appropriate clot formation and their resolution. Coagulopathies frequently are observed in horses with colic[16,72,73] and foals with sepsis[11] and are likely attributable to endotoxemia. Disseminated intravascular coagulation (DIC) results from a widespread activation of the coagulation and fibrinolytic systems and failure of their control mechanisms. Ultimately, this leads to disseminated fibrin deposition in the microvasculature, consumption of platelets and clotting factors, and accumulation of fibrin degradation products (FDPs). Depending on the underlying disease process and the impairment of the systems, DIC can manifest as a diffuse thrombotic syndrome leading to ischemic organ failure, a fibrinolytic syndrome with uncontrolled hemorrhage, or a combination of both.[74] A procoagulant state in which one can detect clinicopathologic abnormalities precedes DIC.

Activation of the coagulation cascade culminates in the cleavage of fibrinogen to fibrin by the serine protease thrombin. Thrombin deposition on endothelial cell surfaces leads to platelet adherence and their activation by surface-bound PAF.[56] The intrinsic and extrinsic arms of the coagulation cascade are activated in endotoxemia. The intrinsic pathway is initiated by activation of coagulation factor XII (Hageman factor), prekallikrein, and high-molecular-weight kininogen, which compose the contact system.[75] Although direct activation of coagulation factor XII by endotoxin has been demonstrated,[76] the extrinsic pathway likely is more important for the development of coagulopathy in endotoxemia and sepsis.[75] Activation of the extrinsic pathway depends on the interaction of coagulation factor VII with tissue factor, which is the only coagulation factor not constitutively present in blood. Tissue factor is present in subendothelial tissues and is exposed on vascular injury but also is expressed on endothelial cells and mononuclear phagocytes in response to lipopolysaccharide.[77,78] Increased expression of monocyte tissue factor (also described as increased procoagulant activity) was found to be associated significantly with coagulopathy and poor prognosis in 829
horses with colic.[79] Furthermore, lipopolysaccharide-induced tissue factor expression by equine peritoneal 830
macrophages may be associated with the development of intraabdominal adhesions.[63]

Regulatory mechanisms of the coagulation cascade include tissue factor pathway inhibitor, antithrombin III (AT III), and the protein C system.[75] Protein C acts as an anticoagulant by inactivating clotting factors V and VIII and promotes fibrinolysis by inactivating plasminogen activator inhibitor (PAI).[80] Protein C activation by thrombin-thrombomodulin complexes is important for the anticoagulative properties of normal endothelium,[75] and downregulation of endothelial thrombomodulin expression by TNF and IL-1 and decreased expression of AT III and tissue factor pathway inhibitor by damaged endothelial cells contribute to the procoagulant state in endotoxemia and sepsis.[81–83] In addition, activation of vascular endothelial cells leads to a loss of prostacyclin and NO production and an increased release of thromboxane A_2 (TXA_2). As a result, platelets are stimulated to aggregate and release TXA_2 and PAF, thereby further promoting clot formation.[17]

The crucial step in the fibrinolytic cascade is the conversion of plasminogen to plasmin, a fibrin-degrading enzyme.[75] Tissue-type (tPA) and urokinase-type (uPA) plasminogen activator are the major initiators of fibrinolysis, whereas PAI and α_2-antiplasmin are the main regulatory components.[84,85] TNF and IL-1 have been shown to induce the release of uPA and tPA and the synthesis of PAI.[75] Activation of fibrinolysis leads to consumption of α_2-antiplasmin and accumulation of FDPs, which if present in high concentrations can

interfere with platelet aggregation, fibrin polymerization, and thrombin formation and can promote bleeding. Additionally, FDPs mediate an increase in vascular permeability. Lipopolysaccharide infusion in rabbits[86] and human beings[87] resulted in an early increase in plasma tPA activity, followed by a later profound rise in PAI activity and fall in tPA activity. Increased plasma PAI concentrations also were found in horses with colic compared with controls.[88,89] Thus although fibrinolysis may compensate initially for accelerated coagulation, its subsequent inhibition contributes to clot formation.

13.7.6.4 COMPLEMENT ACTIVATION

Activation of the complement system in endotoxemia occurs via the alternative pathway through interaction with lipopolysaccharide. Increased concentrations of plasmin and kallikrein (caused by activation of the fibrinolytic and contact system) further promote this pathway by directly activating complement factors C3a and C5a. Aside from being key molecules in the complement cascade, C3a and C5a are anaphylatoxins and cause an increase in vascular permeability via mast cell degranulation. C5a further activates the lipoxygenase pathway in neutrophils and monocytes, acts as a chemotaxin for leukocytes and monocytes, and promotes neutrophil adhesion to endothelial cells.

13.7.6.5 ACUTE PHASE RESPONSE

In response to acute inflammation, synthesis and secretion of a number of proteins called the acute phase proteins increases in hepatocytes, whereas synthesis of albumin decreases. The primary function of this acute phase response may be to suppress and contain inflammatory responses.[56] IL-6 and IL-1 are the most important cytokines that induce the acute phase response,[90] which typically begins within a few hours of the insult and subsides within 24 to 48 hours,[91] unless the initiating cause persists. In horses, fibrinogen (the most commonly evaluated), haptoglobin, transferrin, ferritin, ceruloplasmin, coagulation factor VIII:C, serum amyloid A protein, C-reactive protein, α_1-acid glycoprotein, and phospholipase A_2 are considered part of the acute phase response.[92] One must consider the effect of acute inflammation on the serum concentration of several coagulation factors when evaluating coagulation profiles. Serum fibrinogen concentration is determined primarily by the acute phase response, although fibrinogen is consumed increasingly on activation of the clotting cascade.

13.7.6.6 HEMODYNAMIC CHANGES, DEVELOPMENT OF SHOCK, AND ORGAN FAILURE

Shock is characterized by a loss of homeostasis attributable to breakdown of hemodynamic control mechanisms, decreases in cardiac output and the effective circulating volume, and inadequate perfusion of vital organs. Shock caused by endotoxemia is classified as distributive shock[93] and is largely initiated by vascular dysfunction in the periphery. Peripheral vascular beds are of major importance for the regulation of local tissue perfusion and affect systemic blood pressure by regulating total peripheral resistance. Normally, vascular smooth muscle tone is regulated by endothelin-1 (vasoconstriction), NO, and prostacyclin (vasodilation) released from vascular endothelial cells.[94] As mentioned before, detrimental effects of NO are attributable to induction of *i*NOS in macrophages and other cell types, rather than endothelial-derived NO. Peripheral vasomotor effects of endotoxin manifest as vasodilation and vasoplegia and are mediated by PGI_2, NO, and mediators such as bradykinin. Widespread vasodilation leads to vascular blood pooling, decentralization of blood flow, decreased venous return, and in effect decreased effective circulating volume and cardiac output.[93] Compensatory responses in the form of an initial hyperdynamic phase include

tachycardia, increased cardiac output and central venous pressure, pulmonary hypertension, peripheral vasoconstriction, and increased peripheral vascular resistance.[93,95,96] The early vasoconstrictive phase corresponds to an increased serum concentration of TXA_2,[17] but additional vasoconstrictors such as arginine vasopressin, angiotensin II, serotonin, endothelin, and norepinephrine likely are implicated in the pathogenesis of shock and organ failure.[56] With progression of disease, the animal enters a stage of decompensated shock and progressive systemic hypotension, which correspond to increased plasma concentrations of prostacyclin, PGE_2 and bradykinin.[17,56] Inadequate blood flow and oxygen delivery to tissues caused by hypotension is confounded by direct myocardial suppression via NO,[93] increased vascular permeability,[17] intravascular microthrombosis, and impaired tissue oxygen extraction[93] and results in progressive metabolic acidosis and inhibition of normal cellular metabolism. 830 831

13.7.7 Clinical Signs and Diagnosis

Quantification of endotoxin in plasma samples is possible. The *Limulus* amebocyte lysate assay is an activity assay based on the endotoxin-sensitive hemolymph coagulation cascade in the horseshoe crab *Limulus polyphemus*. In *Limulus* this reaction is thought to be a defense mechanism against gram-negative infection.[97] Although frequently used as a research tool, the assay is not convenient enough to become a routine clinical test. The clinician therefore must appreciate the primary disease processes associated with a high risk of endotoxemia and rely on clinical signs and clinicopathologic data to achieve a diagnosis. In some cases, endotoxemia may be the first indication of disease or may be the most overt of otherwise subtle clinical manifestations. With colitis or proximal enteritis, for example, one may detect signs of endotoxemia before the development of colic, diarrhea, or gastric reflux, which more specifically indicate the nature of the primary illness. Diseases such as peritonitis or pleuritis, however, may show nonspecific clinical findings including fever, anorexia, and depression. Findings such as neutropenia, which indicate endotoxemia, should urge the clinician to search for a septic process.

In vivo experiments in horses clearly show that many of the clinical signs associated with acute gastrointestinal disease and sepsis are attributable to the activities of lipopolysaccharide and cytokines such as TNF. On administration of sublethal doses of lipopolysaccharide the clinical response can be divided into the early hyperdynamic and the later hypodynamic or shock phases. Clinical signs during the first phase, which begins within 15 to 45 minutes after lipopolysaccharide administration, include anorexia, yawning, sweating, depression, evidence of abdominal discomfort, muscle fasciculation, and recumbency. Heart and respiratory rates increase, and decreased borborygmi suggest ileus. Hyperemia of the mucous membranes and an accelerated capillary refill time indicate the hyperdynamic state.[68] If one administers large amounts of lipopolysaccharide or if exposure is ongoing, depression worsens progressively, anorexia persists, and feces develop diarrheic character. Signs of colic typically abate after the initial stage. Fever develops as a result of direct action of TNF on the thermoregulatory center and IL-1-induced local production of PGE_2 in or near the hypothalamus.[98,99] Because of compromised peripheral perfusion, mucous membrane color changes to brick red or purple, a dark "toxic" line appears, and capillary refill time is prolonged.[68] Inadequate peripheral perfusion and compromised organ function finally characterize the hypodynamic shock phase. Body temperature may become subnormal and the skin, especially on extremities, is cool to the touch. The arterial pulse weakens and venous fill is decreased. Vascular endothelial damage and increased capillary permeability result in a muddy mucous membrane color and diffuse scleral reddening.

Hemostatic abnormalities can manifest in the form of thrombosis, such as of the jugular vein, or increased bleeding tendency with mucosal petechiation or ecchymoses and prolonged bleeding from venipuncture sites.[74]

Bleeding also may occur in spontaneous epistaxis or prolonged hemorrhage after nasogastric intubation.[17] Additional clinical signs typically reflect the development of organ failure. Renal failure and laminitis appear to be common complications of endotoxemia in horses. Renal failure results from ischemic cortical necrosis and acute tubular necrosis caused by coagulopathy-induced afferent arteriolar obstruction. Clinical signs may include oliguria, anuria, or hematuria caused by renal infarction. Laminitis may lead to lameness, increased digital arterial pulsation, increased warmth of the hoof wall, and sensitivity to hoof tester pressure. Other signs of organ failure include icterus, anorexia and depression caused by liver failure,[74] tachypnea and dyspnea caused by pulmonary failure, colic and bleeding caused by ischemia-induced gastrointestinal ulceration and abnormal motility patterns,[17] and persistent tachycardia or cardiac arrhythmia in cases of cardiac failure. In pregnant mares, fetal death and abortion can occur because of increased production of $PGF_{2\alpha}$ and decreased serum progesterone concentrations.[100,101]

13.7.8 Alterations in the Hemogram and Serum Biochemistry Profile

Alterations in the hemogram and serum biochemical profile are nonspecific and mainly reflect the underlying 831
disease process and the occurrence of organ failure. Leukopenia caused by neutropenia may be the most specific 832
indicator of acute bacterial sepsis or endotoxemia.[68] In prolonged cases, an increased proportion of immature neutrophil forms (bands) and toxic changes are observable. Toxic changes resulting from neutrophil activation include vacuolation, cytoplasmic granulation, basophilic cytoplasm, and Döhle's bodies. Because neutropenia occurs early in the development of endotoxemia, it also may be a useful parameter for monitoring horses at risk.[68] On recovery, neutropenia typically is followed by a pronounced rebound neutrophilia.

An elevated hematocrit and total serum protein concentration are evidence of dehydration; however, splenic contraction caused by increased sympathetic stimulation and protein losses also influence these parameters. A normal or only slightly decreased serum protein and albumin concentration in the face of an elevated hematocrit and clinical signs of dehydration should alert the clinician to the possibility of protein loss. Hypoproteinemia and hypoalbuminemia can occur because of loss via the gastrointestinal or urinary tract or with pleural or peritoneal cavity effusion. Increased vascular permeability and edema formation contribute to hypoproteinemia.

Serum electrolyte abnormalities primarily depend on the nature and duration of underlying disease processes and need to be evaluated individually. Common sources of electrolyte loss are gastrointestinal secretions, urine, and sweat; however, severe effusion into body cavities may contribute. In anorexic patients, lack of dietary intake is a confounding factor that warrants consideration. In human patients, gram-negative sepsis frequently is associated with hypocalcemia, more specifically a decrease in serum ionized calcium concentration. Endotoxin is thought to be a causative factor, and proposed mechanisms include acquired parathyroid gland insufficiency, dietary vitamin D deficiency, impaired calcium mobilization, and renal 1α-hydroxylase insufficiency leading to decreased 1,25-hydroxylation of vitamin D. Hypocalcemia in septic human patients was found to be associated with hypotension and poor outcome.[102] In horses with surgically managed gastrointestinal disease, decreased serum ionized calcium concentration was a common finding and was most severe in patients with strangulating or nonstrangulating infarctions. In some horses, ionized calcium concentration decreased further throughout surgery. Treatment with calcium gluconate resulted in normalization of serum ionized calcium concentrations in all cases.[103]

Septic neonatal patients are frequently hypoglycemic. Aside from decreased oral intake and generally increased metabolism, glucose use by the infecting bacteria, inhibition of gluconeogenesis by endotoxin, and insulin-like activity produced by macrophages are responsible for hypoglycemia.[17] Interestingly, experimental endotoxin

administration results in transient hyperglycemia in adult horses,[95] whereas profound hypoglycemia occurs in foals.[104]

One should evaluate coagulation parameters if coagulopathy is suspected. The most significant changes can be expected with severe inflammatory disease such as colitis,[72,73] devitalized intestine as with strangulating obstruction,[73,105] and with increased duration of disease. In 30 horses with acute gastrointestinal disease, coagulation profiles were considered normal in only 2 horses.[72] Although coagulation times may be shortened during the procoagulant state, commonly observed abnormalities with developing DIC include an increased concentration of FDPs and soluble fibrin monomer, prolonged prothrombin time indicative of factor VII consumption, prolonged activated partial thromboplastin time indicative of factor VIII:C and IX consumption, prolonged thrombin time, decreased AT III activity, thrombocytopenia, and decreased protein C and plasminogen activities. Fibrinogen concentration frequently increases and reflects the acute phase response rather than coagulation abnormalities.[79] Some clinicians make a diagnosis of DIC if three or more coagulation parameters (specifically AT III, FDPs, platelet count, prothrombin time, and activated partial thromboplastin time) are abnormal,[105] whereas others require overt clinical signs of hemorrhage and concomitant thrombosis in addition to classic laboratory findings.[73] The prognostic value of coagulation parameters has been evaluated.[16,73,89] Overall, persistence or worsening of abnormalities in the face of treatment appears to be more indicative of poor outcome than alterations in any specific parameter. In one study, decreased serum AT III concentration was the parameter most commonly associated with fatal outcome in mature horses with colic.[72]

One should further evaluate the serum biochemical profile regarding compromise or failure of specific organ systems. Increases in serum creatinine and urea nitrogen concentration can have prerenal, renal, or postrenal causes. In cases of endotoxemia and sepsis, prerenal azotemia caused by dehydration and decreased renal blood flow and renal azotemia caused by organ failure are most likely to occur. One can use urine specific gravity and the response to fluid therapy to differentiate renal from prerenal causes of azotemia. Although ideally one should perform urinalysis before initiating fluid therapy, one should never delay treatment to obtain a sample and instead should use the first available urine sample. With prerenal azotemia, urine specific gravity is increased, urinalysis is normal in other respects, and azotemia resolves with adequate fluid therapy. Azotemia in the face of normal or decreased urine specific gravity, however, indicates compromised renal function. Depending on the 832
extent of renal damage, proteinuria and hematuria also may be present. Bacteriuria and an elevated urine 833
leukocyte count may occur if urinary tract infection is the underlying cause for the development of endotoxemia. In these cases, urine culture and sensitivity testing are indicated to aid appropriate antimicrobial therapy.

Increased serum activity of liver enzymes (aspartate aminotransferase, γ-glutamyltransferase, sorbitol dehydrogenase, alkaline phosphatase) are common in endotoxemic patients; however, liver failure caused by endotoxemia is rare. Sorbitol dehydrogenase is the most liver-specific of the enzymes and a sensitive indicator of acute hepatocellular necrosis; however, sorbitol is unstable and routine assays may not be available. One should evaluate liver enzymes and function tests (serum indirect and direct bilirubin concentration, serum bile acids and blood ammonia) in cases of prolonged and profound depression to rule out hepatoencephalopathy.

One should evaluate arterial blood gases in patients with primary respiratory disease or with clinical evidence of respiratory failure and in profoundly depressed, recumbent patients, especially neonates. Hypoxemia observed in response to endotoxin infusion is thought to be caused by an increase in ventilation-perfusion mismatch rather than pulmonary edema as occurs in human patients with acute respiratory distress syndrome. The lung is not a major shock organ in horses; however, pulmonary edema may occur in patients with associated sepsis or complications such as DIC.[106]

13.7.9 Management

The ideal treatment for endotoxemia is prevention. If one possibly can recognize and closely monitor patients at risk, one can provide treatment proactively and may reverse the effects of endotoxin before the inflammatory response has developed a dynamic of its own. Unfortunately, endotoxemia can develop rapidly, and horses are exquisitely sensitive to the effects of endotoxin; therefore, many equine patients are not evaluated until reaching more severe stages of endotoxemia or shock. Prognosis and patient outcome then frequently depend on the severity of complications associated with endotoxemia.[17]

Treatment of endotoxemia involves multiple aspects, and the following strategies have been proposed[107]:

- Inhibition of endotoxin release into the circulation
- Scavenging of lipopolysaccharide molecules to prevent direct effects and interaction with inflammatory cells
- Inhibition of cellular activation by lipopolysaccharide
- Inhibition of mediator synthesis
- Interference with the effects of inflammatory mediators
- General supportive care

In addition, complications such as renal failure, one also must address liver failure, cardiac failure, laminitis, and abortion in pregnant mares.

When evaluating reports concerning the efficacy of any one treatment, one should keep in mind differences in underlying disease processes and the complexity of the inflammatory cascade. A “one for all” treatment most likely will not be found, and similarly, any one treatment can only address one or few pathophysiologic aspects of endotoxemia. Understanding the rationale for different treatment strategies is important to be able to tailor treatment to the needs of the patient.

13.7.9.1 INHIBITION OF ENDOTOXIN RELEASE INTO THE CIRCULATION

Inhibition of endotoxin release requires identification and removal of its source. Therefore whenever endotoxemia is evident, the clinician should strive to reach a diagnosis of the underlying disease and ascertain whether ischemic or inflamed bowel or a gram-negative septic process is present. Aside from history, physical examination, and routine laboratory tests, evaluation may include exploratory laparotomy in colic patients, roentgenologic and ultrasonographic evaluation of the pleural and peritoneal cavity and organs, ultrasonographic evaluation of umbilical remnants in neonatal foals, evaluation of passive transfer and calculation of a sepsis score in foals, and repeated culturing of blood or other specimens. If one suspects an infectious process, one should pursue identification of the responsible microorganisms and their antimicrobial sensitivity spectrum; however, one should not delay treatment to obtain culture results. Specimen containers with removal devices for antimicrobials are available and are useful in cases for which one has initiated treatment before specimen collection. Once one reaches a diagnosis, one must take appropriate measures to correct the primary disease process. Examples are removal of devitalized sections of bowel or infected umbilical remnants, drainage of infected pleural or peritoneal fluid, and gastric lavage followed by

administration of mineral oil and/or activated charcoal in cases of grain overload to prevent further absorption of endotoxin. One must address any septic process with appropriate antimicrobial therapy. Initially, broad-spectrum coverage of the most likely organisms is recommended; one then should specify therapy according to results of culture and sensitivity testing. Sepsis in foals is caused predominantly by gram-negative organisms, of which *E. coli, Actinobacillus* spp., *Klebsiella* spp., *Salmonella* spp. and *Pasteurella* spp. frequently are identified.[108] The reader is referred to other texts for review of general principles of antimicrobial therapy. Regarding endotoxemia specifically, antimicrobial therapy has been suggested to 833
increase the amount of circulating endotoxin by inducing endotoxin release on cell death of gram-negative 834
bacteria. A recent in vitro study compared endotoxin release and inflammatory mediator activity between antimicrobials commonly used to treat *E. coli* septicemia in foals and specifically evaluated amikacin, ampicillin, amikacin plus ampicillin, ceftiofur, and imipenem. Although these antimicrobials showed no difference in the ability to kill bacteria, amikacin and the amikacin/ampicillin combination resulted in the lowest and ceftiofur in the greatest release of endotoxin. Endotoxin release appeared to be dose-dependent in that lesser amounts were released at higher antimicrobial concentrations.[109] Based on these results and clinical experience, combining antimicrobial therapy with endotoxin-binding agents such as polymyxin B may be beneficial, especially when using β-lactam antimicrobials.

Antimicrobials frequently are given perioperatively to colic patients to lower the risk of peritonitis, incisional infection, and generalized sepsis and endotoxemia. Because antimicrobial therapy has been implicated in the development of colitis, the duration of treatment should be minimal. Unless evidence of sepsis, such as fever or changes in the leukogram, is present, perioperative administration of antimicrobials should not exceed a 24 to 48 hours. Conversely, antimicrobial therapy frequently is used in cases of infectious colitis to treat the inciting cause and to prevent sepsis from translocation of bacteria.

13.7.9.2 SCAVENGING OF LIPOPOLYSACCHARIDE MOLECULES

Endotoxin typically has a short plasma half-life and is removed rapidly by mononuclear phagocytes or neutralized by binding to serum proteins and lipoproteins. Many conditions responsible for the development of endotoxemia in horses, however, are associated with an ongoing release of endotoxin. Examples include severe gastrointestinal inflammation as in proximal enteritis or colitis, grain overload, or uncontrolled sepsis. Therapy directed against endotoxin itself may be able to interrupt the continuous activation of the inflammatory cascade in these cases. Further benefits of antiendotoxin treatment may be derived if large amounts of endotoxin have been released before the inciting cause can be addressed.

13.7.9.2.1 Immunotherapy

An important consideration regarding the efficacy of immunotherapy is the region of the lipopolysaccharide molecule against which antibodies are raised. The O-chain of lipopolysaccharide acts as a potent antigen on infection with gram-negative bacteria[6]; however, antibodies directed against the O-chain are serotype specific and cannot afford significant cross-protection against heterologous bacterial strains. The core and lipid A region, both of which show a much higher degree of homology between lipopolysaccharide derived from different bacterial strains, offer a more promising target for immunotherapy. Active immunization against endotoxin has been reported for horses. Vaccination with a bacterin/toxoid vaccine prepared from rough mutants of *Salmonella typhimurium* or *S. enteritidis* protected horses against homologous and heterologous endotoxin challenge[110,111] and carbohydrate overload.[111] Despite these encouraging results and the current availability of a vaccine for use in horses (Endovac-Equi, Immvac Inc., Columbus, Missouri), active immunization against endotoxin does not appear to be a common practice. In comparison,

passive immunization with antilipopolysaccharide antibodies is used widely. Rough bacterial mutants, most commonly J5 of *E. coli* O111:B4 and *S. minnesota* Re595, are used to immunize donor horses and subsequently prepare serum or plasma products. Proposed mechanisms of action after binding of the antibodies to lipopolysaccharide include steric blockade of lipid A interaction with cellular receptors and enhanced bacterial clearance by opsonization.[112–114] Studies concerning the efficacy of antibody administration in equine patients vary in their results. Beneficial effects have been described in experimental models of endotoxemia, acute gastrointestinal disease, and neonates with sepsis,[111,115–117] whereas in other studies, antibodies failed to protect foals and horses against endotoxin effects.[118–120] Furthermore, administration of a *S. typhimurium* antiserum to foals was associated with an increased respiratory rate and higher serum activities of IL-6 and TNF.[118]

Various equine serum and plasma products are currently commercially available. An antiserum raised against the lipopolysaccharide-core of *S. typhimurium* (Endoserum) is available for administration to endotoxemic horses at a recommended dose of 1.5 ml/kg body mass. Diluting the serum ten- to twentyfold in crystalloid intravenous solutions, administering it slowly over 1 to 2 hours, and monitoring the patient for adverse reactions is advisable. Although the product is marketed for use in foals with failure of passive transfer, adverse effects have been reported,[118] and one should use caution when administering it to neonates. Plasma from donors inoculated with J5 *(E. coli)* and *S. typhimurium* (Re mutant) is available under a restricted license (Polymune-J, Vet Dynamics, Inc., San Louis Obispo, California). The manufacturer recommends administration of at least 1 to 2 L in cases of endotoxemia. In addition, hyperimmune plasma, which has a guaranteed minimum immunoglobulin G content but does not contain specific antiendotoxin antibodies (Hi-Gamm Equi, Lake Immunogenics, Inc., Ontario, New York; Polymune and Polymune-Plus, Vet Dynamics, Inc.), is marketed for treatment of failure of passive transfer, 834
and many clinicians use it to treat endotoxemia and sepsis. In addition to antibodies and protein, plasma 835
contains active constituents such as complement components, fibronectin, clotting factors, and AT III[116] and therefore may be particularly useful in patients with endotoxemia-induced coagulopathy. Volumes of 2 to 10 ml/kg body mass of hyperimmune plasma have been recommended for use in endotoxemic patients.[56,121]

13.7.9.2.2 Polymyxin B

Polymyxin B is a cationic polypeptide antibiotic that binds to the anionic lipid A portion of lipopolysaccharide and neutralizes its endotoxin capacity.[122] At dosages required for antimicrobial activity, polymyxin B carries the risk of respiratory paralysis and ototoxic, nephrotoxic, and neurotoxic side effects; however, a much lower dose is required for endotoxin-binding activity. The effects of polymyxin B in horses have been evaluated in different experimental models.[118,122,123] In an in vivo study in foals, treatment with polymyxin B at a dosage of 6000 U/kg body mass before infusion with *S. typhimurium* lipopolysaccharide resulted in significantly less severe elevations of body temperature, respiratory rate, and serum activities of TNF and IL-6 compared with untreated controls.[118] Similarly, polymyxin B treatment of adult horses given endotoxin significantly ameliorated clinical signs and decreased plasma TNF activity.[124] In the latter study, benefits of treatment were also evident at lower dosages of polymyxin B (1000 and 5000 U/kg body mass) and administration of polymyxin B 1 hour after the start of endotoxin infusion. Conversely, polymyxin B failed to ameliorate clinical signs of endotoxemia or prevent the development of coagulopathy, acidosis, lameness, and shock in experimental carbohydrate overload.[125] Side effects suggestive of neurotoxicity appeared after repeated administration of 5 mg/kg body mass (36,000 U/kg) and

in milder form, 2.5 mg/kg body mass (18,000 U/kg) polymyxin B. Nephrotoxicity was not observed. Currently, use of polymyxin B in equine patients is recommended at dosages of 1000 to 5000 U/kg body mass every 8 to 12 hours.[126] One should initiate treatment as early in the disease process as possible, because the beneficial effects of lipopolysaccharide scavenging are limited to the first 24 to 48 hours after the onset of endotoxemia, before tolerance develops. Side effects in the form of neuromuscular blockade and apnea, which necessitate slow infusion of the drug in human patients, have not been observed in horses. One therefore can administer the entire dose as a slow bolus. If one uses polymyxin B in horses with hypovolemia, dehydration, or azotemia, one should attempt to improve peripheral tissue perfusion, minimize the polymyxin B dose, and closely monitor patients for nephrotoxicity. Close monitoring is also important if one administers medications such as aminoglycoside antibiotics, which share a similar spectrum of potential side effects, concurrently. Azotemic neonates have been reported to be more susceptible to the nephrotoxic effects of polymyxin B than adult horses.[124]

In an attempt to decrease the risk for adverse effects while preserving lipopolysaccharide-neutralizing ability, a conjugate of polymyxin B with dextran has been developed.[127] In conjugated form, polymyxin B is prevented from extravasation into tissues, where it exerts toxic effects by interaction with cell membranes. In addition, conjugation increases the residence time of polymyxin B in the circulation and therefore should prolong the antiendotoxic effect. The polymyxin B–dextran combination was evaluated at a total dose of 5 mg/kg body mass of polymyxin B in 6.6 g/kg body mass dextran given 15 minutes before administration of endotoxin in horses.[128] Treatment was found to block the development of tachycardia, tachypnea, fever, and neutropenia completely and to prevent increases in serum concentrations of TNF, IL-6, TXB_2 (a TXA_2 metabolite), and the prostacyclin metabolite 6-keto-PGF_{1a}. Although mild adverse effects in the form of tachypnea, sweating, and increased systolic blood pressure were observed, these were transient and could be prevented by pretreatment with ketoprofen. The polymyxin B–dextran combination is not commercially available at this time.

13.7.9.2.3 Natural Endotoxin-Binding Substances

Natural endotoxin-binding proteins such as LBP, lipoproteins, and sCD14 have been evaluated experimentally. Results of these studies are controversial, and detrimental effects occurred in some cases. [129] A protein receiving much attention regarding potential therapeutic efficacy is the bactericidal permeability-increasing protein (BPI). This protein is structurally similar to LBP but is expressed exclusively in myeloid precursors of polymorphonuclear leukocytes.[130] BPI is stored in primary granules of mature neutrophils and during inflammation is expressed on their cell membranes and secreted into the extracellular environment.[131] BPI has an even higher affinity for lipopolysaccharide than LBP[132] and shows antibacterial activity specific for gram-negative bacteria.[65] Binding of BPI to the gram-negative bacterial membrane results in growth arrest and is an important factor in the antibacterial activity of intact neutrophils. Furthermore, BPI binding disrupts normal membrane organization and makes bacteria more susceptible to hydrophobic substances, including antimicrobials.[133] Experimentally, recombinant BPI has been shown to protect against the toxic and lethal effects of isolated lipopolysaccharide and intact gram-negative bacteria, and clinical trials in human patients show promising results concerning its therapeutic use.[134] The biology and potential use of BPI in horses has not been evaluated. 835

13.7.9.3 INHIBITION OF CELLULAR ACTIVATION BY LIPOPOLYSACCHARIDE

Treatments aimed at inhibiting lipopolysaccharide interaction with cells or turning off intracellular signaling pathways are under investigation. Nontoxic lipopolysaccharide or lipid A structures can act as endotoxin antagonists, if they competitively inhibit binding to LBP or cellular receptors or inhibit cellular activation by other mechanisms. Of the potential antagonists that have been evaluated experimentally, lipopolysaccharide and lipid A from the phototrophic bacterium *Rhodobacter sphaeroides,* and a synthetic compound (E5531) the structure of which is based on *R. sphaeroides* lipopolysaccharide, have been most promising.[135–139] Unfortunately, species differences exist regarding cellular response to these structures, and *R. sphaeroides* lipopolysaccharide acts as a potent inducer of cytokine expression in equine cells.[140] Based on results of receptor transfection studies in other species,[141,142] TLR4 is likely responsible for this phenotypic variation. Additional compounds including lipopolysaccharide derived from nitrogen-fixing plant bacteria of the species *Rhizobium* are being evaluated to reveal further insight into structural requirements for endotoxin antagonists in horses.

13.7.9.4 INHIBITION OF MEDIATOR SYNTHESIS

13.7.9.4.1 Nonsteroidal Antiinflammatory Drugs

Nonsteroidal antiinflammatory drugs (NSAIDs) are probably the most commonly used drugs to treat endotoxemia. The rationale for their use is inhibition of prostaglandin endoperoxide H synthase, that is, COX, and thereby inhibition of prostanoid production (see Figure 13.7-5). Additional beneficial effects may include scavenging of oxygen-derived free radicals and iron chelation; however, side effects may occur at dosages required to achieve these effects.[143] Prostanoids have been identified as important mediators in the inflammatory response in a number of studies, and inhibition of their synthesis is associated with beneficial effects. Two COX isoforms are recognized: constitutively expressed COX-1 and inducible COX-2. Upregulation of COX-2 expression results from various proinflammatory stimuli, including lipopolysaccharide, TNF-α, and IL-1.[144] Constitutively expressed COX products are likely important for maintenance of homeostasis, whereas increased production of prostanoids by COX-2 is thought to be responsible for detrimental effects during inflammation and shock. Research has focused on the development of selective COX-2 inhibitors; however, none of these products are currently available for use in horses. In horses the most commonly used NSAID to treat endotoxemia is flunixin meglumine. Beneficial effects of flunixin meglumine have been described in experimental models of endotoxemia[145–147] and in clinical cases. In equine colic patients, treatment with flunixin meglumine before exploratory surgery resulted in reduced plasma concentrations of TXB_2 and PGE_2 and had a favorable effect on cardiovascular parameters.[148] Flunixin meglumine was shown further to maintain cardiac output and systemic arterial blood pressure, improve blood flow to vital organs, reduce pulmonary endothelial damage, and improve survival on endotoxin challenge.[149–152]

NSAID use in horses carries the risk of side effects, most importantly the development of gastrointestinal ulceration and renal papillary necrosis (renal crest necrosis). In a study comparing the side effects of flunixin meglumine (1.1 mg/kg body mass), phenylbutazone (4.4 mg/kg body mass), and ketoprofen (2.2 mg/kg body mass) given 3 times daily for 12 days, lesions of the gastric glandular mucosa occurred most commonly. Phenylbutazone resulted in the most severe side effects, which included small intestinal edema,

erosions, and ulcers in the large colon and decreased serum albumin concentration. Renal crest necrosis occurred more frequently in horses treated with phenylbutazone but also occurred with flunixin meglumine treatment.[153] Despite the higher risk of side effects, use of phenylbutazone has been suggested for certain cases. In colic patients, phenylbutazone may provide analgesia and ameliorate endotoxin-induced ileus without masking cardiovascular effects of endotoxin, which are used to determine the necessity of surgical exploration.[154] For similar reasons and to minimize side effects a reduced dose of flunixin meglumine (0.25 mg/kg body mass) has been suggested and is used widely in horses.[155] At this dosage, flunixin meglumine was shown to inhibit eicosanoid synthesis efficiently in an in vivo model of endotoxemia.[156] Reduction of clinical signs, however, was dose dependent, and therefore one should choose the appropriate dose based on the circumstances of each case.

Ketoprofen has been suggested to have superior effects because of a proposed dual inhibitory effect on COX and lipoxygenase and may carry a decreased risk of side effects compared with flunixin meglumine and phenylbutazone. A comparison of cytokine and eicosanoid production by lipopolysaccharide-stimulated isolated monocytes in vitro, however, showed no significant difference between horses pretreated with flunixin meglumine (1.1 mg/kg body mass) or ketoprofen (2.2 mg/kg body mass), respectively.[157]

Eltenac has been evaluated in an experimental endotoxemia model in horses.[158] Given 15 minutes before lipopolysaccharide infusion, eltenac at a dose of 0.5 mg/kg protected against changes in clinical, hemodynamic, and hematologic parameters and blunted the lipopolysaccharide-induced rise in plasma cytokine concentrations in comparison with controls. Some parameters, however, including heart rate, 836
leukocyte count, lactate concentration, and plasma TNF activity, were not improved. 837

Ibuprofen may have beneficial effects superior to the other NSAIDs, because it may be possible to achieve tissue concentrations safely that allow iron chelation to occur. According to a study in healthy foals, dosages of ibuprofen up to 25 mg/kg every 8 hours can be given safely for up to 6 days.[143]

13.7.9.4.2 Corticosteroids

The use of corticosteroids for antiinflammatory therapy in sepsis and endotoxemia has been controversial in human and equine patients, and beneficial effects superior to the ones achieved by NSAIDs have not been demonstrated consistently overall. Corticosteroids inhibit the activity of phospholipase A_2 and the release of arachidonic acid from cell membrane phospholipids, as well as the production of TNF, IL-1, and IL-6 in response to a lipopolysaccharide stimulus. Experimentally, beneficial effects of dexamethasone in equine endotoxemia have been demonstrated.[159,160] To inhibit TNF production by equine peritoneal macrophages, however, the required concentration of dexamethasone was high and corresponded to an in vivo dosage (approximately 3 mg/kg body mass) greatly exceeding current recommendations.[159] Although single doses of corticosteroids are unlikely to carry a disproportionate risk of side effects, one should consider the suggested association of laminitis with corticosteroid use in horses. In cases of sepsis, further immunosuppressive effects could be detrimental.

In human patients with certain types of septic shock, dysfunction of the hypothalamic-pituitary-adrenal axis has been recognized and successfully treated with hydrocortisone replacement therapy.[161] Use of corticosteroids for this indication has not been evaluated in horses.

13.7.9.4.3

Pentoxifylline

Pentoxifylline, a methylxanthine derivative and phosphodiesterase inhibitor, has been suggested for use in endotoxemia because of its effects on neutrophil function and its ability to inhibit the production of various cytokines, interferons, and thromboplastin. Decreased production of TNF, IL-6, TXB_2, and thromboplastin in response to endotoxin was shown in an equine ex vivo model.[162] In horses given endotoxin followed by treatment with pentoxifylline (7.5 mg/kg body mass followed by continuous infusion of 3 mg/kg/hr for 3 hours), however, only minimal beneficial effects were observed.[163] Treatment significantly improved body temperature, respiratory rate, and whole blood recalcification time, but no effect was observed regarding heart rate, blood pressure, leukocyte count, plasma fibrinogen concentration, and serum cytokine concentrations. The conclusion was that benefits of treatment with pentoxifylline might be restricted to administration of high bolus doses or continuous infusion early in the pathophysiologic process. In an in vivo endotoxemia model in horses, combination of pentoxifylline (8 mg/kg body mass) and flunixin meglumine (1.1 mg/kg body mass) was found to have greater benefit than each treatment on its own.[164] The currently recommended dosage for oral administration of pentoxifylline is 8 mg/kg every 8 hours. Because of its rheologic properties, that is, the ability to increase erythrocyte deformability and microvascular blood flow, pentoxifylline may be particularly useful in endotoxemic patients showing evidence of laminitis. An intravenous preparation of pentoxifylline is not commercially available.

13.7.9.4.4

Antioxidants

Dimethyl sulfoxide (DMSO) is used frequently in an attempt to scavenge oxygen-derived radicals. The treatment may be most appropriate in cases of ischemia-induced intestinal damage and associated reperfusion injury. However, DMSO failed to show beneficial effects in an experimental model of intestinal ischemia when administered on reperfusion of the ischemic intestine.[165] DMSO at the commonly used dose of 1 g/kg body mass was shown to increase mucosal loss after ischemia and reperfusion of the large colon,[166] and hence a reduced dose of 0.1 g/kg body mass has been proposed for cases of intestinal ischemia. For intravenous administration, DMSO needs to be diluted in polyionic solutions to a concentration not exceeding 10%. Oral administration of a 10% to 20% solution via nasogastric intubation is also possible. Aside from DMSO the xanthine oxidase inhibitor allopurinol has been suggested as a treatment to prevent oxygen radical–induced tissue damage. During periods of ischemia, tissue xanthine dehydrogenase is converted to xanthine oxidase, which on reperfusion catalyzes the generation of superoxide radicals.[167,168] Evaluation in horses showed beneficial effects of 5 mg allopurinol per kilogram body mass administered 12 hours before endotoxin challenge.[169] In another study, mucosal damage attributable to oxygen-derived free radicals was not attenuated by allopurinol in an experimental ischemia-reperfusion model.[166]

13.7.9.4.5

Lidocaine

Lidocaine given intravenously has been suggested as an antiinflammatory, analgesic, and prokinetic agent, and some clinicians use it to treat colic and laminitis in horses. In an experimental endotoxemia model in rabbits, lidocaine was found to inhibit hemodynamic and cytokine responses to endotoxin profoundly if given immediately following lipopolysaccharide infusion.[170] Use of lidocaine therefore may have additional merit in endotoxemic patients. A common regimen for lidocaine use in horses is administration 837

of an initial bolus (1.3 mg/kg body mass) followed by continuous infusion at a rate of 0.05 mg/kg/min. One should monitor patients for toxic neurologic effects associated with a lidocaine overdose. 838

13.7.9.4.6

ω-3 Fatty Acids

High concentrations of ω-3 fatty acids can alter the phospholipid composition of cellular membranes toward a decreased ratio of ω-6 to ω-3 and thereby can affect membrane functions such as phagocytosis, receptor binding, and activities of membrane-bound enzymes.[68] Most importantly for the treatment of endotoxemia, ω-3 fatty acid incorporation into cell membranes decreases the availability of arachidonic acid (an ω-6 fatty acid) for eicosanoid synthesis[171] and provides alternative substrates. Metabolism of ω-3 fatty acids via the COX and lipoxygenase pathway leads to the production of 3-series prostaglandins and 5-series leukotrienes, which have less biologic activity than their 2-series and 4-series counterparts derived from arachidonic acid. Aside from these mechanisms, ω-3 fatty acids prevent lipopolysaccharide-induced upregulation of CD14 in monocytic cells and therefore may be able to block transmembrane signaling of lipopolysaccharide.[172] Cells from horses given linseed oil (high in ω-3 fatty acids) for 8 weeks before blood collection showed significantly decreased expression of procoagulant activity, TXB_2, and TNF in response to lipopolysaccharide stimulation.[173,174] In an in vivo experimental model of endotoxemia in horses, treatment resulted in prolonged activated partial thromboplastin time and whole blood recalcification time, suggesting an anticoagulant effect; however, a significant beneficial effect on clinical response and serum eicosanoid concentrations was not observed.[175] Because dietary addition of ω-3 fatty acids requires several weeks of treatment, intravenous infusion was evaluated and shown to alter the composition of cell membrane phospholipids rapidly.[176] Further evaluation of this treatment for use in horses is necessary before dosage recommendations can be made.

13.7.9.5

INTERFERENCE WITH THE EFFECTS OF SPECIFIC INFLAMMATORY MEDIATORS

13.7.9.5.1

Antibodies Directed Against Tumor Necrosis Factor

Monoclonal and polyclonal antibodies against equine TNF have been evaluated.[177–179] Administration of a monoclonal antibody preparation before lipopolysaccharide infusion resulted in significantly reduced plasma TNF-activity, improved clinical abnormality scores, lower heart rate, and higher leukocyte count compared with controls.[178] Furthermore, plasma concentrations of lactate and 6-keto-PGF_{1a} were reduced significantly, whereas TXA_2 production was not affected.[177] In another study,[179] administration of a rabbit polyclonal antibody against recombinant human TNF was unable to improve clinical and hematologic parameters when given shortly (15 minutes) after lipopolysaccharide infusion, although inhibition of TNF activity was present in vitro.[179,180] Findings in horses are in agreement with studies in other species and suggest that beneficial effects of TNF inhibition may be limited to administration before lipopolysaccharide exposure. Widespread clinical use therefore is unlikely to become feasible. Clinical trials in human patients have not shown significant benefits of TNF antibody treatment.[181,182]

13.7.9.5.2

Platelet-Activating Factor Receptor Antagonists

The effects of selective PAF receptor antagonists have been evaluated. PAF is implicated in the development of systemic hypotension,[183] lipopolysaccharide-induced platelet aggregation,[184] ileus,[185] and

increased vascular permeability[186] and may mediate recruitment of leukocytes to inflamed tissues.[187,188] A study in horses using the PAF receptor antagonist SRI 63-441 before lipopolysaccharide infusion showed significant decreases in heart rate and shorter elevation of lactate concentrations in response to the treatment. Although not statistically significant, additional beneficial effects included delayed onset of fever, a shortened period of neutropenia, and reduced maximal platelet aggregation.[189]

13.7.9.6 SUPPORTIVE CARE

13.7.9.6.1 Fluid Therapy and Cardiovascular Support

Whenever possible, the clinician should correct volume and electrolyte deficits, or at least improve them, before anesthetizing a patient for a surgical procedure. For initial resuscitation, polyionic solutions such as lactated Ringer's solution given at rates of 10 to 20 ml/kg/hr are appropriate. Patients with severe hypovolemia and shock may require higher fluid volumes. A viable alternative to large-volume resuscitation with isotonic fluids is the use of small volumes of hypertonic solutions, which transiently raise plasma osmolality, thereby causing a fluid shift from the interstitial space into the vasculature and rapidly restoring circulating volume. Hypertonic saline solution (7.5% sodium chloride) is the most commonly used hypertonic solution and has been shown to have beneficial effects in endotoxemic horses.[190] A dose of 4 ml/kg is recommended, which one should give as a bolus infusion over 10 to 15 minutes, followed by administration of an isotonic solution to restore total body fluid volume. One should use hypertonic saline with caution in patients with sodium and/or chloride derangements and should monitor serum electrolyte concentrations in the case of repeated administration. Improvement of the cardiovascular status in response to fluid therapy is indicated by normalization of heart rate, mucous membrane color, and capillary refill 838
time. Failure of urination to occur despite appropriate fluid resuscitation should result in critical evaluation 839
of renal function.

Once one has stabilized the patient, one should choose a maintenance fluid rate to maintain adequate hydration and plasma volume. For adult horses, the maintenance fluid rate is approximately 2 ml/kg/hr, whereas neonatal foals that are not nursing may require larger volumes (4 ml/kg/hr). One should monitor fluid administration carefully in endotoxemic patients, because lowered plasma oncotic pressure caused by hypoproteinemia along with an increased vascular permeability increase the risk of tissue edema formation. Furthermore, a rapid increase in total body fluid volume may be detrimental in patients with compromised cardiac and peripheral vasomotor function and may increase the severity of vascular pooling in peripheral organs. In these patients, hypertonic saline or colloids may be more appropriate means of stabilization than large volumes of crystalloid solutions.

Plasma is an ideal colloid and should be administered to maintain a serum total protein concentration above 4.2 g/dl.[121] To raise plasma protein concentration and colloid osmotic pressure significantly, however, horses often require large volumes of plasma (7 to 10 L or more in a 450-kg horse), and one should consider alternative colloids. Furthermore, high-molecular-weight polymers are thought to provide superior oncotic effects in cases of sepsis and endotoxemia, when vascular permeability is increased. Hetastarch, or hydroxyethyl starch, (Hespan) is commercially available as a 6% solution in 0.9% sodium chloride. Hetastarch molecules have very high molecular weight, and degradation must occur before renal excretion.[191] These properties result in a longer plasma half-life and prolonged oncotic effects compared with other colloids; persistence of the oncotic effect for 24 hours was found in hypoproteinemic horses.[192] A dosage of 5 to 15 ml/kg given by slow intravenous infusion along with an equal or greater volume of crystalloid fluids

is recommended.[191,193] In human patients, prolonged activated partial thromboplastin time, decreased factor VIII activity, and decreased serum fibrinogen concentration have been described in association with hetastarch use.[194] In the limited number of equine studies, bleeding times were not affected[195,196]; however, one should monitor patients treated with hetastarch for coagulopathy.

One should base correction of serum electrolyte concentrations on the results of laboratory evaluation. Ideally, one should evaluate serum electrolyte concentrations of patients receiving fluid therapy daily. One should take ongoing losses and lack of dietary intake into account, especially when serum concentrations, as in the case of potassium, poorly reflect total body electrolyte stores. Potassium supplementation is recommended in patients experiencing prolonged (greater than 48 hours) periods of anorexia. One can add calcium in the form of calcium gluconate, which is available as a 23% solution. Based on a study in healthy horses, rates of administration for calcium gluconate in the range of 0.1 to 0.4 mg/kg/min are recommended, [197] and as a guideline, one should administer 0.5 to 1 ml/kg body mass per day of a 23% solution. One can add potassium in the form of potassiu m chloride or potassium gluconate to intravenous solutions at a dose of 20 to 40 mEq/L given at a maintenance rate. Administration of potassium should not exceed a rate of 0.5 to 1 mEq/kg/hr.

Metabolic acidosis in endotoxic shock is attributable to lactic acidemia and inadequate tissue perfusion.[198] Acid-base balance often improves considerably after fluid resuscitation (preferably with alkalinizing solutions such as lactated Ringer's solution) alone; however, additional sodium bicarbonate may be required in cases in which serum bicarbonate concentration remains below 15 mEq/L. For adult horses, the bicarbonate deficit (in mEq HCO_3) is calculated as 0.3 × body mass (kg) × base deficit, whereas for foals one should use a factor of 0.5. As a general rule, one should administer half the required amount as a bolus followed by the remaining half over 12 to 24 hours. Because endotoxemia is a dynamic process and losses are ongoing, one should reevaluate acid-base status at least once daily.

Foals with sepsis are frequently hypoglycemic, and 5% dextrose solutions are useful as initial resuscitation fluids. One should reduce the glucose concentration of intravenous solutions according to the blood glucose concentration to avoid prolonged hyperglycemia. Administration of hyperimmune plasma (20 to 40 ml/kg body mass) is highly recommended in foals with evidence of partial or complete failure of passive transfer.

One should consider positive inotropic and vasomotor agents in patients with persistently inadequate tissue perfusion. Lower dosages of dopamine (0.5 to 2 μg/kg/min) result in vasodilation of the renal, mesenteric, coronary, and intracerebral vasculature via dopaminergic effects, whereas higher dosages (up to 10 μg/kg/min) also exer t stimulation of β_1-adrenergic receptors, resulting in increased myocardial contractility and heart rate.[199] Dobutamine is a direct β_1-adrenergic agonist and does not appear to have significant vasodilator properties. Dosages for dobutamine of 1 to 5 μg/kg/min as continuous intravenous infusion have been recommended for use in horses. In addition, norepinephrine was evaluated in hypotensive critically ill foals that were refractory to the effects of dopamine and dobutamine.[200] At dosages up to 1.5 μg/kg/min administered concurrently with dobutamine, six out of seven foals showed an increase in mean arterial pressure, and all foals had increased urine output. Because of the risk of cardiac side effects, close 839
monitoring of heart rate and rhythm should accompany infusion of inotropes. Indirect blood pressure 840
measurements using a tail cuff may be used to monitor the effects of treatment.

13.7.9.6.2

Management of Coagulopathy

More frequently than overt thrombosis or bleeding attributable to DIC, hemostatic abnormalities occur in the form of alterations in the coagulation profile. A procoagulant state with shortened bleeding times or prolonged bleeding times caused by consumption of clotting factors may be evident. One should address abnormalities in the coagulation profile as early as possible but especially if they persist more than 24 hours after initiation of therapy. Because of the complex interactions of coagulation and fibrinolysis during endotoxemia, one should combine anticoagulant therapy with the administration of fresh frozen plasma to replace clotting and fibrinolytic factors. Heparin acts as an anticoagulant by activation of AT III and subsequent inhibition of thrombin, release of tissue factor pathway inhibitor from endothelial cells, and inhibition of platelet aggregation.[201] Because endogenous AT III levels frequently are decreased in patients with coagulopathy, addition of heparin to fresh frozen plasma may be the most effective route of administration. An initial dose of 100 IU/kg body mass followed by 40 to 80 IU/kg body mass 3 times daily has been recommended.[121] Anemia caused by erythrocyte agglutination occurs in some patients during therapy with unfractionated heparin[202,203] but typically resolves within 96 hours if therapy is discontinued.[121] Because of the risk of microthrombosis associated with erythrocyte agglutination, use of low-molecular-weight heparin (50 IU/kg body mass subcutaneously every 24 hours) has been recommended[204] but may be cost-prohibitive. One may give aspirin orally (10 to 20 mg/kg body mass, every 48 hours), which irreversibly inhibits platelet COX activity, to inhibit platelet aggregation and microthrombosis. Platelet hyperaggregability has been implicated in the pathogenesis of carbohydrate-induced laminitis,[205] and heparin and aspirin have been recommended to prevent development of laminitis. In an in vitro study, however, aspirin did not inhibit endotoxin-induced platelet aggregation.[206]

13.7.9.6.3

Prevention of Abortion in Pregnant Mares

Luteolysis caused by increased concentrations of $PGF_{2\alpha}$ leads to pregnancy loss in endotoxemic mares before day 55 of pregnancy.[207] Daily administration of altrenogest (Regu-Mate, Hoechst-Roussel Agri-Vet, Somerville, New Jersey) at a dose of 44 mg orally consistently prevented fetal loss in mares if administered until day 70 of pregnancy.[100] Treatment with flunixin meglumine, by blockade of $PGF_{2\alpha}$ release,[101] also may contribute to the maintenance of pregnancy in endotoxemic mares. The pathogenesis of fetal loss and abortion caused by endotoxemia, surgery, or systemic disease later in gestation is not understood completely. Proposed mechanisms include direct effects on the fetus, placental function, or placental progesterone production.[208]

13.7.9.6.4

Laminitis

The pathophysiology of laminitis caused by endotoxemia is understood incompletely; however, decreased digital blood flow[209,210] and intravascular microthrombosis have been implicated. Decreased NO production by vascular endothelial cells in response to endotoxin has been suggested as a mechanism for vasoconstriction and decreased blood flow[211]; however, use of NO donors remains controversial. Maintenance of adequate peripheral perfusion and anticoagulant and antiinflammatory therapy may be helpful in preventing and treating laminitis caused by endotoxemia.

13.7.10 Summary

Although the innate immune response to endotoxin (lipopolysaccharide) is crucially important for the preservation of homeostasis and health, large amounts of endotoxin can evoke an excessive and uncontrolled inflammatory response and result in a dysfunction of hemostatic and circulatory control mechanisms, loss of vascular integrity, and finally tissue damage. Conditions commonly associated with the development of endotoxemia in horses are acute gastrointestinal diseases, especially of ischemic and severe inflammatory nature, and localized or generalized infections. Although measuring endotoxin concentrations in equine plasma is possible, this is not feasible in a clinical setting, and one typically reaches a diagnosis of endotoxemia based on clinical signs and clinicopathologic data. Successful treatment of endotoxemia requires resolution of the primary disease process in addition to neutralization of circulating endotoxin, interference with the activities of inflammatory mediators, and general supportive care. Newer treatments, such as blockade of endotoxin-interaction with cells or interruption of cell signaling pathways, are under investigation. Possible sequelae of endotoxemia include DIC, multiple organ failure, circulatory failure, and death. Frequently, the outcome of conditions associated with endotoxemia in horses depends on the severity of associated complications; for example, renal compromise, laminitis, and abortion.

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13.8 13.8—Oral Diseases

Samuel L. Jones

The word *mouth* is used commonly to signify the first part of the alimentary canal or the entrance to it.[1] The mouth is bounded laterally by the cheeks, dorsally by the palate, and ventrally by the body of the mandible and by the mylohyoideus muscles. The caudal margin is the soft palate. The mouth of the horse is long and cylindric, and when the lips are closed, the contained structures almost fill the cavity. A small space remains between the root of the tongue and the epiglottis and is termed the *oropharynx*. The cavity of the mouth is subdivided into sections by the teeth. The space external to the teeth and enclosed by the lips is termed the *vesicle of the mouth,* and in the resting state the lateral margins of the vesicle, that is, the buccal mucosa, are in close contact with the cheek teeth. Caudally, the external space communicates with the pharynx through the aditus pharyngis. The mucous membrane of the mouth is continuous at the margin of the lips with the skin and during life is chiefly pink but can be more or less pigmented, depending on the skin color and the breed type.

13.8.1 Morphology and Function

The lips are two muscular membranous folds that unite at angles close to the first cheek teeth. Each lip presents an outer and an inner surface. The upper lip has a shallow median furrow (philtrum); the lower lip has a rounded prominence or chin (mentum). The internal surface is covered with a thick mucous membrane that contains

small, pitted surfaces that are the openings of the ducts of the labial glands. Small folds of the mucous membrane called the frenula labii pass from the lips to the gum. The free border of the lip is dense and bears short, stiff hairs. The arteries of the mouth are derived from the maxillary, mandibular, labial, and sphenopalatine arteries of the major palatine artery. The veins drain chiefly to the lingual facial vein. Sensory nerves originate from the trigeminal nerve (cranial nerve V) and the motor nerves from the facial nerve (VII). The cheeks spread back from the lips and form both sides of the mouth and are attached to the alveolar borders of the bones of the jaws. The cheeks are composed of skin and muscular and glandular layers and then the internal mucous membrane. The skin is thin and pliable. In contrast, the oral mucous membrane is dense and in many areas of the oral cavity is attached firmly to the periosteum so that construction of oral mucosal flaps can be achieved only by horizontal division of the periosteal attachment. Such a feature is important in reconstructive techniques applied to the oral cavity. The blood supply to the cheeks comes from the facial and buccal arteries and the sensory nerves from the trigeminal and motor nerves from the facial nerve.

The hard palate (palatum durum) is bounded rostrally and laterally by the alveolar arches and is continuous with the soft palate caudally. The hard palate has a centra l raphe that divides the surface into two equal portions. From the line of the rostral cheek tooth, the hard palate is concave to the line of the caudal cheek tooth. Paired transverse ridges (about 18) traverse the concavity and have their free edges directed caudally. The incisive duct is a small tube of mucous membrane that extends obliquely through the palatine fissure. The dorsal component
communicates by a slitlike opening in the rostral portion of the ventral nasal meatus and its palatine end is blind 846
and lies in the submucosa of the palate. When stallions display their flehmen response, watery secretions enter 847
the nose from the glands of the vomeronasal duct. To what extent these secretions aid in pheromone reception is not known.[2]

That portion of the palatine mucosa immediately behind the incisor teeth frequently is swollen (lampas) during eruption of the permanent teeth. This swelling is physiologic and not pathologic.

The tongue is situated on the floor of the mouth between the bodies of the mandible and is supported by the sling formed by the mylohyoideus muscles. The root of the tongue is attached to the hyoid bone, soft palate, and pharynx. The upper surface and the rostral portion of the tongue are free; the body of the tongue has three surfaces. The apex of the tongue is spatulate and has a rounded border. The mucous membrane adheres intimately to the adjacent structure and on the dorsum is dense and thick. From the lower surface of the free part of the tongue, a fold of mucous membrane passes to the floor of the mouth forming the lingual frenulum. Caudally, a fold passes on each side of the dorsum to join the soft palate, forming the palatoglossal arch. Dorsally from the soft palate the palatopharyngeal arch attaches and circumvents the aditus laryngis and attaches to the roof of the nasopharynx. The mucous membrane of the tongue presents four kinds of papillae:

1. *Filiform papillae* are fine threadlike projections across the dorsum of the tongue. They are absent on the root of the tongue and are small on the rostral portion of the tongue.
2. The *fungiform papillae* are larger and easily seen at the rounded free end. They occur principally on the lateral portion of the tongue.
3. *Vallate papillae* are usually two or three in number and are found on the caudal portion of the dorsum of the tongue. The free surface bears numerous small, round secondary papillae.
4. Foliate papillae are situated rostral to the palatoglossal arches of the soft palate where they form a rounded eminence about 2 or 3 cm in length marked by transverse fissures.

Foliate, vallate, and fungiform papillae are covered with taste buds and secondary papillae.

The lingual and sublingual arteries supply the tongue from the linguofacial trunk and matching veins. The linguofacial trunk drains into the linguofacial vein. The lingual muscles are innervated by the hypoglossal nerve (XII) and the sensory supply is from the lingual and glossopharyngeal (IX) nerves.

13.8.2 Equine Dentition

The formula for the deciduous teeth of the horse is 2 times I3-3 C0-0 P3-3 for a total of 24. The permanent dental formula is 2 times I3-3 C1-1 P3-3 or P4-3 M3-3 for a total of 40 or 42. In the mare the canine teeth are usually small or do not erupt, hence reducing the number to 36 or 38. The first premolar tooth (wolf tooth) is often absent and has been reported as occurring in only 20% of the upper dentition of Thoroughbred horses.[3] The teeth of the horse are complex in shape and are compounded of different materials (dentin, cementum, and enamel). They function as grinding blades to masticate and macerate cellulose food in the important first stage of the digestive process. The cheek teeth in the horse are a well-documented feature of the evolution of *Equus caballus*.

13.8.2.1 DECIDUOUS TEETH

The first incisor is present at birth or the first week of life. The second incisor erupts at 4 to 6 weeks of age; the third incisor, at 6 to 9 months of age; the first and second premolars, at birth to 2 weeks of age; and the third premolar, 3 months of age.

13.8.2.2 PERMANENT TEETH

The eruption times for the permanent teeth are as follows: first incisor, 2½ years of age; second incisor, 3½years of age; third incisor, 4½ years of age; the canine tooth, 4 to 5 years of age; the first premolar (wolf tooth), 5 to 6 months of age; the second premolar, 2½ years of age; the third premolar, 3 years of age; the fourth premolar, 4 years of age; the first molar, 10 to 12 months of age; the second molar, 2 years of age; and the third molar, 3½ to 4 years of age. This eruption sequence clearly indicates that the eruption of the second and third permanent premolar teeth give the potential for dental impaction.

The modern horse has six incisor teeth in each jaw that are placed close together so that the labile edges form a semicircle. The occlusal surface has a deep enamel invagination (infundibulum) that is filled only partially with cementum. As the incisor teeth wear, a characteristic pattern forms in which the infundibulum is surrounded by rings of enamel, dentin, enamel, and crown cementum in a concentric pattern. Each incisor tooth tapers from a broad crown to a narrow root so that as the midportion of the incisor is exposed to wear, the cross-sectional diameters are about equal; that is, at 14 years of age, the central incisor tooth of the horse has an occlusal surface that is an equilateral triangle. Observations on the state of eruption, the angles of incidence of the incisor teeth, and the pattern of the occlusal surfaces are used as guides for aging of horses. The canine teeth are simple teeth without complex crowns and are curved. The crown is compressed and is smooth on its labial aspect but carries two ridges on its lingual aspect. No occlusal contact occurs between the upper and lower canine teeth. 847

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When erupted, the six cheek teeth of the horse function as a single unit in the mastication of food. Each arcade consists of three premolar and three molar teeth. The maxillary arcade is slightly curved, and the teeth have a square occlusal surface. The occlusal surfaces of the mandibular teeth are more oblong, and each arcade is straighter. The horse is anisognathic, that is, the distance between the mandibular teeth is narrower (one-third)

than the distance between the upper cheek teeth. This anatomic arrangement affects the inclination of the dental arcade as the jaws slide across each other in the food preparation process. The unworn upper cheek tooth presents a surface with two undulating and narrow ridges, one of which is lateral and the other medial. On the rostral and lingual side of the medial style is an extra hillock. The central portion of these surfaces is indented by two depressions that are comparable with, but much deeper than, the infundibula of the incisor teeth. When the teeth have been subjected to wear, the enamel that closed the ridges is worn through and the underlying dentin appears on the surface. Thus after a time the chewing surface displays a complicated pattern that may be likened to the outline of an ornate letter *B,* the upright stroke of the *B* being on the lingual aspect. Dentin supports the enamel internally, cementum supports the enamel lakes, and the peripheral cementum fills in the spaces between the teeth so that all six teeth may function as a single unit, that is, the dental arcade. Transverse ridges cross each tooth so that the whole maxillary arcade consists of a serrated edge. The serrations are formed so that a valley is present at the area of contact with adjacent teeth. These serrations match fitting serrations on the mandibular arcade.

The true roots of the cheek teeth are short compared with the total length of the tooth. Cheek teeth have three roots: two small lateral roots and one large medial root. By custom, that portion of the crown embedded within the dental alveolus is referred to as the *reserve crown,* and the term *root* is confined to that area of the tooth that is comparatively short and enamel free. Wear on the tooth gradually exposes the reserve crown, and the roots lengthen. In an adult 1000-lb horse the maxillary cheek teeth are between 8.0 and 8.5 cm in length. Dental wear accounts for erosion and loss of tooth substance at a rate of 2 mm/yr.

The pulp chambers of the teeth are also complex. The incisors and canines have a single pulp chamber. The mandibular cheek teeth have two roots and two separate pulp chambers. The maxillary cheek teeth, although they have three roots, have in fact five pulp chambers. As occlusal wear proceeds, deposition of secondary dentin within the pulp chambers protects the chambers (e.g., the dental star, medial to the infundibulum on the incisor teeth).

In the mandibular cheek teeth the transverse folding of the enamel anlage (during morphogenesis of the tooth) does not take place, and the occlusal surface is a simple surface of central dentin surrounded by enamel. Each tooth then is conformed to a single arcade by the presence of peripheral crown cementum.

13.8.3 Mouth Diseases

The oral cavity and oropharynx are subject to a variety of diseases. However, many conditions affecting the first portion of the alimentary system produce the same clinical signs, regardless of their cause. The clinical signs may include inappetance or reluctance to eat, pain on eating or swallowing, oral swelling, oral discharge, and fetid breath. Affected animals may show some interest in food but hesitate to eat it. Salivation may be excessive and may be contaminated with purulent exudate or blood. The occurrence of bruxism (i.e., grinding of teeth) can indicate discomfort in other areas of the alimentary tract; for example, bruxism and frothing oral saliva are characteristic features of gastric ulceration in the horse.

The clinician needs to be aware that considerable weight loss can occur rapidly with inability to feed and swallow. Diseases that result in denervation of the pharynx and inappropriate swallowing can have the complication of inhalation pneumonia.

13.8.3.1 EXAMINATION AND CLINICAL SIGNS

After a complete physical examination and ascertaining the history, the clinician should approach examination of the mouth systematically in all cases. One can examine a considerable portion of the mouth and teeth from the outside by palpation of the structures through the folds of the cheek. Most horses allow an oral examination without sedation or the use of an oral speculum. In many cases, however, one best achieves the detailed oral examination by sedation and the use of an oral speculum and a light source. One should irrigate the mouth to wash out retained food material so as to be able to inspect and palpate the lips, cheeks, teeth, and gums.

The classic signs of dental disease in the horse include difficulty and slowness in feeding, together with a progressive unthriftiness and loss of body condition. In some instances, the horse may quid, that is, it may drop poorly masticated food boluses from the mouth, and halitosis may be obvious. Additional problems reported by owners include bitting and riding problems and headshaking or head shyness. Facial or mandibular swelling may occur. Nasal discharge can result from dental disease associated with maxillary sinus empyema. Mandibular fistulae frequently are caused by lower cheek tooth apical infections. Some correlation exists between the age of the animal and clinical signs (Table 13.8-1). 848

TABLE 13.8-1 Correlation Between Dental Disease and Dental Therapy

AGE	EXAMINE FOR	NECESSARY DENTISTRY
2–3 years	1. First premolar vestige (wolf teeth)	1. Remove wolf teeth if present.
	2. First deciduous premolar (upper and lower)	2. Remove deciduous teeth if ready; if not, file off corners and points of premolars.
	3. Hard swelling on ventral surface of mandible beneath first premolar	3. Obtain x-ray film; extract retained temporary premolar if present.
	4. Cuts or abrasions on inside of cheek in region of the second premolars and molars	4. Lightly float or dress all molars and premolars if necessary.
	5. Sharp protuberances on all premolars and molars	5. Rasp protuberances down to level of other teeth in arcade.
3–4 years	1. (1), (2), (4), and (5) above	1. (1), (2), (4), and (5) above
	2. Second deciduous premolar (upper and lower)	2. Remove if present and ready.
4–5 years	1. (1), (4), and (5) above	1. (1), (4), and (5) above
	2. Third deciduous premolar	2. Remove if present and ready.
5 yrs and older	1. (1), (4), and (5) above	1. (1), (4), and (5) above
	2. Uneven growth and "wavy" arcade	2. Straighten if interfering with mastication.
	3. Unusually long molars and premolars	3. Unusually long molars and premolars may have to be cut if they cannot be filed down.

From Baker GJ: Diseases of the teeth. In Colohan PT, Mayhew IG, Merritt AM et al, editors: *Equine medicine and surgery,* ed 4, vol 1, Goleta, Calif, 1991, American Veterinary Publications.

13.8.3.2

ANCILLARY DIAGNOSTIC TECHNIQUES

Ancillary aids for a complete examination of the oral cavity of the horse may include radiology, endoscopic examination, fluoroscopy, biopsy, and culture. One should take care always during endoscopic evaluation of the oral cavity using a flexible endoscope. The author recommends sedation and the use of an oral speculum to prevent inadvertent mastication of the endoscope. If one uses general anesthesia as part of the diagnostic workup, then endoscopic evaluation of the oral cavity is much easier. In selected cases, advanced imaging technologies such as computed tomography, magnetic resonance imaging, or nuclear scintigraphy may be beneficial.

13.8.4 Dysphagia

13.8.4.1 CLINICAL SIGNS AND DIAGNOSIS

The lips of the horse are mobile and prehensile. In many ways they function like the tip of the elephant's trunk in that they test, manipulate, and sample the environment for potential nutritive value. Consequently, loss of motor function (e.g., facial palsy) affects the efficiency of the prehensile system. The lips grasp food in grazing or browsing, and the incisor teeth section the food. With mastication and lubrication with saliva, the bolus of food forms and is manipulated from side to side across the mouth, assisted by the tight cheeks of the horse and the palatine ridges. Swallowing begins as the food bolus contacts the base of the tongue and the pharyngeal walls. During swallowing, the soft palate elevates to close the nasopharynx, the base of the tongue elevates, and the hyoid bone and the larynx move rostrally following contraction of the hyoid muscles. During this process, the rima glottidis closes and the epiglottis tilts dorsally and caudally to protect the airway so that food is swept through lateral food channels around the sides of the larynx into the laryngoesophagus. Fluoroscopic studies in nursing foals in the dorsoventral view showed that contact occurs between the lateral food channels in the midline so that in outline the food bolus achieves a bow tie shape.[4]

Dysphagia is defined as a difficulty or inability to swallow. Anatomic classifications for dysphagia include prepharyngeal, pharyngeal, and esophageal (postpharyngeal) dysphagias. The site of the cause for dysphagia influences the clinical signs. Prepharyngeal dysphagia is characterized by dropping food (quidding) or water from the mouth, reluctance to chew, hypersalivation, or abnormalities in prehension. Pharyngeal and esophageal dysphagias are characterized by coughing; nasal discharge containing saliva, water, or food material; gagging; anxiousness; and neck extension during attempts to swallow. The following section describes esophageal dysphagia in more detail. Causes of dysphagia can be divided into four types: painful, muscular, neurologic, or obstructive (Table 13.8-2). Pain and obstruction cause dysphagia by interfering with 849
the mechanics of prehension, bolus formation and transfer to the pharynx, and deglutition. Muscular and 850
neurologic causes of dysphagia impede prehension and swallowing by affecting the motor function of the lingual or buccal musculature, muscles of mastication (temporal and masseters), and pharyngeal and cranial esophageal muscles. Sensory loss to the lips, buccal mucous membranes, pharynx, or tongue also may cause dysphagia. Neurologic causes of dysphagia may affect the forebrain, brainstem, or peripheral nerves that control prehension (cranial nerves Vm, Vs, VII, and XII), transfer of the food bolus to the pharynx (cranial nerves Vs and XII) and swallowing (cranial nerves IX and X).

TABLE 13.8-2 Differential Diagnoses for Dysphagia

CLASS OF DYSPHAGIA	DIFFERENTIAL DIAGNOSES
Painful	Tooth root abscess or periodontal disease
	Broken teeth
	Abnormal dentition or wear
	Stomatitis, glossitis, or pharyngitis
	Nonsteroidal antiinflammatory drug toxicity
	Chemical irritation
	Thrush (candidiasis)
	Influenza
	Streptococcus equi
	Vesicular stomatitis virus
	Actinobacillus lignieresii
	Buccal, gingival, or glossal trauma (bits or chains)
	Foreign bodies
	Retropharyngeal lymphadenopathy or abscess
	Mandibular trauma
	Stylohyoid osteopathy
	Temporomandibular osteopathy
Muscular	Hyperkalemic periodic paralysis
	Nutritional myopathy (white muscle disease)
	Polysaccharide storage disease
	Glycogen branching enzyme deficiency
	Masseter myositis
	Hypocalcemia tetany or ecclampsia
	Myotonia
	Rectus capitis ventralis rupture
	White snakeroot toxicity
	Megaesophagus

Obstructive	Retropharyngeal abscess and lymphadenopathy
	Oral, pharyngeal, retropharyngeal, laryngeal, or esophageal malformations, injury, edema, or neoplasia
	Pharyngeal or epiglottic cysts
	Pharyngeal abscess or foreign body
	Dorsal displacement of the soft palate or rostral displacement of the palatopharyngeal arch
	Cleft palate
	Guttural pouch tympany or empyema
	Follicular pharyngitis
	Esophageal obstruction
	Pharyngeal cicatrix
	Retropharyngeal abscess or neoplasia
Neurologic forebrain disease; generalized neuropathy; disorders of cranial nerves V, VII, IX, X, or XII	Guttural pouch empyema, mycosis, or neoplasia
	Stylohyoid osteopathy
	Lead poisoning
	Petrous temporal bone osteomyelitis or fracture
	Retropharyngeal abscess
	Botulism
	Yellow star thistle toxicity
	Viral encephalitis
	Cerebral edema
	Cerebral or brainstem hemorrhage
	Intracranial masses (hematoma, neoplasia, abscess)
	Meningitis
	Verminous encephalitis
	Equine protozoal myeloencephalitis
	Equine herpesvirus 1
	Equine dysautonomia
	Hepatoencephalopathy
	Tetanus
	Polyneuritis equi

Diagnosis of the cause of dysphagia is based on physical examination including a careful oral examination, neurologic examination, clinical signs, and endoscopy of the pharynx, esophagus, and guttural pouches. Radiology may be useful to assess the bony structures of the head and throat. Ultrasonography is valuable for

examining the retropharyngeal space and esophagus to detect and evaluate masses. One may detect pharyngeal or esophageal causes of dysphagia with routine endoscopic examination or with contrast radiography. Although one also can use endoscopy to assess deglutition, one must remember that sedation adversely affects the deglutition mechanism. One may assess deglutition using fluoroscopy[4] or manometry,[5] but these techniques require specialized equipment. Specific diagnostic procedures for nonalimentary causes of dysphagia are covered elsewhere in this text (see Chapter 3).

13.8.4.2

MANAGEMENT

Specific treatments aimed at resolving the underlying disorder causing dysphagia are discussed in detail elsewhere. One should avoid feeding roughage with long fiber length (hay or grass) to most horses with dysphagia. Dietary modifications that promote swallowing such as feeding slurries made from complete pelleted feeds may be sufficient to manage some cases of partial dysphagia. One must take care to prevent or 850
avoid aspiration pneumonia in horses with pharyngeal or esophageal dysphagia. One can manage foals by 851
feeding mare's milk or a suitable substitute through a nasogastric tube. One also may administer pellet slurries or formulated liquid diets via nasogastric tubes to older horses. Prolonged nutritional management of dysphagic horses may require extraoral feeding using a tube placed through an esophagostomy.[6]

Formulated pelleted diets are often easy to administer through a tube as slurry and are balanced to meet the nutritional requirements for healthy horses. One must feed sufficient quantities to deliver adequate calories (16 to 17 Mcal/day for a 500-kg horse). Adjustments may be necessary for horses that are cachectic or have extra metabolic demand (such as pregnancy). Adding corn oil to the ration (1 cup every 12 or 24 hours) is a common method of increasing fed calories. Liquid diets also have been used for enteral feeding[7] but may not be tolerated as well as pelleted diets. Regardless of the method of nutritional management, one must monitor and replace salivary losses of electrolytes. Saliva contains high concentrations of Na, K, and Cl. A group of ponies with experimental esophagostomies[8] and a horse with esophageal squamous cell carcinoma[9] were fed a complete pelleted diet through esophagostomy tubes but developed metabolic acidosis, hyponatremia, and hypochloremia apparently because of salivary losses. Surprisingly, salivary losses of potassium did not result in hypokalemia in these cases, presumably because of replacement in the diet. However, if the diet is deficient in potassium, hypokalemia may result. One often can accomplish electrolyte replacement by adding NaCl and KCl to the diet. One can maintain horses for months with frequent feedings through an esophagostomy tube.[9] Parenteral nutrition (total or partial) may be useful in the short term but is not often feasible for long-term management.

TABLE 13.8-3 Sites of Apical Infections in Diseased Cheek Teeth

	CHEEK TOOTH*												
	1		2		3		4		5		6		
	R	C	R	C	R	C	R	C	R	C	R	C	NUMBER AFFECTED
Maxillary	2	7	9	9	10	11	7	8	0	0	0	0	
Total	9		18		21		15		0		0		63
Mandibular	0	4	18	22	13	10	5	3	3	1	0	0	
Total	4		40		23		8		4		0		79
													142

From Baker GJ: Diseases of the teeth. In Colohan PT, Mayhew IG, Merritt AM et al, editors: *Equine medicine and surgery,* ed 4, vol 1, Goleta, Calif, 1991, American Veterinary Publications.

* *R,* Rostral root; *C,* caudal root.

13.8.5

Dental Diseases

13.8.5.1

ERUPTION DISORDERS

Tooth eruption is a complex phenomenon involving the interplay of dental morphogenesis and those vascular forces responsible for creating the eruption pathway. These changes are responsible for osteitis and bone remodeling within the maxilla and mandible. Young horses frequently show symmetric bony swelling resulting from these eruption cysts. In some cases, additional clinical signs of nasal obstruction with respiratory stridor or nasal discharges may be apparent.

Pathologic problems associated with maleruption include a variety of dental diseases. Oral trauma can displace or damage erupting teeth or the permanent tooth buds. As a result, teeth may be displaced and erupt in abnormal positions or may have abnormal shapes. Supernumerary teeth, incisors and molars, can develop, as well as palatal displacement of impacted teeth (maxillary P3-3, or third cheek tooth). In almost all of these conditions some form of surgical treatment is necessary.

Significant evidence from the location of apical osteitis in diseased teeth (Table 13.8-3) confirms that dental impaction is a major cause of dental disease in the horse. In a series of 142 extracted teeth, 63 were P3-3 or P4-4 (cheek tooth 2 or 3, respectively).[10] Early observations had indicated that the first molar (M1, or cheek tooth 4) was the most commonly diseased tooth, and an "open infundibulum" in this tooth has been suggested as the cause.[11] Studies on cementogenesis of the maxillary cheek teeth have shown, however, that in fact most maxillary cheek teeth have a greater or lesser degree of hypoplasia of cementum within the enamel lakes and that this "lesion" rarely expands into the pulp. The central infundibular hole is the site of its vascular supply to 851
the unerupted cement lake. On those occasions in which caries of cementum occurs, that is, secondary 852
inflammatory disease and acid necrosis of the cementum, apical osteitis may develop.

13.8.5.2

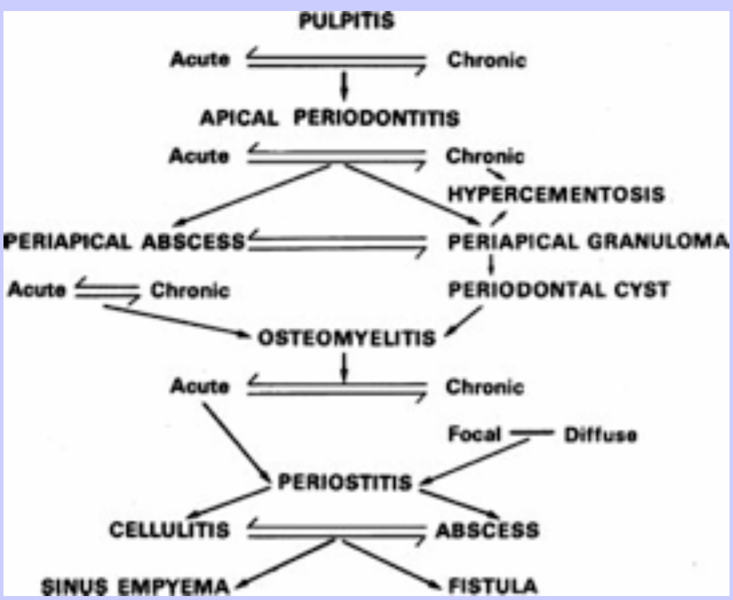
DENTAL DECAY

Pulpitis is key to the pathogenesis of dental decay in the horse. The initiation of inflammatory pulp changes may be a sequela to dental impaction or dental caries or may result from fracture of a tooth. If the onset of the inflammatory process is slow, then formation of secondary dentin within the pulp chambers may protect the pulp and the tooth. Secondary dentin formation occurs from stimulation of odontoblasts within the pulp chamber. Such changes are the normal process of protection during dental wear and attrition as crown substances wear away and the reserve crown comes into wear. In acute disease, however, this defense mechanism is ineffective, and the changes that occur and that are sequelae to pulpitis reflect the location of each affected tooth. For example, pulpitis and apical osteitis of the third mandibular cheek tooth most commonly results in the development of a mandibular dental fistula. Pulpitis of the third maxillary cheek tooth, however, results in an inflammatory disease within the rostral maxillary sinus and in development of chronic maxillary sinus empyema (Figure 13.8-1).

Oblique radiographs greatly assist the diagnosis of dental decay by demonstrating sinus tract formation, sequestration of bone, mandibular osteitis, hyperplasia of cementum, and new bone formation (so-called alveolar periosteitis).[12]

The management of dental decay in the horse usually involves surgical extraction of the diseased tooth. In some cases one can use apicoectomy and retrograde endodontic techniques to save the diseased tooth. One must take care, however, in selection of patients. In most cases of apical osteitis in the horse that result from dental impaction, immature root structures make achieving an apical seal of the exposed pulp difficult.

Figure 13.8-1 Possible sequelae to pulpitis in the horse. (From Baker GJ: Disease of the teeth. In Colohan PT, Mayhew IG, Merritt AM et al, editors: *Equine medicine and surgery*, ed 4, vol 1, Goleta, Calif, 1991, American Veterinary Publications.)



13.8.6 Periodontal Disease

Gingival hyperemia and inflammation occur during the eruption of the permanent teeth and are common causes of a sore mouth in young horses (particularly 3-year-olds as the first dental caps loosen). Such periodontal changes usually resolve as the permanent dental arcade is established. During normal mastication, the shearing forces generated by the occlusal contact of the cheek teeth essentially clean the teeth of plaque and effectively inhibit deposition of dental calculus. Wherever occlusal contact is ineffective, periodontal changes and calculus buildup occur; for example, the deposition of calculus on the canine teeth of mature geldings and stallions is common. Routine dental prophylaxis forms an important component of maintaining normal occlusal contact, and for this reason one should remove arcade irregularities that result in enamel point formation on the buccal edges of the maxillary cheek teeth and the lingual edges of the mandibular cheek teeth. One should remove these edges annually in horses that are at grass and twice yearly in young horses, aged horses, and stabled horses. Horses at grass have been shown to have a greater range of occlusal contact and therefore better periodontal hygiene than stabled horses. In stabled horses the range of occlusal contact is narrower and the formation of enamel points occurs more frequently with subsequent buccal ulceration and the initiation of a cycle of altered occlusal contact and hence irregular arcade formation. This process leads to severe forms of periodontal disease and wave mouth formation.

Periodontal disease occurs with abnormal occlusal contact and initiation of the cycle of irregular wear and abnormal contact. Such changes progress to loss of alveolar bone, gross periodontal sepsis, and loss of tooth support. In this sense periodontal disease truly is the scourge of the equine mouth and results in tooth loss.[13]

13.8.7 Congenital and Developmental Abnormalities

13.8.7.1 CLEFT PALATE

Palatine clefts may result from an inherited defect and are caused by failure of the transverse palatal folds to
fuse in the oral cavity. Harelip accompanies few palatine clefts in the horse. The degree of palatine clefting 852
depends on the stage at which interruption in the fusion of the palatopalatal folds occurs. Toxic or teratogenic 853
effects are documented in other species, but little data are available in the horse.

In recent years, treatment for repair of uncomplicated palatine defects has been recommended but prognosis is generally poor because of the considerable nursing care required and the high incidence of surgical failures. One should emphasize early surgery and the use of mandibular symphysiotomy in affording surgical exposure. The combination of mandibular symphysiotomy and transhyoid pharyngotomy to approach the caudal margins of the soft palate affords surgical access, and one can construct mucosal flaps to repair the defects. However, the incidence of surgical breakdown is high, and healing by first intention is the exception rather than the rule. A recent surgical report documented the successful closure of a median cleft of the lower lip and mandible in a donkey.[14]

13.8.7.2 CAMPYLORHINUS LATERALIS

Foals born with a severely deviated premaxilla and palate have a wry nose. One can achieve surgical correction of the deviated premaxilla by submucosal division of the premaxilla across the nose at the line of

the first cheek tooth. Circumstantial evidence indicates that such a defect has a genetic cause, and the defect occurs most frequently in the Arabian breed.

13.8.7.3 CYSTS

Other developmental abnormalities are subepiglottic cysts resulting from cystic distortion of remnants of the thyroglossal duct, which may cause dyspnea and choking in foals. Surgical removal of these cysts results in normal function.

13.8.7.4 PARROT MOUTH

The most significant developmental defect of dental origin is a maxilla that is longer than the mandible, that is, the horse is parrot-mouthed. An overbite of 2 cm in the incisor arcade may be present in a horse with a mismatch of less than 1 cm between the first upper and lower cheek teeth. Parrot mouth and monkey or sow mouth are thought to be inherited conditions. Some correction of minor incisor malocclusion occurs up to 5 years of age.

Recognition and detection of parrot mouth are important in the examination of potential breeding stock. Surgical attempts to inhibit overgrowth of the premaxilla by wiring or by the application of dental bite plate procedures have been documented in recent years.[15]

13.8.8 Oral Wounds

As has been indicated, the horse is by nature a curious animal and uses its lips as a means of exploring a variety of objects. Wounds of the lips, incisive bone, and the mandibular incisor area occur commonly in the horse and usually result from the horse getting the lips, jaw, or teeth caught in feeding buckets, in fence posts, or in halters or having a segment of tongue encircled with hair in tail chewing. As the horse panics and pulls away from its oral entrapment, considerable trauma can occur to the lips, teeth, and gums.

Most wounds repair satisfactorily, provided one finds them early and observes the basic principles of wound hygiene, excision of necrotic tissue, and wound closure. One must ensure that oral mucosal defects are closed and that effective oral seals are made before external wounds are closed. In some cases, offering specially constructed diets or even feeding the horse by nasogastric tube or esophagostomy during the healing processes may be necessary.

13.8.9 Stomatitis and Glossitis

Foreign body penetration of the tongue, cheek, or palate has been reported in grazing and browsing horses and in particular in horses that have certain hay sources that contain desiccated barley awns or yellow bristle grass.[16] Other plant material and grass awns also occasionally may penetrate the tongue, gingiva, or cheek, causing inflammation or abscesses. Ulcerative stomatitis also results from the toxicity of phenylbutazone therapy.[17] Vesicular stomatitis is a highly contagious viral blistering disease described in more detail elsewhere. Treatment of glossitis and stomatitis primarily aims at removing the inciting cause.

Actinobacillus lignieresii, the causative agent of actinobacillosis, has been isolated and identified from ulcers on the free border of the soft palate and oral and laryngeal granulomata. The bacterium also was reported in a

sublingual caruncle in a horse with a greatly swollen tongue.[18] Therapy with 150 ml of 20% sodium iodide and 5 g of ampicillin every 8 to 12 hours effected a clinical cure.

13.8.10 Salivary Glands

13.8.10.1 FUNCTION

Saliva is important for lubricating and softening food material. The horse has paired parotid, mandibular, and polystomatic sublingual salivary glands. The parotid gland is the largest of the salivary glands in the horse and is situated in the space between the ramus of the mandible and the wing of the atlas. The parotid duct is formed at the ventral part of the gland near the facial crest by the union of three or four smaller ducts. The duct leaves the gland above the linguofacial vein, crosses the tendon of the sternocephalicus muscle, and enters the 853
mouth obliquely in the cheek opposite the third upper cheek tooth. The parotic duct orifice is small, but some 854
dilation of the duct and a circular mucous fold (the parotid papillae) exist at this point. The mandibular gland is smaller than the parotid gland and extends from the atlantal fossa to the basihyoid bone. For the most part, the mandibular gland is covered by the parotid gland and by the lower jaw. The mandibular duct is formed by union of a number of small duct radicles that emerge along the concave edge of the gland and run rostral to the border of the mouth opposite the canine tooth. The orifice is at the end of a sublingual caruncle. The mandibular gland possesses serous, mucous, and mixed alveolar glandular components. The parotid gland is a compound alveolar serous gland. The parotid salivary gland can secrete saliva to yield rates of 50 ml/min, and a total daily parotid secretion can be as much as 12 L in a 500-kg horse. Parotid secretion only occurs during mastication, and administration of atropine or anesthesia of the oral mucosa can block secretion. Parotid saliva is hypotonic compared with plasma, but at high rates of flow, concentrations of sodium, chloride, and bicarbonate ions increase.

13.8.10.2 SALIVARY GLAND DISORDERS

Parotid saliva of the horse has a high concentration of calcium, and occasionally calculi (sialoliths) form within the duct radicles of the parotid salivary gland.[19] Congenital parotid duct atresia, acquired stricture from trauma to the duct, or obstruction by plant material (sticks or foxtails and other seeds) also may occur. The clinical signs of sialolithiasis or other forms of ductule obstruction include a fluid swelling in the form of a mucocele proximal to the stone and occasionally inflammation of the parotid gland. Ultrasonography is useful to diagnose salivary mucoceles and to detect foreign bodies or sialoliths. Measurement of electrolyte concentrations in aspirates from suspected mucoceles might be helpful to distinguish them from hematomas. Salivary potassium and calcium concentrations are higher than plasma. Treatment may require surgical removal of the stone or plant material in the case of sialolithiasis or foreign body obstructions. Other causes of obstruction may require resection of the affected portion of the duct or chemical ablation of the gland.[20]

Primary sialoadenitis is unusual but can occur in one or both glands. The condition is painful and may be associated with a fever and anorexia. Secondary sialoadenitis is more common and usually is associated with trauma. Infectious sialoadenitis from *Corynebacterium pseudotuberculosis*[21] or other bacterial pathogens also may occur. Diagnosis is by physical examination and by finding an enlarged edematous parotid gland tissue on ultrasonographic examination. Culture and cytologic examination of aspirates may be useful for diagnostic purposes. Treatment in usually palliative, consisting of nonsteroidal antiinflammatory drugs. Appropriate antibiotic therapy is indicated as directed by culture and sensitivity results.

Chemical irritation, glossitis, stomatitis, or other causes of prepharyngeal dysphagia cause ptyalism or excessive salivation in horses. Specific therapy for the ptyalism usually is not required as long as salivary losses are not excessive, resulting in dehydration and electrolyte imbalances. Ingestion of the fungal toxin slaframine also causes hypersalivation in horses.[22] The fungus *Rhizoctonia leguminicola*, which produces slaframine, causes black patch disease in red clover. Slaframine is a parasympathomimetic compound that stimulates exocrine secretion in the parotid gland. Slaframine toxicosis most commonly occurs in the spring or early summer and rarely requires treatment other than removal from the pasture. Mowing removes the source in most cases because regrowth in pastures often has less fungal contamination.[23]

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13.9

13.9—Esophageal Diseases

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13.9.1

Anatomy and Function

The esophagus is a musculomembranous tube that originates from the pharynx dorsal to the larynx and terminates at the cardia of the stomach.[1] In adult Thoroughbred horses the esophagus is approximately 120 cm long. The cervical portion is approximately 70 cm long; the thoracic portion, approximately 50 cm long; and the short abdominal portion, only approximately 2 cm long. The cervical esophagus generally lies dorsal and to the left of the trachea in the cervical region. In the thorax the esophagus courses through the mediastinum lying dorsal to the trachea and crosses to the right of the aortic arch dorsal to the heart base.

The esophagus has no digestive or absorptive functions and serves as a conduit to the stomach for food, water, and salivary secretions. The esophageal mucosa is a keratinized stratified squamous epithelium.[1] The submucosa contains elastic fibers that contribute to the longitudinal folds of the esophagus and confer elasticity to the esophageal wall. A transition occurs in the muscle type composing the tunica muscularis from striated skeletal muscle in the proximal two thirds of the esophagus to smooth muscle in the distal third. In the proximal esophagus the skeletal muscle layers spiral across one another at angles. Within the smooth muscle layers of the distal esophagus the outer layer becomes more longitudinal, whereas the inner layer thickens and becomes circular. The wall of the terminal esophagus can be 1 to 2 cm thick. Deep cervical fascia, pleura, and peritoneum contribute to the thin fibrous tunica adventitia of the esophagus.

Motor innervation to the striated skeletal muscle of the esophagus includes the pharyngeal and esophageal branches of the vagus nerve, which originate in the nucleus ambiguus of the medulla oblongata. Parasympathetic fibers of the vagus nerve supply the smooth muscle of the distal esophagus. Sympathetic innervation of the esophagus is minimal.

Passage of ingesta through the esophagus can be considered part of the swallowing process, which consists of oral, pharyngeal, and esophageal stages. The oral stage is voluntary and involves transport of the food bolus from the mouth into the oropharynx. During the involuntary pharyngeal stage the food bolus is forced through the momentarily relaxed upper esophageal sphincter by simultaneous contractions of the pharyngeal muscles. In the esophageal phase of swallowing the upper esophageal sphincter closes immediately, the lower esophageal sphincter opens, and esophageal peristalsis propels the bolus into the stomach.[2] Unlike a food bolus, liquids do not require peristalsis to reach the lower esophageal sphincter and may precede the food bolus during swallowing.

The upper esophageal sphincter prevents esophagopharyngeal reflux during swallowing and air distention of the esophagus during inspiration. Upper esophageal pressure increases in response to pressure from a food bolus and to increased intraluminal acidity, as would occur with gastroesophageal reflux. The lower esophageal sphincter is a smooth muscle located at the gastroesophageal junction that is morphologically ill defined but forms an effective functional barrier.[2] Normally the lower esophageal sphincter is closed in response to gastric distention to restrict gastroesophageal reflux. Relaxation of the lower esophageal sphincter permits passage of ingested material from the esophagus to the stomach. Distention of the stomach with ingesta mechanically constricts the 855
lower esophageal sphincter. Gastric distention also triggers a vagal reflex that increases lower esophageal 856
sphincter tone, a safety mechanism against gastroesophageal reflux. The mechanical and vagal mechanisms that promote lower esophageal sphincter tone prevent spontaneous decompression of the stomach, which along with a lack of a vomiting reflex in the horse, increases the risk of gastric rupture during episodes of severe distention.

13.9.2 Esophageal Obstruction

Esophageal obstruction has many causes (Table 13.9-1) and most often is manifested clinically by impaction of food material and resulting esophageal dysphagia. Esophageal obstruction may be caused by primary impactions (simple choke) of roughage, particularly leafy alfalfa hay, coarse grass hay, bedding, and even grass. Prior esophageal trauma or poor mastication caused by dental abnormalities may predispose horses to primary esophageal impaction.[3] Wolfing or gulping food may precipitate primary impactions, particularly if the horse is exhausted or mildly dehydrated after a long ride or is weakened from chronic debilitation. Impactions also may result from disorders that physically impede the passage of food material and fluid by narrowing the luminal diameter, reduce the compliance of the esophageal wall, or alter the conformation of the esophageal wall such that food material accumulates in a pocket or diverticulum. Foreign bodies, intra- or extramural masses, or acquired or congenital anomalies cause these so-called secondary impactions. Intramural causes of esophageal obstruction include tumors (squamous cell carcinoma), strictures, diverticula, and cysts.[3–10] Mediastinal or cervical masses (tumors or abscesses) may cause extramural obstructions. Congenital anomalies are covered in detail later.

TABLE 13.9-1 Causes of Complete or Partial Esophageal Obstruction in the Horse

CATEGORY	DIFFERENTIAL
Intraluminal	Foreign body
	Feed material (simple impaction)
Extramural	Neoplasm (squamous cell carcinoma, lymphoma)
	Vascular ring anomaly (persistent right aortic arch)
	Granuloma
Intramural	Esophageal abscess
	Granuloma
	Neoplasm (squamous cell carcinoma, leiomyosarcoma)
	Cysts (intramural cysts, duplication cysts)
	Diverticulum
	Stenosis
Functional disorders	Dehydration
	Exhaustion
	Pharmacologic (acepromazine, detomidine)
	Primary megaesophagus (congenital ectasia)
	Esophagitis
	Autonomic dysautonomia
	Vagal neuropathies

13.9.2.1

CLINICAL SIGNS AND DIAGNOSIS

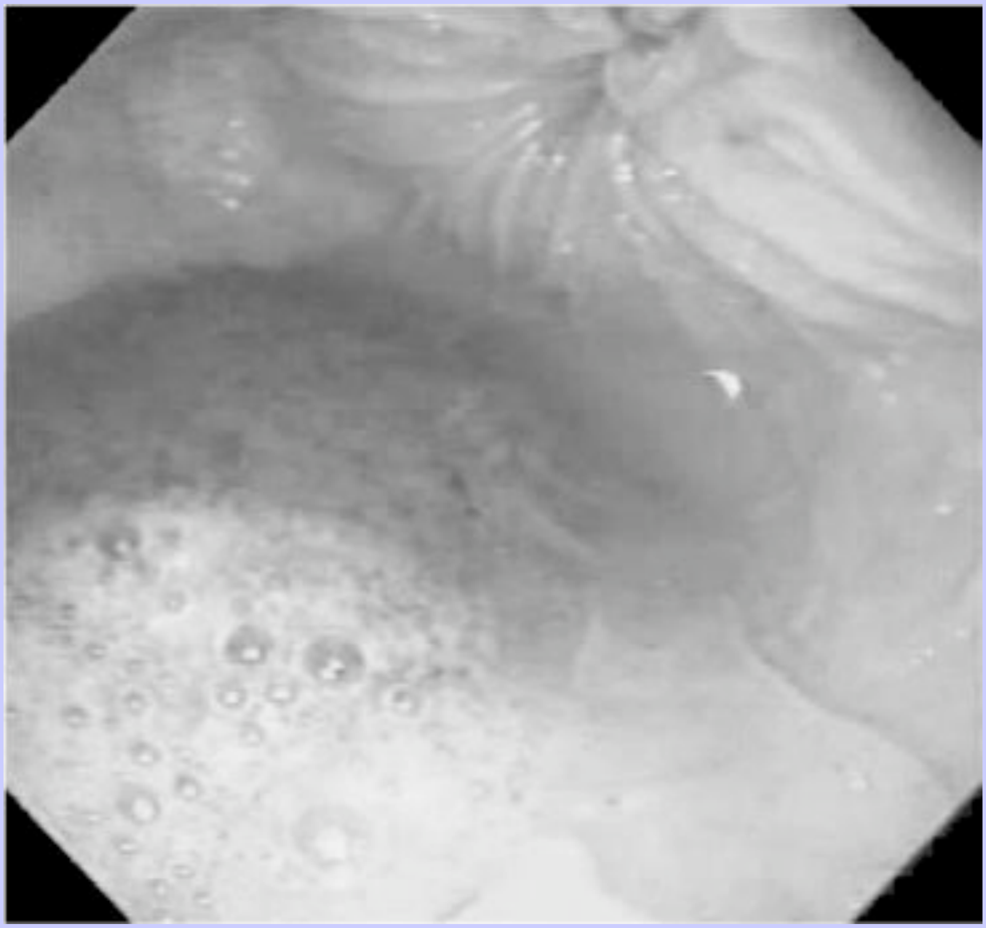
The clinician must perform a thorough physical examination, including complete oral and neurologic examination, to help rule out causes of dysphagia and nasal discharge other than esophageal obstruction. The clinical signs associated with esophageal obstructions are related primarily to regurgitation of food, water, and saliva caused by esophageal (postpharyngeal) dysphagia.[11] Horses with esophageal obstruction are often anxious and stand with their neck extended. One may note gagging or retching, particularly with acute proximal obstructions. Bilateral frothy nasal discharge containing saliva, water, and food material; coughing; odynophagia; and ptyalism are characteristic clinical signs, the severity of which varies with the degree and location of the obstruction. Distention in the jugular furrow may be evident at the site of obstruction. One may observe other clinical signs related to regurgitation of saliva, water, and food material, such as dehydration, electrolyte or acid-base imbalances, weight loss, and aspiration pneumonia. In extreme cases, pressure necrosis from the impaction or trauma to the esophagus may cause esophageal rupture. If the rupture is in the cervical esophagus, crepitus or cellulitis may be evident along with signs of systemic inflammation. Thoracic

auscultation is important to determine whether aspiration pneumonia is present. Intrathoracic esophageal rupture may result in pleuritis and its associated clinical signs.

Passage of a nasogastric tube is an effective way to detect and localize an obstruction but provides little information about the nature of the obstruction or the condition of the esophagus. The most direct method for diagnosis of esophageal obstructions is endoscopic examination. Most cases of esophageal obstruction occur at sites of natural narrowing of the esophageal lumen, such as the cervical esophagus, the thoracic inlet, base of the heart, or the terminal esophagus, thus one may need an endoscope longer than 1 m for complete evaluation. Endoscopic evaluation is useful before relief of an impaction to localize the obstruction and to investigate the nature of the impaction if one suspects a foreign body. Foreign bodies may be retrievable via transendoscopic tethering.[12] One can obtain critical diagnostic and prognostic information following resolution of the impaction. Assessing the affected esophagus for mucosal ulceration, rupture, masses, strictures, diverticula, and signs of functional abnormalities is important (Figure 13.9-1).

Ultrasonography of the cervical region is useful not only to confirm a cervical esophageal impaction but also 856
to provide critical information about the location and extent of the impaction and esophageal wall thickness 857
and integrity. Ultrasonography may provide information about the cause.[13] Radiographic assessment of the esophagus can confirm the presence of esophageal obstruction in cases in which one cannot view the affected area adequately using endoscopy. One can detect impacted food material in the esophagus by a typical granular pattern and often can observe gas accumulation proximal to the obstruction. Air or barium contrast radiographic studies are most useful for evaluating the esophagus following relief of the impaction if one suspects a stricture. One often can detect esophageal dilation, diverticula, rupture, functional disorder (megaesophagus), or luminal narrowing caused by extraluminal compression more easily using contrast radiographic studies instead of endoscopy (Figure 13.9-2).[14–16] One should take care when interpreting radiographic studies in sedated horses, particularly after passage of a nasogastric tube or other esophageal manipulations that may contribute to esophageal dilation.[17]

Figure 13.9-1 Endoscopic view of the cervical esophagus in an adult horse 6 months after an episode of choke that caused circumferential ulceration of the esophageal mucosa. The area of luminal narrowing (stricture) is at the upper right of the image, and the proximal dilation forms an outpouching of the esophageal wall. A contrast esophagram revealed that the outpouching was a pulsion diverticulum.

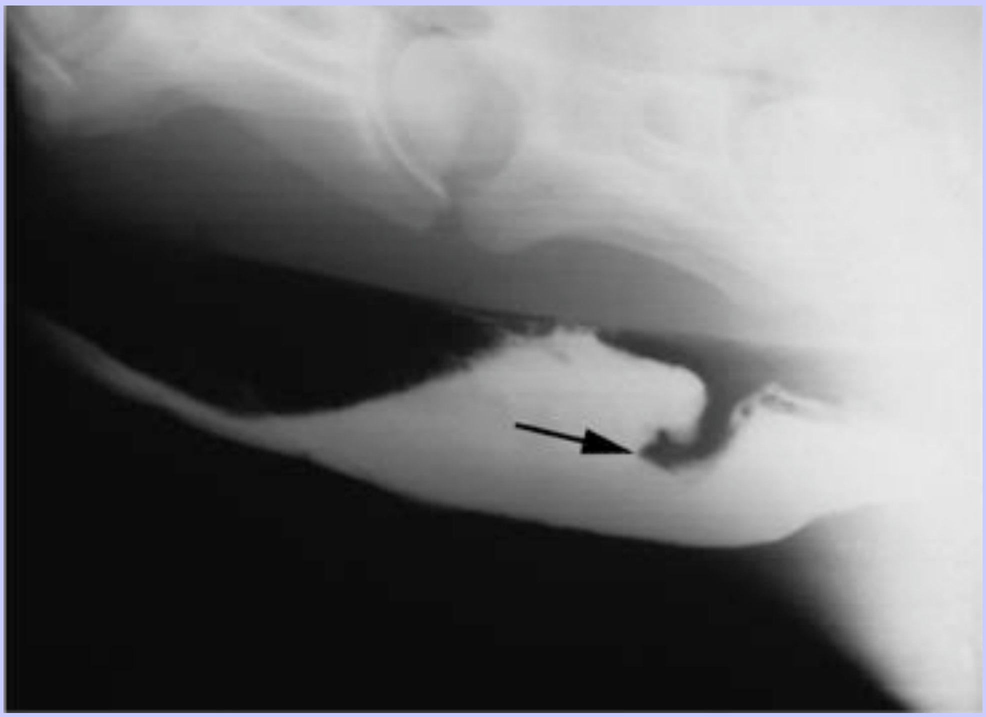


13.9.2.2 TREATMENT

The primary goal of treatment for esophageal impaction is to relieve the obstruction. Parenteral administration of acepromazine (0.05 mg/kg intravenously), xylazine (0.25 to 0.5 mg/kg intravenously) or detomidine (0.01 to 0.02 mg/kg intravenously), oxytocin (0.11 to 0.22 IU/kg intramuscularly), and/or esophageal instillation of lidocaine (30 to 60 ml of 1% lidocaine) may reduce esophageal spasms caused by pain or may decrease esophageal tone.[17–20] Some clinicians advocate parasympatholytic drugs such as atropine (0.02 mg/kg

intravenously) to reduce salivary secretions and lessen the risk of aspiration. However, undesirable effects of atropine including excessive drying of the impaction and inhibition of distal gastrointestinal motility may preclude its use.

Figure 13.9-2 Contrast esophagram in a horse with circumferential esophageal stricture *(arrow)* and a pulsion diverticulum proximal to the stricture.



Resolution of an impaction may require physical dispersal of the material.[18] One can use a nasogastric tube to displace the impacted material along with external massage if the obstruction is in the cervical region. Often, carefully lavaging the esophagus with water via an uncuffed or a cuffed nasogastric tube while the head is lowered is necessary to aid in breaking up the impaction. Some clinicians advocate a dual tube method whereby a tube is placed through each nasal passage into the esophagus for ingress and egress of the lavage fluid. Because of the risk of aspiration of water and food material, esophageal lavage sometimes is done under general anesthesia with a cuffed nasotracheal tube.

In refractory cases, intravenous administration of isotonic fluid containing 0.9% NaCl and KCl (10 to 20 mEq/L) for 24 hours at a rate of 50 to 100 ml/kg/day along with esophageal relaxants such as oxytocin may promote hydration and softening of the impaction and help prevent or alleviate any electrolyte or acid-base imbalances resulting from salivary losses of chloride, sodium, and potassium.[21] One should note that the effects of oxytocin on esophageal tone occur in the proximal two thirds of the esophagus and may not be 857
effective for distal obstructions.[19,20] Rarely, esophageal obstruction ultimately may require esophagotomy to 858
relieve the impaction. One must enforce strict restriction of food and water, including access to bedding material, until the obstruction is resolved and the esophagus has regained function.

Systemic effects of dysphagia associated with esophageal impaction include dehydration, hyponatremia, hypochloremia, and metabolic alkalosis from prolonged loss of salivary free water and electrolytes.[21] If the duration of a complete esophageal obstruction is 48 hours or longer, one should correct dehydration and electrolyte and acid-base imbalances. One can restore fluid and electrolyte balance with oral electrolyte solutions if the patient is less than 6% to 7% dehydrated and the esophageal obstruction is resolved. Horses that are greater than 6% to 7% dehydrated or those that have a refractory obstruction or moderate to severe electrolyte imbalances may require intravenous fluid therapy with solutions containing 0.9% NaCl and KCl (10 to 20 mEq/L).

One should perform esophageal endoscopy after relief of the impaction to determine whether any complications of the impaction have developed or if a primary cause of the obstruction is present. Endoscopic examination is critical to determine the postobstruction treatment plan and for follow-up evaluation of esophageal healing. One should reevaluate the horse every 2 to 4 weeks following resolution of the impaction if one notes esophageal dilation or mucosal injury. Additional evaluation via radiography may be warranted to assess motility and transit times.

Dilation proximal to the site of obstruction, mucosal injury from trauma, stricture formation, formation of a diverticulum, megaesophagus, and esophagitis are sequelae to esophageal obstruction that predispose patients to reobstruction. The rate of reobstruction may be as high as 37%. Depending on the duration of the obstruction and the degree of trauma or dilation, the risk of reobstruction is high for 24 to 48 hours or longer, thus one should withhold food for at least 24 to 48 hours after resolution of the obstruction. Sucralfate (20 mg/kg orally every 6 hours) may hasten healing if esophageal ulceration is evident, but the efficacy of sucralfate for this purpose is not established. Some clinicians suggest that administration of a nonsteroidal antiinflammatory drug (NSAID) such as flunixin meglumine (1 mg/kg orally or intravenously every 12 hours) or phenylbutazone (1 to 2 mg/kg orally or intravenously every 12 to 24 hours) for 2 to 4 weeks after resolution of the impaction may reduce the development of strictures. Judicious use of NSAIDs is recommended to prevent NSAID-induced worsening of esophageal mucosal injury. One should avoid orally administered NSAIDs if esophagitis is present. After 48 to 72 hours or when the esophageal mucosa has recovered as assessed by endoscopy, one can feed the horse soft food (moistened pellets and bran mashes). One can return the patient gradually to a high-quality roughage diet over 7 to 21 days, depending on the degree of esophageal damage induced by the impaction and the nature of any underlying disease. The prognosis for survival is good (78%), but some horses may require permanent dietary modification if persistent chronic obstruction is a problem.[3]

Aspiration pneumonia and perforation are potential complications of severe or prolonged esophageal obstructions. If aspiration is suspected, administration of broad-spectrum antibiotics that are effective against gram-positive and gram-negative organisms, including metronidazole (20 mg/kg orally every 8 hours) for anaerobes is advisable. A subsequent section describes treatment of esophageal perforation or rupture.

13.9.3 Esophagitis

Esophagitis refers to a clinical syndrome of esophageal inflammation that may or may not be ulcerative. The major protective mechanisms of the esophageal mucosa include salivary and food material buffers, normal peristaltic motility, and the barrier formed by the gastroesophageal sphincter. Reflux esophagitis is caused by repeated episodes of gastric fluid regurgitation into the distal esophagus and subsequent chemical injury to the mucosa (Figure 13.9-3).[22] Esophageal mucosal ulceration also can occur if the clearance of gastric fluid from the 858
esophagus is delayed, such as in functional disorders of the esophagus. Like ulceration of the squamous portion 859

of the stomach in horses, gastric acid and bile salt chemical injury is a major mechanism of esophageal squamous epithelial ulceration.[22,23] Reflux esophagitis may occur along with gastric ulcer disease, motility disorders, increased gastric volume from gastric outflow obstructions, gastric paresis, intestinal ileus, or impaired lower esophageal sphincter function.[7,22] Other causes of esophagitis in horses include trauma (foreign bodies, food impactions, nasogastric tubes), infection (mural abscesses), or chemical injury (pharmaceuticals, cantharidin) (Figure 13.9-4).[24–27]

Figure 13.9-3 Endoscopic view of the distal esophagus in a 7-month-old foal with duodenal obstruction. The ulcerative lesions in the distal esophagus and the generalized hyperkeratosis of the esophageal mucosa are notable.

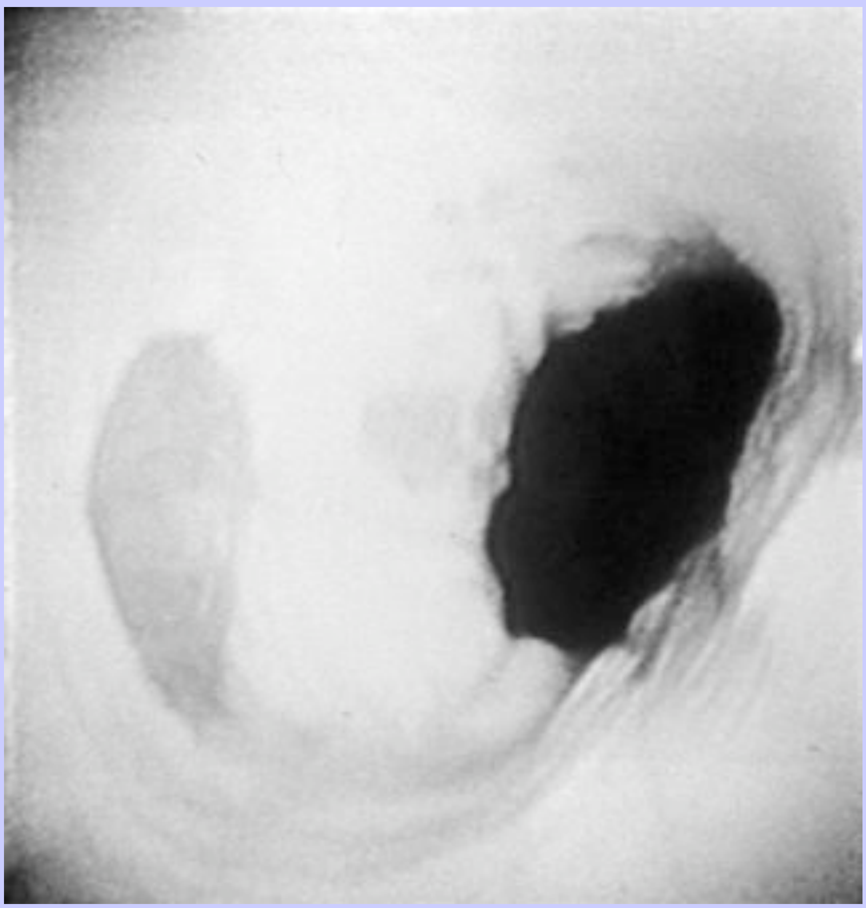
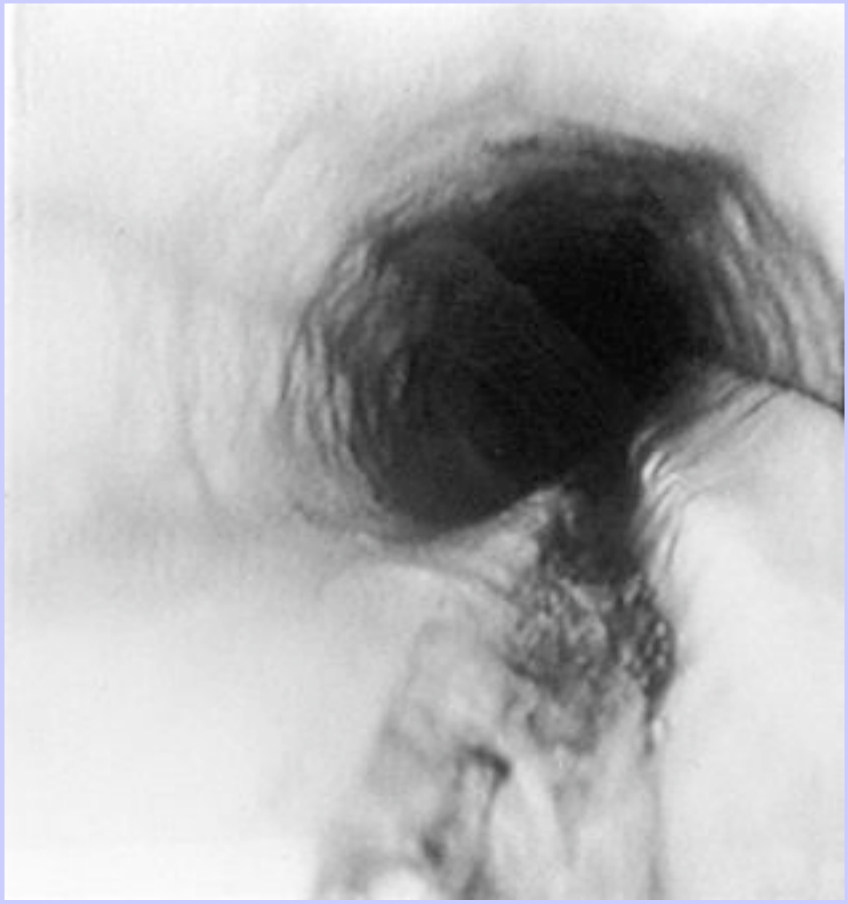


Figure 13.9-4 Endoscopic view of the cervical esophagus of a horse that had repeated passages of a stiff nasogastric tube. The deep, linear ulceration of the esophageal mucosa is notable.



13.9.3.1

CLINICAL SIGNS AND DIAGNOSIS

The clinical signs of esophagitis are nonspecific and similar to esophageal obstruction and gastric ulcer diseases. Gagging or discomfort when swallowing may be evident, and hypersalivation and bruxism are signs of esophageal pain. Esophageal (postpharyngeal) dysphagia may be evident. One may note partial or complete anorexia such that horses with chronic esophagitis may have significant weight loss. Esophageal hypomotility dysfunction caused by the inflammatory process may result in esophageal impaction. Clinical signs of underlying diseases that predispose to esophagitis may predominate or mask the signs of esophagitis. Horses with gastrointestinal motility disorders such as proximal enteritis or gastric outflow obstruction are at a high risk of developing reflux esophagitis because of the presence of gastric acid and bile salts in the fluid reflux. Foals with gastric, pyloric, or duodenal strictures caused by chronic ulceration commonly have reflux esophagitis.

Diagnosis requires endoscopic examination of the esophagus. One may note diffuse, patchy, linear, or coalescing erosion or ulcerations (see Figures 13.9-3 and 13.9-4). One also may observe significant edema or hyperemia. Determining whether an underlying disease, such as infection, neoplasia, esophageal strictures, or diverticula, is present is important. In addition, one must examine the stomach to determine whether the esophagitis is associated with gastritis, gastric obstruction, or gastric ulcer disease. Contrast radiography may be helpful to detect esophageal ulceration and is useful to assess esophageal motility and transit time.[14]

13.9.3.2 TREATMENT

The principles of therapy for reflux esophagitis include control of gastric acidity, mucosal protection, and correction of any underlying disorder contributing to gastroesophageal reflux. Reduction of gastric acid production with H_2 histamine receptor blockers such as ranitidine or proton pump antagonists such as omeprazole is critical for resolution of the esophagitis. Some clinicians advocate using sucralfate to promote healing of ulcerated esophageal mucosa. However, the ability of sucralfate to bind ulcerated esophageal mucosa is not proven, nor is the efficacy of sucralfate for hastening esophageal ulcer healing.

Horses with reflux esophagitis following delayed gastric outflow caused by gastroduodenal ulcer disease, gastric paresis, or proximal enteritis may benefit from prokinetic drugs that act on the proximal gastrointestinal tract. Metoclopramide (0.02 to 0.1 mg/kg subcutaneously every 4 to 12 hours) reduces gastroesophageal reflux by increasing lower esophageal sphincter tone, gastric emptying, and gastroduodenal coordination. One should exercise caution when giving metoclopramide to horses because they are prone to extrapyramidal neurologic side effects of the drug. Cholinergic drugs such as bethanechol (0.025 to 0.035 mg/kg subcutaneously every 4 to 24 hours or 0.035 to 0.045 mg/kg orally every 6 to 8 hours) may improve gastric emptying and are effective for treating reflux esophagitis. For esophagitis from trauma or pressure injury after esophageal impaction, judicious use of NSAIDs may be warranted to reduce esophageal inflammation and pain.

Dietary modification may be necessary for patients with esophagitis, depending on the degree of ulceration or if motility is impaired. One should feed horses with mild esophagitis frequent small meals of moistened pellets and fresh grass. Severe esophagitis may necessitate withholding food and complete esophageal rest for several 859
days. Although the prognosis for esophagitis is good in the absence of underlying disease, the risk of stricture 860
formation is high if severe circumferential or coalescing ulcerations are present. Esophagitis from severe trauma or infection may be prone to stricture formation.

13.9.4 Motility Disorders

Motility dysfunction of the equine esophagus is caused most commonly by hypomotility resulting in esophageal dilation (ectasia) or megaesophagus. Although megaesophagus in horses most commonly is acquired, reports indicate idiopathic megaesophagus in young horses may be congenital.[28–31] Acquired megaesophagus in horses may be a consequence of chronic or recurrent esophageal obstruction.[3,7] Esophageal impactions of a short duration cause a proximal dilation of the esophagus that is generally reversible.[14] However, if the duration of the obstruction is long enough, the motility of the esophagus proximal to the site of obstruction may be impaired permanently. Other causes of acquired megaesophagus include extraesophageal obstruction by tumors or abscesses, pleuropneumonia, and vascular ring anomalies.[3,8]

Acquired megaesophagus also may result from neurologic, neuromuscular, and muscular disorders. Neurologic diseases that cause vagal neuropathy—such as equine protozoal myeloencephalitis, equine herpesvirus myeloencephalitis, and idiopathic vagal neuropathy—have been associated with megaesophagus in horses. Pleuropneumonia may be associated with a vagal neuropathy resulting in megaesophagus. Megaesophagus is an early sign of equine dysautonomia[32] and may be observable in patients with botulism. Myasthenia gravis is a well-known cause of megaesophagus in nonequine species but has not been reported in horses. Also in other species, electrolyte disorders, cachexia, primary myopathies, myositis, and Addison's disease may affect esophageal motility but have not been associated with megaesophagus in horses. One can induce iatrogenic megaesophagus by the α_2-adrenergic agonist detomidine, but this is transient and reversible.[17,33] Nonetheless, the use of this drug may complicate clinical evaluation of esophageal motility.

Esophageal inflammation, particularly reflux esophagitis, may affect motility and cause megaesophagus. However, because esophageal hypomotility affects the tone and function of the lower esophageal sphincter, reflux esophagitis also may be a complication of a primary functional disorder. Thus assessing esophageal motility in horses with esophagitis that is not responding appropriately to treatment is important.

13.9.4.1 CLINICAL SIGNS AND DIAGNOSIS

Along with a complete physical examination one should include a careful neurologic examination to help rule out primary neurologic causes of megaesophagus. Because esophageal hypomotility is a functional obstruction, the clinical signs of esophageal hypomotility or megaesophagus are similar to esophageal obstruction. Unlike mechanical obstruction the onset of clinical signs is insidious rather than acute. The clinical signs include those associated with esophageal dysphagia.[7,8,28–31] The cervical esophagus may be dilated enough to be evident externally. Weight loss is a common sign. Signs attributable to an underlying disease may be evident.

Diagnosis of esophageal hypomotility requires transit studies. One can measure the transit time of a bolus from the cervical esophagus to the stomach by fluoroscopy or contrast radiography.[14,32] Other signs of esophageal hypomotility and megaesophagus include pooling of contrast material and an absence of peristaltic constrictions.[7,14,28,32] Endoscopy may reveal a dilated esophagus and an absence of peristaltic waves.[7,28] One may observe evidence of underlying disease causing obstruction or esophageal dilation.[3,7] One should evaluate the esophagus for evidence of esophagitis that is causing esophageal motility dysfunction or is a result of impaired esophageal clearance of gastric fluid. Esophageal manometry may be useful to document abnormal postdeglutition contraction pressures, contraction time, and propagation times but is not often available for routine clinical application.[28,34] One should perform other diagnostic tests such as a complete blood count and chemistry to help determine a possible underlying cause. Cerebral spinal fluid analysis may be indicated to rule out neurologic disorders. Specialized testing such as electromyography to detect neuromuscular disorders may also be indicated.

13.9.4.2 TREATMENT

Treatment of esophageal hypomotility or megaesophagus should aim at treating the underlying cause. Dietary modification should aim at improving esophageal transit of food. One should feed the horse slurries of pellets, and feeding from an elevated position to promote transit may be beneficial. Metoclopramide or bethanechol may benefit patients with reflux esophagitis associated with megaesophagus by increasing lower esophageal

tone, gastric emptying, and reducing gastroesophageal reflux. The prognosis depends on the underlying cause and the degree of dilation. Although many cases of megaesophagus associated with reflux esophagitis respond well to treatment, many other forms of megaesophagus including congenital megaesophagus have a poor prognosis.

13.9.5 Esophageal Stricture

Strictures most commonly are caused by pressure necrosis from esophageal impactions that induce circumferential erosion or ulceration of the esophageal mucosa, although esophageal injury caused by oral administration of corrosive medicinal agents and trauma to the neck may also result in stricture formation.[35] Congenital strictures also have been reported.[36] Strictures caused by mucosal and submucosal trauma are termed *esophageal webs* or *rings*. Strictures may also originate in the muscular layers and adventitia of the esophagus (mural strictures) or in all of the layers of the esophagus (annular stenosis).[36,37] Horses with these lesions have a presentation similar to those with simple obstructions, because strictures result in partial obstruction and impaction of food material in the lumen. One can detect esophageal webs or rings with endoscopy (see Figure 13.9-1), whereas identification of mural strictures or annular stenosis may require a double-contrast esophogram (see Figure 13.9-2).

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In a retrospective study of horses with esophageal stricture following simple obstruction, maximal reduction in esophageal lumen diameter occurred within 30 days of the esophageal obstruction. Although surgery has been used to relieve such strictures, initial medical management is warranted because strictures may resolve with conservative therapy, and the esophagus continues to remodel for up to 60 days following ulceration. In one report, seven horses with esophageal obstruction-induced stricture were treated conservatively by feeding a slurry diet and administering antiinflammatory and antimicrobial medications, and five of seven were clinically normal within 60 days.[35] One of the five successfully treated horses had a 10-cm area of circumferential ulceration, suggesting that the potential exists for extensive mucosal injury to resolve without permanent stricture formation.

If resolution of strictures within 60 days is insufficient, one should investigate other methods to increase esophageal diameter. Bougienage has been used successfully in small animal patients and human beings. The technique involves passage of a tubular dilatable instrument down the esophagus and stretching of the stricture. One may perform the technique by passing a nasogastric tube with an inflatable cuff. However, one has to perform the procedure frequently to have any success, and horses do not tolerate it well.[36] Alternatively, a number of surgical techniques have been used to resolve strictures, including resection and anastomosis,[38,39] temporary esophagostomy with fenestration of the stricture,[37] esophagomyotomy for strictures of the muscularis and adventitia,[40,41] or patch grafting with local musculature.[42] However, such surgeries are fraught with complications, largely because of the propensity of the traumatized esophagus to restricture.[3,35] The esophagus lacks a serosal layer and does not rapidly form a fibrin seal as does the remainder of the intestinal tract, so anastomoses tend to leak.[38] In addition, tension on the esophagus during swallowing and movement of the neck impairs healing of anastomoses.[37,39] In spite of these difficulties, the long-term prognosis for horses with chronic esophageal strictures treated surgically is better than for those treated nonsurgically.[3]

13.9.6 Esophageal Diverticula

Two types of diverticula are traction (true) diverticula and pulsion (false) diverticula. Traction diverticula result from wounding and subsequent contraction of periesophageal tissues, with resultant tenting of the wall of the esophagus. Pulsion diverticula arise from protrusion of esophageal mucosa through defects in the muscular wall of the esophagus and usually result from trauma or acute changes in intraluminal pressure.[36] Traction diverticula appear as a dilation with a broad neck on contrast esophagography, whereas pulsion diverticula typically have a flask shape with a small neck on an esophagram (see Figure 13.9-2).[10,43] Although traction diverticula are usually asymptomatic and of little clinical significance, pulsion diverticula may fill with feed material, ultimately leading to esophageal obstruction.[43–45] A movable mass in the midcervical region may be noticeable before onset of complete obstruction.[36] Pulsion diverticula may be corrected surgically by inverting or resecting prolapsed mucosa and closing the defect in the wall of the esophagus.[10,43,45] Inversion of excessive mucosa may reduce the diameter of the esophageal lumen and predispose horses to esophageal obstruction and therefore should be reserved for small diverticula.[10]

13.9.7 Congenital Disorders

Congenital disorders of the esophagus are rare. Reported congenital abnormalities include congenital stenosis,[46] persistent right aortic arch,[8] esophageal duplication cysts,[47–49] intramural inclusion cysts,[9,50] and idiopathic megaesophagus.[28,30,31] In the one report of congenital stenosis, double-contrast radiography revealed concentric narrowing of the thoracic esophagus in the absence of any vascular abnormalities at the base of the heart. Successful treatment included having the foal stand with the forelimbs elevated off the ground following each feeding.[46]

Persistent right aortic arch is a congenital anomaly in which the right fourth aortic arch becomes the definitive aorta instead of the left aortic arch, which results in constriction of the esophagus by the ligamentum arteriosum as it extends between the anomalous right aorta and the left pulmonary artery. Clinical signs may include those associated with esophageal (postpharyngeal) dysphagia, drooling, and distention of the cervical esophagus resulting from partial obstruction of the thoracic esophagus.[8,51] Endoscopic examination typically reveals 861
dilation of the esophagus cranial to the obstruction with evidence of diffuse esophagitis. Successful surgical 862
treatment of persistent right aortic arch has been reported in one foal.[51]

Esophageal duplication cysts and intramural inclusion cysts cause typical signs of esophageal obstruction, including salivation, esophageal dysphagia, and swelling of the cervical esophagus as the cysts enlarge.[47,49,50] Such signs can make them difficult to differentiate from other forms of esophageal obstruction (choke). Endoscopic examination may reveal compression of the esophageal lumen and communication with the esophageal lumen if it exists. Ultrasonographic examination may be the most useful method of antemortem diagnosis if the cyst is in the cervical esophagus. Examination of an aspirate of the mass may aid in the diagnosis by revealing the presence of keratinized squamous cells.[47,50] Surgical treatments have included complete surgical resection and surgical marsupialization.[47,49,50] The latter appears to be more successful and results in fewer complications.[49,50] Complications of surgical resection have included laryngeal hemiplegia following surgical trauma to the recurrent laryngeal nerve in the region of the esophagus and esophageal fistula formation.[50]

13.9.7.1 ESOPHAGEAL PERFORATION

Perforation typically occurs in the cervical region in response to external trauma, necrosis of the esophageal wall caused by a food impaction, or rupture of an esophageal lesion such as an impacted diverticulum. The esophagus is particularly vulnerable to external trauma in the distal third of the neck because only a thin layer of muscle covers it at this point.[52] Iatrogenic perforation may occur in response to excessive force with a stomach tube against an obstruction or a compromised region of the esophagus.[24] Esophageal perforations may be open or closed and tend to cause extensive cellulitis and necrosis of tissues surrounding the wound because of drainage of saliva and feed material within fascial planes. Systemic inflammation associated with endotoxemia from septic cellulitis may occur. Closed perforations of the esophagus are particularly troublesome because food material, water, saliva, and air may migrate to the mediastinum and pleural space via fascial planes.[24,52] Because of the leakage of air into the tissues surrounding the rupture, extensive subcutaneous and fascial emphysema frequently develops and is usually evident clinically and on cervical radiographs. Pneumomediastinum and pneumothorax are potentially fatal complications of esophageal ruptures.

Treatment should include converting closed perforations to open perforations if possible,[53] extensive debridement and lavage of affected tissues, broad-spectrum antibiotics, tetanus prophylaxis, and esophageal rest. The clinician may achieve the latter by placing a feeding tube into the esophagus via the wound. Alternatively, one may place a nasogastric tube using a small tube (12-F diameter).[24] For open perforations, once the wound has granulated and contracted to a small size, one may attempt peroral feeding.[52] Extensive loss of saliva via esophageal wounds may lead to hyponatremia and hypochloremia. In addition, transient metabolic acidosis occurs because of salivary bicarbonate loss, followed by progressive metabolic alkalosis.[21] Although reports of esophageal wounds healing well by second intention exist, healing takes a prolonged time.[54] In addition, some perforations never completely heal and form permanent esophagocutaneous fistulae that may require surgical correction. The development of esophageal strictures is not common because wounds are usually linear and not circumferential. However, traction diverticula may develop. Other complications of esophageal wounds include Horner's syndrome and left laryngeal hemiplegia.[52]

In a retrospective study on esophageal disorders, only 2 of 11 horses with esophageal perforations survived long-term[3]; in a report of esophageal trauma following nasogastric intubation, 4 of 5 horses were euthanized.[24] The prognosis is therefore poor in horses with esophageal perforations, largely because of the extent of cellulitis, tissue necrosis, shock, and local wound complications.

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13.10 13.10—Diseases of the Stomach

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Specialized endoscopic equipment allowing visual inspection of the entire adult equine stomach has become 863
increasingly available to veterinarians in academia and private practice. Thus gastric disease in horses recently has 864
gained increasing awareness among veterinarians, owners, and trainers.

13.10.1 Gastroduodenal Ulceration

Peptic ulcer disease is defined as erosions or ulcers of any portion of the gastrointestinal tract normally exposed to acid.[1] Mucosal damage can include inflammation, erosion (disruption of the superficial mucosa), or ulceration (penetration of the submucosa). In severe cases, full-thickness ulceration can occur, resulting in perforation. The proximal (orad) portion of the equine stomach is lined by stratified squamous mucosa similar to the esophageal lining. The distal (aborad) portion of the stomach is lined with glandular mucosa, and the distinct junction between the two regions is deemed the *margo plicatus*. Ulceration can occur in either or both gastric regions, although different clinical syndromes and pathophysiologic mechanisms apply. As a result, the broad term *equine gastric ulcer syndrome* (EGUS) has been used to encompass the wide array of associated clinical syndromes. EGUS develops in horses of all ages and continues to be of major clinical and economical importance.[2]

13.10.1.1 PREVALENCE

The prevalence of gastric ulceration has been reported for a variety of breeds and types of horses; however, most current data involve Thoroughbreds in race training. The prevalence of squamous ulceration in horses in race training varies from 70% to 94%[3–8] and can be as high as 100% when limited to animals actively racing.[4] In a survey of 50 active show horses, 58% had gastric ulceration, with only 1 horse having ulceration of the glandular fundus.[9] In one large retrospective study (3715 adult horses from 1924 to 1996) evaluating

incidence of gastric ulceration identified at necropsy, an overall prevalence of 10.3% was found. The highest prevalence was found in Thoroughbreds (including Arabians) and Standardbred trotters, and cold-blooded horses were affected significantly less. Lesions were located most commonly in the squamous mucosa along the margo plicatus, followed by the glandular body, proximal squamous mucosa, and antrum.[10]

Many studies investigating prevalence of gastric ulceration do not differentiate between squamous and glandular lesions or evaluate only squamous disease. In a recent study in which the gastric antrum and pylorus were evaluated in 162 horses in a hospital setting, 58% had antral or pyloric erosions or ulcerations, 58% had squamous mucosal lesions, and 8% had lesions involving the glandular body.[11] A correlation between the presence or severity of squamous disease and antral/pyloric disease was not identified.

The reported prevalence of gastric ulceration in foals varies from 25% to 57%.[12–14]

13.10.1.2 PATHOPHYSIOLOGY

An imbalance between inciting and protective factors in the mucosal environment can result in ulcer formation.[15,16] The major intrinsic factors promoting ulcer formation include hydrochloric acid, bile acids, and pepsin, with hydrochloric acid being the predominant factor. Various intrinsic factors protect against ulcer formation such as the mucus-bicarbonate layer, maintenance of adequate mucosal blood flow, mucosal prostaglandin E_2 and epidermal growth factor production, and gastroduodenal motility. In human beings, extrinsic ulcerogenic factors include nonsteroidal antiinflammatory drugs, *Helicobacter pylori,* stress, changes in diet, or gastrointestinal disorders, especially those resulting in delayed gastric emptying.[1] In human neonates, physiologic stress associated with a major primary illness seems to be associated strongly with gastric ulcers.[17] Many of the other factors mentioned previously are believed to be important in horses, but clear evidence of an infectious agent has not yet been identified in horses or foals with EGUS.[18,19] Recently, the possibility of *Helicobacter* infection in horses has reemerged with the identification of polymerase chain reaction products from Urel, a proton-gated urea channel unique to gastric-dwelling *Helicobacter* species, in the squamous epithelium of three horses, two of which had squamous erosions.[20]

The specific factors involved in injury and the protective mechanisms vary between regions of the proximal gastrointestinal tract. The pathophysiology of squamous mucosal ulceration in the horse appears similar to that in gastroesophageal reflux disease in human beings and ulceration of the nonglandular mucosa in pigs. Excess acid exposure is the predominant mechanism responsible for squamous mucosal ulceration, although many details remain unclear.[21] Hydrochloric acid is secreted by parietal cells in the gastric glands via a hydrogen-potassium adenosine triphosphatase (H^+,K^+-ATPase) pump on the luminal side. Horses secrete acid continuously, and measured pH of equine gastric contents varies from less than 2 to greater than 6 depending on the dietary state of the horse (fed or fasted).[22,23] A protocol of repeated 24-hour periods of fasting and feeding has been shown to induce squamous erosion and ulceration.[24] Because this protocol results in periods of prolonged gastric acidity (pH <2.0) and because concurrent administration of the histamine$_2$ (H_2) receptor antagonist ranitidine reduces lesion severity, the protocol supports the role of acid exposure in the pathogenesis of squamous ulcer disease.

Several peptides can stimulate or inhibit parietal cell secretion of acid. The predominant stimuli for hydrochloric acid secretion are gastrin, histamine, and acetylcholine via the vagus nerve.[1] G cells release gastrin within the antral mucosa, whereas mast cells and enterochromaffin-like cells release histamine in the 864

gastric gland. Histamine binds to type 2 receptors on the parietal cell membrane, causing an increase in cyclic adenosine monophosphate and resulting in phosphorylation of enzymes that activate the proton pump. Gastrin and acetylcholine can act via calcium-mediated intracellular pathways and also stimulate histamine release directly.[25] Isolated equine parietal cells respond maximally to histamine stimulation and only minimally to carbachol and pentagastrin.[26] Gastrin release is controlled primarily by gastrin-releasing peptide, which is stimulated by gastric distention and increased luminal pH, but the interaction between gastrin and histamine has not been elucidated fully in the horse. 865

Somatostatin, released by fundic and antral D cells, is the primary inhibitor of gastric acid secretion by parietal cells. The inhibitory effect of somatostatin is primarily paracrine, but plasma levels of somatostatin negatively correlate with gastric luminal acidity.[27] Epidermal growth factor, a peptide produced in saliva, also inhibits gastric acid secretion.[28]

Foals can produce significant amounts of gastric acid by the second day of life, with consistent periods of acidity (pH <2.0) in clinically normal animals.[29,30] In one study, foals tended to have a high gastric pH at day 1 of age,[30] but in a study of critically ill foals, some foals demonstrated periods of gastric acidity on the first day of life.[31] Suckling was associated with an immediate rise in gastric pH, whereas periods of rest in which foals did not suck for more than 20 minutes were associated with prolonged periods of acidity.[29] Whereas premature human infants are capable of gastric acid production at 28 weeks of gestation,[32] only 1 of 7 premature foals demonstrated an acidic pH recording in a study of gastric pH profiles in critically ill foals.[31] However, multiple factors likely were involved in critically ill foals of this study, and the true ontogeny of gastric acid production in foals is currently unknown.

Equine squamous mucosa is thin at birth but becomes hyperplastic and parakeratotic within days.[33] The parallel between decreasing pH and proliferation of squamous epithelium correlates with that observed in other species.[34] The combination of a thin gastric epithelium with a high acid output may leave neonatal foals susceptible to ulcer formation at a young age. In addition, one must remember the difference in normal appearance of the squamous mucosa when interpreting gastric endoscopy in a neonatal population.

In esophageal squamous mucosa, intercellular tight junctions and bicarbonate secretion are the major factors involved in protection against acid injury in other species, although squamous bicarbonate secretion had not been documented in the horse.[35–37] The principal barrier is a glycoconjugate substance secreted by cells in the *stratum spinosum,* with a contribution from the tight junctions in the *stratum corneum*.[37] This barrier function is considered weak at best, and thus a functioning lower esophageal sphincter, normal salivary flow, and salivary mucins contribute to the prevention of acid injury in human gastroesophageal reflux disease. In horses a mechanical barrier like the lower esophageal sphincter is not available to protect the gastric squamous mucosa from acid exposure. The normal gastric fill line rests just below the cardia, so only the squamous mucosa along the lesser curvature adjacent to the margo plicatus should receive exposure to acidic gastric contents regularly. Not surprisingly, this correlates with the most common location of squamous mucosal ulceration.

Bile salts and pepsin have been implicated as contributing factors to ulcer disease in many species. In rabbit esophageal mucosa, bile salt absorption occurs and is correlated directly with mucosal barrier disruption. The unconjugated bile salts cholate and deoxycholate have a pK_a (negative logarithm of the ionization constant of an acid) of 5 and 5.3, respectively, and therefore cannot remain in solution and cause mucosal damage in the presence of acid. Alternatively, the conjugated bile salt taurocholate (pK_a 1.9) can cause mucosal injury in the

ionized salt form at pH 7 or the un-ionized acid form at pH 1 to 2.[38] In the pig, bile salts or acid alone cause squamous mucosal damage, whereas a combination of the two result in extensive damage in vitro.[39] In the horse a similar synergistically damaging effect was found with the addition of bile salts and acid (pH 2.5) to stratified squamous mucosa in vitro in one study.[40] In addition, the investigators were able to document levels of bile salts and acid sufficient to cause mucosal damage in gastric contents within 14 hours of feed deprivation. This is not surprising, given that duodenogastric reflux occurs normally in the horse.[41] In a separate in vitro study of equine squamous mucosa, prolonged exposure to acid alone (pH 1.5) had a damaging effect, and synergism with exposure to a combination of acid and pepsin or taurocholate was not found.[42] The lack of synergism likely is caused by the lower pH used in this study and stresses the importance of acid exposure in squamous ulcer disease.

Pepsinogens are secreted primarily by chief cells, although secretion by neck cells, cardiac glands, and antral pyloric glands also occurs.[43] In an acidic environment (pH <3.0), pepsinogen is converted to the active pepsin. Although the proteolytic activity of pepsin normally is directed toward dietary protein, it also can act on the gastric mucosa.[44] Thus acid remains the major contributing factor to squamous mucosal damage, although other factors such as pepsin and bile salts may play an important role as well in the initiation or perpetuation of disease.

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Several mechanisms help protect the glandular mucosa from acid injury. The mucus-bicarbonate layer serves to titrate H^+ ion from the gastric lumen to CO_2 and H_2O. Cellular restitution and prostaglandins of the E series, which enhance mucosal blood flow and secretion of mucus and bicarbonate in the glandular mucosa have not been documented in squamous epithelium.[21,36] Of these mechanisms, mucosal blood flow is likely the most important contributor to overall gastric mucosal health. Nitric oxide is a key regulator of mucosal blood flow and prostaglandin synthesis and thus may play a role in mucosal protection.[45]

Dietary factors also have been implicated in ulcer disease. Horses in race training have a high incidence of gastric ulceration and frequently are fed high-concentrate, low-roughage diets. In one study, higher volatile fatty acid (acetic, propionic, and isovaleric acid) concentrations, higher gastric juice pH, and lower number and severity of nonglandular ulceration were documented after feeding an alfalfa hay-grain diet compared with a bromegrass hay diet.[46] However, many factors differed between the diets, such as digestible energy, bulk, crude protein, and mineral content (especially calcium). Thus dietary factors represent an important area of further investigation in the pathophysiology of EGUS, particularly squamous ulceration.

The pathophysiologic correlation between exercise and squamous ulcer disease has not yet been defined despite the high prevalence of ulceration in performance horses. Preliminary work suggests that gastric compression occurs during treadmill exercise, presumably because of an increase in intraabdominal pressure.[47] Such contracture could result in increased acid exposure to the squamous mucosa by raising the fill line of gastric contents. Further studies in this laboratory have provided support for this theory by demonstrating a high pH in the proximal stomach, immediately distal to the lower esophageal sphincter, during resting conditions that decreases during treadmill exercise (M. Lorenzo-Figueras and A.M. Merritt, personal communication, 2002).

Risk factors associated with gastric ulceration include gender and age, and the reported prevalence of gastric ulcers has increased over time. In one study, ulcers were found more commonly in stallions, and the prevalence of gastric ulceration decreased with age, independent of gender, although this trend was only significant in the population of Standardbred trotters.[10] Interestingly, the frequency of gastric ulceration

increased from less than 6% before 1945 to approximately 18% after 1975. In a study of Thoroughbred horses in race training, an increase in squamous ulcer severity was noted in horses 3 years old or older and in those horses that had raced.[4] In the same study, severity of glandular lesions did not change between examinations, and age (>3 years) was the only factor associated with glandular lesion severity.

Several studies have failed to document a correlation between nonsteroidal antiinflammatory drug (NSAID) administration and naturally occurring ulcer disease.[3,4,6,7,10] However, NSAID administration is a well-known cause of gastric ulceration under experimental conditions.[48–52] NSAID-related ulceration typically is described as predominantly glandular, although nonglandular ulceration also can occur by a mechanism that has not yet been characterized fully. NSAIDS cause a decrease in prostaglandin E_2 synthesis because of inhibition of the cyclooxygenase pathway. Therefore a resultant decrease in glandular mucosal protection, most notably via decreased mucosal blood flow and mucus production, is the most likely mechanism of action. In one study, however, phenylbutazone administration resulted in ulceration of the glandular mucosa at the pyloric antrum but did not alter mucosal prostaglandin E_2 concentration significantly.[52]

13.10.1.3 CLINICAL SYNDROME: NEONATAL FOALS

Clinical signs typically associated with gastric ulceration in foals include poor appetite, diarrhea, and colic. Many foals probably never exhibit clinical signs, and some do not exhibit clinical signs until ulceration is severe or fatal perforation has occurred. Glandular ulceration typically is considered the most clinically significant type of disease in this population.

The physiologic stress of a concurrent illness has been associated with gastric ulceration in foals. Retrospectively, 14 (23%) of 61 foals up to 85 days of age with a clinical disorder were found to have lesions in the gastric glandular mucosa,[13] and prospectively 8 (40%) of 20 foals up to 30 days of age with a clinical disorder had glandular ulceration.[53] By contrast, only 4% to 9% of clinically normal foals examined in endoscopic surveys had lesions observed in the gastric glandular mucosa.[14,54]

Critically ill neonatal foals can have a greatly different pH profile compared with that in clinically normal foals, potentially because of alterations in gastric motility and acid secretion.[31] Gastric ulceration was not identified in any animals at necropsy in that study; however, ulceration has been documented in a similar population.[12] Thus factors other than acid exposure, most notably mucosal blood flow, may play an important role in the stress-related ulceration in neonates. Subjectively, gastric ulceration and rupture in the hospitalized neonatal population occurs less commonly now than in previous reports. Advances in overall neonatal care, especially supportive care, likely have contributed to this decline.

13.10.1.4 CLINICAL SYNDROME: SUCKLINGS/WEANLINGS

In suckling foals less than 50 days old, lesions typically originate in the squamous mucosa adjacent to the margo plicatus along the greater curvature. Such lesions can occur in foals as young as 2 days of age and have 866
been observed in 50% of foals less than 50 days old. Histologic examination of these lesions has revealed 867
disruption of the epithelial layers of the mucosa and a neutrophilic infiltration. Another phenomenon that occurs in young foals is the shedding, or desquamation, of squamous epithelium, which appears as flakes or sheets of epithelium. Desquamation occurs without ulceration in up to 80% of foals less than 35 days of age, and this process typically is not associated with clinical signs.[13,14,54]

In older foals, lesions become more prevalent in the squamous mucosa, particularly along the lesser curvature.[53] Lesions also are found in the squamous mucosa of the fundus and adjacent to the margo plicatus. These lesions can be severe and often are associated with clinical signs such as diarrhea, poor appetite, and poor growth and body condition. Diarrhea is the most frequent sign in symptomatic foals with squamous mucosal lesions and is associated with more diffuse erosion or ulceration of the squamous mucosa than that which occurs in asymptomatic foals. In some foals, poor growth, rough hair coat, a potbelly appearance, or all of those occur along with moderate to severe squamous mucosal ulceration. In horses with severe or diffuse squamous ulceration, bruxism or colic may occur.

Gastroduodenal ulcer disease occurs almost exclusively in suckling and early weanling foals. Clinical signs of duodenal ulceration are similar to those described for gastric ulceration (bruxism, colic, salivation, diarrhea), but the consequences are often more severe. Lesions occur primarily in the proximal duodenum and range from diffuse inflammation to severe ulceration. Foals with duodenal ulceration often have delayed gastric emptying and may have gastroesophageal reflux. Complications can include gastric or duodenal rupture, duodenal stricture, and ascending cholangitis. Severe squamous and esophageal ulceration and aspiration pneumonia can occur following gastroesophageal reflux.[15,55–58]

The gastroduodenal ulcer disease syndrome can occur in outbreaks and most commonly is identified in intensive breeding operations. The cause of duodenal lesions in foals is not known. One theory is that the problem begins with diffuse duodenal inflammation that can coalesce down to a focal area of ulceration (G.D. Lester and A.M. Merritt, personal communication, 2002). A temporal relationship between gastroduodenal ulcer disease and rotaviral diarrhea has been suggested, but an infectious cause remains unproven. Although lesion location and severity associated with rotaviral infection varies among species, duodenal ulceration has not been reported.[59]

13.10.1.5 CLINICAL SYNDROME: YEARLINGS AND ADULT HORSES

Clinical signs attributable to EGUS in older horses vary and classically include anorexia and chronic or intermittent colic of varying severity.[60] Many horses with endoscopic evidence of disease may appear to be clinically normal or have vague signs that include decreased consumption of concentrates, postprandial episodes of colic, poor performance or failure to train up to expectations, poor-quality hair coat, and decreased condition or failure to thrive. Diarrhea typically is not associated with gastric ulceration in adult horses, although ulceration can occur concurrently with other causes of diarrhea. Horses actively racing are more likely to have squamous ulceration than those solely in training.[4]

Lesions occur predominantly in the squamous mucosa, particularly adjacent to the margo plicatus (Figure 13.10-1). In more severe cases, lesions can extend dorsally into the squamous fundus. Clinically relevant lesions typically affect a greater portion of the squamous mucosa and can be deep enough to cause bleeding. However, bleeding from ulcers in the gastric squamous mucosa typically is not associated with anemia or hypoproteinemia.

According to a recent study, the incidence of glandular lesions, particularly within the pyloric region, may be higher than previously reported,[11] which emphasizes the importance of a thorough endoscopic examination and proper documentation of lesion location when reporting or discussing EGUS, especially the differentiation between squamous and glandular disease.

Figure 13.10-1 Endoscopic view of the right side of the stomach of a horse with recurrent colic and poor appetite. The large area of ulceration on the squamous mucosa adjacent to the margo plicatus is notable.

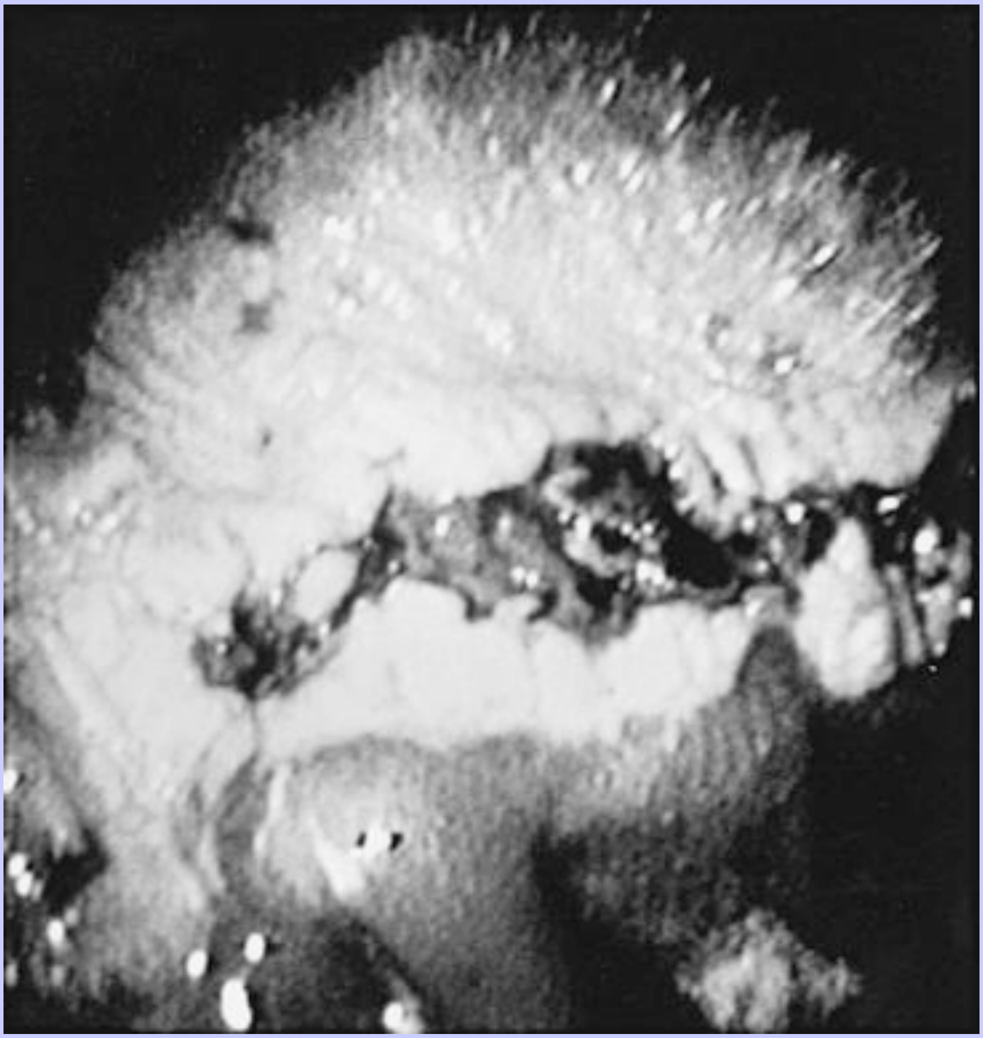

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TABLE 13.10-1 Equine Gastric Ulcer Syndrome Lesion Scoring System

LESION GRADE	DESCRIPTION
0	Intact epithelium with no appearance of hyperemia or hyperkeratosis
1	Intact mucosa with areas of reddening or hyperkeratosis (squamous)
2	Small single or multifocal lesions
3	Large single or multifocal lesions or extensive superficial lesions
4	Extensive lesions with areas of deep ulceration

From Andrews FM, Bernard WV, Byars TD et al: Recommendations for the diagnosis and treatment of equine gastric ulcer syndrome (EGUS), *Equine Vet Educ* 1:122–134, 1999.

13.10.1.6 DIAGNOSIS

Although one may suspect a diagnosis of EGUS based on clinical signs and response to treatment, the only current method of confirmation is via gastroendoscopy, which one can perform easily in the standing horse or foal with mild sedation. In adult horses a 3-m endoscope allows for visual inspection of the entire stomach, pylorus, and proximal duodenum. Shorter scopes permit examination of the gastric body and fundus, but not the pyloric antrum in most cases. One should use an endoscope with a maximum external diameter of 9 mm for neonatal foals. Numerous scoring systems for lesion severity have been described, but a recent consensus has been published by the Equine Gastric Ulcer Council (Table 13.10-1).[2]

Duodenal ulceration can be difficult to confirm. Duodenoscopy is the most specific means of diagnosis, although the procedure is more difficult than gastroscopy. Additionally, an endoscope at least 200 cm in length is needed for foals up to 5 to 7 months old, and a longer endoscope usually is required for older animals. Diffuse reddening or inflammation may be the only recognizable lesion in cases of early duodenal disease.

Excessive enterogastric reflux of bile through the pylorus suggests duodenal dysfunction. However, the pylorus frequently appears open, and some degree of enterogastric reflux is common under normal conditions. Ulceration at the pylorus or pyloric antrum also suggests the presence of a duodenal lesion. If one can perform gastroendoscopy, but not duodenoscopy, the severity of lesions, particularly in the glandular mucosa and in the squamous mucosa of the lesser curvature dorsal to the pyloric antrum, usually will be severe when duodenal ulcers are present.

TABLE 13.10-2 Therapeutic Options for Treating Equine Gastric Ulcer Syndrome

DRUG	DOSE (mg/kg)	DOSING INTERVAL (HOURS)	ROUTE OF ADMINISTRATION
Ranitidine	6.6	8	Orally
Ranitidine	1.5–2	6–8	Intravenously or intramuscularly
Cimetidine	20–25	8	Orally
Cimetidine	6.6	6–8	Intravenously or intramuscularly
Omeprazole	2–4	24	Orally
Sucralfate	20–40	8	Orally
Aluminum/magnesium antacids	0.5	4	Orally

13.10.1.7

TREATMENT

Multiple pharmacologic treatments have been suggested for treating EGUS. Because acid has been implicated as the most important pathophysiologic component of squamous ulcer disease, most antiulcer therapy centers on suppression or neutralization of gastric acid. Severity and location of gastric lesions and severity and duration of clinical signs, as well as medication cost, can play a role in the therapeutic management of EGUS (Table 13.10-2).

If gastroendoscopy is unavailable, some guidelines to therapy can be used, but the efficacy of the treatment is based on clinical signs, which are often vague or nonspecific. Signs of colic or diarrhea that result from gastric ulcers often resolve within 48 hours. One can note improvements in appetite, bodily condition, and attitude within 1 to 3 weeks. If one does not observe improvement in clinical signs, treatment has not been effective or gastric ulceration was not the primary problem.

The principal therapeutic options for ulcer treatment include H_2 antagonists (cimetidine, ranitidine, famotidine, nizatidine), proton pump blockers (omeprazole, pantoprazole, rabeprazole, esomeprazole), the mucosal adherent sucralfate, and antacids.

The H_2 antagonists suppress hydrochloric acid secretion through competitive inhibition of the parietal cell histamine receptor that can be overcome partially with exogenous pentagastrin.[61] Use of H_2 antagonists has been successful in raising gastric pH and resolving gastric lesions in foals and adult horses.[29,55,62]

Clinical and experimental evidence has demonstrated greater individual variability with lower dosages of H_2 antagonists.[63] Thus dosage recommendations are based on levels necessary to increase gastric pH and promote ulcer healing in a majority of horses. Commonly recommended dosages are 20 to 30 mg/kg orally every 8 hours or 6.6 mg/kg intravenously every 6 hours for cimetidine and 6.6 mg/kg orally every 8 hours or 1.5 to 2 868 869

mg/kg intravenously every 6 hours for ranitidine. Famotidine has been used less extensively in the horse, but a dose of 10 to 15 mg/kg/day has been recommended.

Because gastric perforation caused by glandular ulcer disease has been reported in hospitalized neonates, many clinicians routinely use prophylactic antiulcer therapy in this population. Although clinically normal foals respond predictably to ranitidine,[29] sick neonates have shown variability in pH response to intravenously administered ranitidine, with a much shorter duration of action and in some cases no noticeable response.[31] Thus currently used dosing schedules for hospitalized foals may be inadequate. Because some critically ill foals have a predominantly alkaline gastric pH profile and because gastric acidity may be protective against bacterial translocation in neonates, the need for prophylactic ulcer therapy is controversial. In critically ill human neonates, intravenous administration of ranitidine raises gastric pH and gastric bacterial colonization but does not increase the risk of sepsis.[64] In a retrospective study of 85 hospitalized foals less than 30 days of age, no difference in the frequency of gastric ulceration at necropsy was found between those foals that received prophylactic treatment for gastric ulcers and those that did not.[65] Because the study was retrospective, specific details regarding lesion location and severity were not available; however, none of the foals in the study died because of gastric ulcer disease.

H_2 antagonist therapy should continue for 14 to 21 days, but complete ulcer healing may take 30 to 40 days. If an animal is kept in race training during therapy, clinical signs may resolve but the lesions may not. Currently, cimetidine and ranitidine are available in injectable, tablet, and liquid forms. Famotidine and nizatidine are available in tablets.

Proton pump inhibitors block secretion of H^+ at the parietal cell membrane by irreversibly binding to the H^+,K^+-ATPase proton pump of the cell. These agents have a prolonged antisecretory effect, which allows for once-daily dosing. Omeprazole, the first proton pump inhibitor to be developed, is the only currently approved agent for the treatment of EGUS.

Several studies have documented the safety of orally administered omeprazole in foals and adult horses.[66,67] Omeprazole has demonstrated efficacy in the healing of NSAID-induced ulcers in horses and in naturally occurring cases of EGUS.[68,69] More importantly, omeprazole has been shown to eliminate or reduce the severity of gastric ulcers in Thoroughbreds maintained in race training.[70]

The available equine preparation of omeprazole (GastroGard, Merial, Ltd., Duluth, Georgia) is recommended at a dose of 4 mg/kg orally every 24 hours. Initial reports suggested that 3 to 5 days of omeprazole therapy were necessary to achieve maximum acid suppression; however, an increase in gastric pH and a decrease in acid output are evident 5 to 8 hours after omeprazole paste administration.[71] After initial treatment (28 days), treatment with 2 or 4 mg/kg every 24 hours has been shown to decrease or prevent the recurrence of disease in animals maintained in training.[72] The powder form of omeprazole degrades rapidly in an acidic environment, thus one must use an enteric-coated capsule (as used in the human preparation) or a specially formulated paste (such as GastroGard) to allow delivery of the active drug to the small intestine for absorption. Many compounding pharmacies prepare omeprazole in liquid or paste formulation for use in horses, but their efficacy has not been evaluated to date.

Other proton pump inhibitors have been developed recently for use in human beings, including rabeprazole, lansoprazole, esomeprazole, and pantoprazole. In gastro esophageal reflux disease treatment in human beings, esomeprazole has demonstrated a higher rate of healing at 4 and 8 weeks compared with omeprazole, but

rabeprazole, lansoprazole, and pantoprazole have similar efficacy.[73] An intravenous formulation of pantoprazole recently became available commercially and may prove beneficial for patients in need of antiulcer therapy that cannot be treated orally. Research regarding the pharmacokinetics and efficacy of other proton pump inhibitors in horses is not currently available.

Sucralfate is effective in treating peptic ulcers and preventing stress-induced ulcers in human beings. The mechanism of action likely involves adherence to ulcerated mucosa, stimulation of mucus secretion, enhanced prostaglandin E synthesis, and increased concentration of growth factor at the site of ulceration, although the prostaglandin effects may not play an important role in ulcer healing.[74] These are factors relevant to glandular mucosa, and the efficacy of sucralfate in treating ulcers in the equine gastric squamous mucosa remains undetermined. Sucralfate may be effective in preventing stress-induced ulcers in neonatal foals, because these occur in the glandular mucosa, although no clinical evidence directly supports this concept. In human beings, sucralfate provides protection against stress-induced ulcers with a decreased risk of pathogenic gastric colonization.[75] One should give sucralfate at a dosage of 10 to 20 mg/kg every 6 to 8 hours. The efficacy of sucralfate in an alkaline pH is controversial but appears likely.[76–78] Moreover, at the time of administration of 869
an H_2 antagonist, the gastric pH likely will have returned to an acidic pH since the last dosage and will remain 870
so for 30 to 60 minutes depending on the route of administration; thus one likely can administer the agents simultaneously if so desired.

The use of antacids to treat gastric ulcers has not been examined critically in the horse. Research in horses has shown that 30 g aluminum hydroxide per 15 g magnesium hydroxide results in an increase in gastric pH above 4 for approximately 2 hours.[79] Thus although antacids may be useful for treating ulcers in horses, a dose of approximately 180 to 200 ml at least every 4 hours is necessary for a standard adult horse.

The use of synthetic prostaglandin E_1 analogs, such as misoprostol, has been effective in treating gastric and duodenal ulcers in human beings, and the proposed mechanism of action involves inhibition of gastric acid secretion and mucosal cytoprotection.[80] Frequently reported adverse effects of intestinal cramping and diarrhea in human beings have precluded the use of misoprostol in horses.

One should consider prokinetic drugs in foals with duodenal disease and gastroesophageal reflux and when one suspects delayed gastric emptying without a physical obstruction. The cholinergic drug bethanechol has been shown to increase the rate of gastric emptying in horses.[81] In cases of acute gastric atony, bethanechol 0.025 to 0.030 mg/kg administered subcutaneously every 3 to 4 hours has been effective in promoting gastric motility and emptying, followed by oral maintenance dosages of 0.35 to 0.45 mg/kg 3 to 4 times daily. Adverse effects can include diarrhea, inappetance, salivation, and colic, but at the dosages stated, adverse effects have been infrequent and mild. A complete review of ileus and prokinetic therapy is available in Chapter 13.6.

For foals with severe gastroduodenal ulcer disease that have developed duodenal stricture, surgical therapy is necessary.[57,82] These animals require a serious financial commitment because intensive perioperative medical therapy is critical for a successful outcome. Even with surgical therapy, these foals often warrant a guarded prognosis.

13.10.2 Other Disorders of the Stomach

13.10.2.1 PYLORIC STENOSIS AND DELAYED GASTRIC EMPTYING

Pyloric stenosis is a structural resistance to gastric outflow. Congenital pyloric stenosis has been reported in foals and one yearling and results from hypertrophy of the pyloric musculature.[83–85] Acquired pyloric stenosis can result from neoplasia or duodenal ulceration.[86–89] Clinical signs depend on the degree of obstruction and include abdominal pain, salivation, and teeth grinding. Complete or near complete obstruction can result in gastric reflux and reflux esophagitis. In foals with congenital pyloric hypertrophy, clinical signs may begin with the consumption of solid feed. In foals one can make a presumptive diagnosis via gastric endoscopy and radiography (plain and contrast studies). Depending on the cause and severity of disease, gastric endoscopy may provide a presumptive diagnosis in the adult horse. Measurement of gastric emptying can aid the diagnosis. Several methods of measurement are currently available, including nuclear scintigraphy, acetaminophen absorption, and postconsumption [^{13}C] octanoic acid blood or breath testing.[81,90,91] Exploratory laparotomy shows a distended stomach and thickened pylorus accompanied by a relatively empty intestinal tract.

If complete obstruction is not present, medical therapy with a prokinetic such as bethanechol can increase the rate of gastric emptying.[81] Phenylbutazone and cisapride also have been shown to attenuate the delay in gastric emptying caused by endotoxin administration.[90,92] Surgical repair is necessary for definitive treatment of complete or near-complete obstruction and consists of gastroenterostomy or pyloroplasty.[57,82]

13.10.2.2 GASTRIC DILATION AND RUPTURE

Gastric dilation can be classified as primary, secondary, or idiopathic. Causes of primary gastric dilation include gastric impaction, grain engorgement, excessive water intake after exercise, aerophagia, and parasitism.[86,93] Secondary gastric dilation occurs more commonly and can result from primary intestinal ileus or small or large intestinal obstruction. Time to development of gastric reflux is proportional to the distance to the intestinal segment involved, with duodenal obstruction resulting in reflux within 4 hours.[94] Clinical signs of gastric dilation include those associated with acute colic and in severe cases, ingesta appearing at the nares. Associated laboratory abnormalities include hemoconcentration, hypokalemia, and hypochloremia.[86]

The most common reported cause of gastric rupture in horses varies between reports. In a retrospective study of 54 horses, gastric rupture occurred most commonly as a secondary phenomenon (65%), usually because of small intestinal obstruction, with primary gastric dilation and idiopathic rupture occurring almost equally (15% and 17%, respectively).[93] In another retrospective study of 50 horses in combination with a search of the Veterinary Medical Database (VMDB), 60% of the gastric rupture cases were classified as idiopathic.[95] Risk factors for gastric rupture include feeding grass hay, not feeding grain, gelding, and a nonautomatic water source.[93,95] Nasogastric intubation does not preclude the possibility of gastric rupture, and the amount of 870
reflux obtained before rupture varies greatly.[93] Because these reports were retrospective, one cannot rule out 871
confounding factors with certainty.

Regardless of the initiating cause, gastric rupture usually occurs along the greater curvature. In horses with rupture caused by gastric dilation, tears in the seromuscular layer are frequently larger than the corresponding

tears in the mucosal layer, indicating that the seromuscularis likely weakens and tears before the mucosa.[93,95] In contrast, horses with gastric rupture following gastric ulceration usually demonstrate full-thickness tears of equal size in all layers. Gastric rupture is usually fatal because of widespread contamination of the peritoneal cavity, septic peritonitis, and septic shock. Initial clinical signs vary with the primary disease; however, when rupture occurs, a previously painful animal can exhibit signs of relief. Subsequent signs are consistent with peritonitis and shock, including tachypnea, tachycardia, sweating, and muscle fasciculations. Surgical repair is thus limited but has been reported for partial-thickness tears,[96] and in one case of a combined tear of the mucosa and muscularis with only a focal serosal tear, a full-thickness repair was performed with a favorable outcome.[97]

13.10.2.3 GASTRIC IMPACTION

Gastric impaction can result in acute or chronic signs of colic in the horse. Although a specific cause is not always evident, ingestion of coarse roughage (straw bedding, poor-quality forage), foreign objects (rubber fencing material), and feed that may swell after ingestion or improper mastication (persimmon seeds, mesquite beans, wheat, barley, sugar beet pulp) have been implicated. Possible predisposing factors include poor dentition, poor mastication and rapid consumption of feedstuffs, and inadequate water consumption. Clinical signs can vary from anorexia and weight loss to those consistent with severe abdominal pain. In severe cases, spontaneous reflux may occur, with gastric contents visible at the nares. In cases of acute severe abdominal pain, one often makes a diagnosis during exploratory celiotomy. In animals not exhibiting signs of colic warranting surgical intervention, an endoscopic finding of a full stomach after a normally adequate fast (18 to 24 hours) often can confirm the diagnosis. Abdominal radiographs are reserved for smaller horses and ponies. In addition to pain management, specific treatment consists of gastric lavage via nasogastric intubation or massage and injection of fluid to soften the impaction during laparotomy.[98–100]

13.10.2.4 MISCELLANEOUS CAUSES OF GASTRITIS

Nonulcerative gastritis rarely occurs in the horse; however, a single case of emphysematous gastritis caused by *Clostridium perfringens* has been reported.[101]

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13.11
13.11—Duodentitis–Proximal Jejunitis (Anterior Enteritis, Proximal Enteritis)

Rebecca S. McConnico

Duodenitis–proximal jejunitis (DPJ) is an inflammatory condition affecting the upper small intestine and resulting in distention, abdominal pain, gastric reflux caused by excessive fluid and electrolyte secretion, and increased peritoneal fluid protein concentration without a significant elevated nucleated cell count. Clinical signs of DPJ 873

874 mimic that of a strangulating or nonstrangulating small intestinal obstruction, so distinguishing between the two syndromes is important because appropriate treatment of small intestinal obstruction usually requires surgical intervention. Studies suggest that the survival rate for horses with DPJ that endured surgical exploratory laparotomy was poor compared with those treated medically, although differences in disease severity may have accounted for the results in these early reports.[1,2] The clinical syndrome of DPJ was well described in the 1980s, and although recognized by its classical presentation, varying degrees of focal intestinal and systemic illness may occur.[1–4] DPJ usually occurs alone but can occur along with gastritis, ileitis, typhlitis, and or colitis.

13.11.1 Pathophysiology

Typical pathologic findings in horses with DPJ include involvement of the duodenum and usually the proximal jejunum.[3] The ileum and large colon usually are determined to be grossly normal. Gastric distention is a common finding and is thought to be caused by hypersecretory mechanisms in the proximal small intestine and a functional ileus of affected enteric segments. The small intestine may be 5 to 7 cm in diameter because of fluid distention with malodorous, red to brown-red intralumenal fluid accumulation. Duodenal (and jejunal) serosal surfaces may have varying degrees and distribution of bright-red to dark-red petechial and ecchymotic hemorrhages and yellow to white streaks. The enteric mucosal surfaces are usually hyperemic and have varying degrees of petechiation and ulceration.

Microscopically, the most severe lesions have been located in the duodenum and proximal jejunum but may extend proximally to the gastric mucosa and aborally to the large intestinal mucosa and submucosa.[3] Microscopic lesions consist of varying degrees of mucosal and submucosal hyperemia and edema. More severe lesions include villus degeneration with necrosis and more severely, sloughing of villous epithelium. The lamina propria, mucosa, and submucosa may have varying degrees of granulocyte infiltration (predominantly neutrophils), and the muscular layers and serosal surfaces contain small hemorrhages. Proximal small intestinal serosal fibrinopurulent exudate is a common finding in the more severe cases; therefore the term *hemorrhagic fibrinonecrotic duodenitis–proximal jejunitis* has been suggested as a more descriptive name for this syndrome.

Horses with DPJ often have evidence of multiple organ involvement such as hepatic changes including congestion and varying degrees of biliary duct hyperplasia. Additional systemic involvement likely is caused by endotoxin absorption, metabolic imbalances such as acidemia, and circulatory changes.

The cause of this syndrome remains an enigma (much like the cause of other inflammatory conditions affecting the intestinal tract). Several microorganisms have been implicated as playing a role in triggering DPJ, including *Clostridium* spp., *Salmonella* spp., and some mycotoxins, but efforts to reproduce the syndrome experimentally have been futile.[5] A recent dietary change with an abrupt increase in dietary concentrate level has been suggested to predispose a horse to developing DPJ because of intraluminal microbial imbalances.

Two intracellular processes control intestinal secretion, the cyclic nucleotide (cyclic adenosine monophosphate and cyclic guanosine monophosphate) and the calcium systems.[6] Agents (inflammatory mediators, microorganisms, toxic agents) can activate adenyl cyclase (vasoactive intestinal peptide, prostaglandin E_2) or guanyl cyclase (bacterial enterotoxins) and induce increases in cyclic adenosine monophosphate and cyclic guanosine monophosphate, respectively. This reaction causes phosphorylation of specific protein kinases, which induce the actual mucosal membrane transport events. Increases in intracellular free calcium may arise from cyclic nucleotide–dependent release of stored calcium within the cell or from increased calcium entry across the cell membrane.[7] Calcium may act through calmodulin, which then can activate membrane-phosphorylating protein kinases. The net effect is increased movement of sodium and chloride into the mucosal cell from the interstitium, with secretion of sodium and chloride into the intestinal lumen. Water follows the directional flux of sodium and chloride through highly permeable intercellular spaces. Several bacterial toxins and endogenous mediators can cause active secretion and contribute to a synergistic mucosal secretory response. Passive secretion of protein-rich fluid into the lumen occurs following damage to the mucosal epithelium, capillary endothelium, and submucosal inflammation in the proximal small intestine. The clinically relevant events that result from active and passive fluid secretion are proximal small intestinal distention and nasogastric reflux, dehydration, and circulatory shock.

The concentration of protein in the peritoneal fluid from horses with DPJ is usually higher than in horses with small intestinal obstruction. A disproportionate increase in total protein concentration relative to nucleated cell count occurs probably by leakage of blood or plasma into the peritoneal cavity without a significant stimulus for leukocyte chemotaxis. Suggested mechanisms for increased abdominal fluid protein concentration include serositis associated with inflamed intestine and small intestinal distention causing passive congestion and increased capillary hydrostatic pressure of visceral peritoneal vessels.[8]

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Small intestinal ileus is another hallmark sign of DPJ and the pathophysiology is complicated, involving primary and secondary dysfunction of the central, autonomic, and enteric nervous systems and their purported roles in governing intestinal motility.[9] Primary role-players in DPJ-associated ileus include peritoneal inflammation, inflammatory cell migration/activation within the muscularis, small intestinal mechanical distention, and effects of endotoxin absorption. The use of prokinetic agents for treating ileus and gastric/small intestinal distention in horses with DPJ is becoming more common, but veterinarians should realize that a potential restriction on their use is the need for normal intestinal integrity. In spite of that, one may use motility modifiers judiciously. 875

13.11.2 Clinical and Clinicopathologic Signs

The veterinarian has the challenge of differentiating horses with DPJ from horses with small intestinal obstructive lesions so as to avoid surgical intervention (Table 13.11-1). Horses with DPJ typically show signs of acute abdominal pain initially, and then after gastric decompression, volume replacement, and analgesic therapy, the colic signs subside, but signs of lethargy and malaise become more apparent. In contrast, horses with obstructive lesions of the small intestine usually show signs of abdominal pain until the affected viscus is repaired via surgical intervention or the viscus ruptures. Another differentiating characteristic is the large volume (>4 to 20 L with each decompressive effort) of nasogastric reflux that is often malodorous and orange-brown or red-brown. DPJ-affected horses have moderate to severe small intestinal distention palpated on rectal examination, temperature of 38.6° to 39.1° C (101.5° to 102.5° F), dehydration, brick-red mucous membranes, lethargy and absent borborygmi, prolonged capillary refill time, tachycardia (>60 beats/min), and tachypnea. Although the signs of abdominal pain usually resolve after gastric decompression, most horses remain severely lethargic. Without periodic removal of the fluid that accumulates in the proximal intestinal tract, signs of abdominal pain usually recur. Horses with DPJ often require gastric decompression at 2-hour intervals, with 2 to 10 L of fluid recovered each time. Nasogastric tubes left in place for long periods of time cause varying degrees of pharyngitis, laryngitis, and esophagitis.

TABLE 13.11-1 Physical and Laboratory Examination Findings in Horses With Duodenitis–Proximal Jejunitis (DPJ) and an Operable Small Intestinal Lesion

EXAMINATION	DPJ	STRANGULATING OBSTRUCTION	NONSTRANGULATING OBSTRUCTION
Abdominal pain	Acute onset	Acute onset	Acute onset
Attitude	Depressed, painful	Painful	Painful
Heart rate (beats/min)	40–80	≥80	40–80
Respiratory rate (breaths/min)	16–28	24–40	15–35
Rectal temperature (°F)	101.5–102.5	≤101	99–101
Capillary refill time	>3 seconds	≥3 seconds	1.5–3.0 seconds
Intestinal sounds	Mildly to greatly depressed	Greatly depressed to absent	Mildly depressed to absent
Nasogastric reflux	3–4 gallons orange-brown	2–4 gallons, ± malodorous	2–3 gallons with ingesta character, ± malodorous
Rectal examination	Dilated to moderately distended	Moderately to greatly distended	Mild to moderately distended, ± ileal impaction
Response to nasogastric decompression	Depressed, quiet	Temporary to no pain relief	Temporary to no pain relief
Complete blood count	± Increased white blood cells; mature neutrophilia	± Increased white blood cells; slight neutrophilia	Normal to increased white blood cells
Peritoneal fluid protein	>3.0 g/dl and may be >4.5 g/dl	2.5–4.5 g/dl	2.5–3.5 g/dl
Peritoneal fluid nucleated cell count	≤5000/μ l	3000 to >20,000/μl	3000 to 12,000/μl

*Findings in which differences between DPJ and obstructive disorders are often present are italicized.

Typical clinical laboratory findings include an increased packed cell volume and total plasma protein reflective of volume depletion, a metabolic acidosis (with elevated anion gap) in longstanding or severe cases, an increased peritoneal fluid protein concentration (often >3.5 g/dl), and a mild to moderate elevation of the peritoneal white 875
blood cell count, although the count usually is less than 10,000 cells per microliter.[3,4] The peritoneal fluid is 876
usually yellow and turbid, but in severe cases diapedesis occurs resulting in a serosanguinous color. The white blood cell count in the peripheral blood may be normal, decreased, or increased. In addition, hyponatremia, hypochloremia, hypokalemia, and acid-base alterations (elevated anion gap) are often evident. The loss of enteric bicarbonate through evacuation of enterogastric reflux and poor tissue perfusion from hypovolemia can lead to metabolic acidosis. One makes a definitive diagnosis of DPJ in most cases by gross examination of the duodenum and proximal jejunum at surgery or at necropsy. Some equine practitioners have observed an apparent

geographic relationship in the incidence and severity of the syndrome, with more cases occurring in the southeastern United States.

13.11.3 Treatment

Horses with DPJ appear to share a common characteristic clinical presentation, and the mechanisms leading to electrolyte imbalances, fluid loss, ileus, and endotoxemia and septicemia are similar. Treatment regimens are supportive and aim at plasma volume replacement (usually in the form of crystalloid fluid replacement), analgesia and antiinflammatory therapy, gastric decompression, antiendotoxin therapy, antimicrobial therapy if indicated, nutritional support, and nursing care.

13.11.3.1 FLUID THERAPY

One should institute aggressive intravenous polyionic fluid therapy immediately in a horse with DPJ. One should calculate the total fluid deficit based on clinical assessment of dehydration (e.g., for 8% or moderate dehydration, 0.08 × 450 kg body mass = 36 L) and should administer replacement fluids rapidly (up to 6 to 10 L per hour per 450-kg adult horse). Administering intravenous hypertonic saline (7%) may be useful to treat hypovolemic shock in horses with severe circulatory shock. The use of 1 to 2 L of hypertonic saline (7% NaCl) improved systemic blood pressure and cardiac output in horses with hemorrhagic shock and in a model of equine endotoxemia.[10] If one chooses this treatment option, intravenous administration of replacement isotonic fluids must follow immediately to maintain tissue integrity. One should not allow horses with significant volumes of gastric reflux to ingest foodstuffs or liquids orally.

Once one has administered replacement fluids and the horse is well hydrated, one should administer maintenance fluid amounts, which may be as high as 120 ml/kg/day. Unfortunately, the intravenous fluid therapy itself may accelerate the flux of fluid from the vasculature into the intestinal lumen because of a reduction in intravascular oncotic pressure and an increased capillary perfusion pressure, which can result in an increased volume of gastrointestinal reflux. However, the veterinarian should not consider reducing the volume of intravenous fluid therapy because excessive fluid losses continue to occur. One should monitor plasma protein concentration, overall hydration, and the volume of reflux and then determine the rate of intravenous fluid administration. During the initial hours of therapy, even aggressive intravenous fluid administration results in only moderate clinical improvement. The clinical response, as evidenced by improved hydration status, decreased nasogastric reflux, improved attitude, and improvement in values reflecting kidney function (decreased blood urea nitrogen and creatinine), correlates with improvement of intestinal damage.

13.11.3.2 HYPOPROTEINEMIA

Horses with DPJ that continue to reflux large volumes of enterogastric fluid frequently for more than 36 to 48 hours most likely will experience protein loss from the inflamed and disrupted intestinal mucosal barrier and from systemic protein catabolism. Decreased colloid oncotic pressure leads to decreased effective circulating fluid volume and edema. Total plasma protein may decline to below 4 g/dl and the albumin may decrease to below 2.0 g/dl. Fresh or thawed frozen plasma is ideal for replacement of functional proteins. One should consider treatment with intravenous plasma therapy or a combination of plasma and synthetic colloid (e.g., synthetic amylopectin) as soon as one sees evidence of a consistent decline in total plasma protein or albumin (<2.0 g/dl) or if the horse is developing dependent edema. Fresh plasma (preferred) or fresh frozen plasma is the treatment of choice if coagulation disorders accompany protein loss. An average-size horse (450 kg)

requires 6 to 10 L of plasma (albumin 3.0 g/dl) or synthetic colloid to improve plasma oncotic pressure. Administration of additional aliquots of 2 to 10 L of a balanced colloidal solution may be necessary if the DPJ crisis continues. In addition to albumin (the major colloid component), plasma contains other components that provide overall systemic support (e.g., fibronectins, complement inhibitors, elastase and proteinase inhibitors, antithrombin III). One may administer a 6% solution of hydroxyethyl starch (Hetastarch (6%), Abbott Laboratories, North Chicago, Illinois), a synthetic colloid, at 5 to 10 ml/kg. Because of the large size of the starch molecules, this solution is an effective plasma volume expander, resulting in sustained dose-dependent decreases in packed cell volume and plasma protein concentration with increased oncotic pressure. The cost of an appropriate amount of commercial plasma or synthetic colloid solution for treatment of adult horses with DPJ may be prohibitive but can be life-saving. 876

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13.11.3.3 ANTIENDOTOXIN THERAPY

Horses with enteritis frequently absorb large amounts of endotoxin from the disrupted intestinal mucosal barrier, therefore putting these horses at a high risk for laminitis. One should monitor digital pulses every 4 to 6 hours until systemic signs of enteritis have abated (fever, leukopenia, etc.). Treatment to combat endotoxemia is critical, and several therapeutic approaches are available. Choice of treatment options is based on severity of disease, renal function, hydration status, and economics. The reader is referred to Chapter 13.7 for a thorough discussion of endotoxemia pathophysiology, treatment, and prevention.

13.11.3.4 ANTIINFLAMMATORIES AND ANALGESIA

Nonsteroidal antiinflammatory drugs are the most frequently used group of drugs for treatment of abdominal pain in horses (flunixin meglumine 1.1 mg/kg intravenously every 12 hours or phenylbutazone 2.2 mg/kg orally or intravenously every 12 hours). The clinician must weigh the benefit of the analgesic effect of nonsteroidal antiinflammatory drugs with the possibility of further damage to the intestine by potentially blocking the protective effects of intestinal mucosal prostaglandins.

One should consider other classes of drugs for treating colic associated with DPJ. Butorphanol (Torbugesic; an opioid analgesic) at 0.06 to 0.1 mg/kg with detomidine (Dormosedan; an α-agonist) at 0.01 to 0.02 mg/kg given intramuscularly every 6 to 8 hours is a useful combination that has minimal effects on gastrointestinal motility.

13.11.3.5 ANTIMICROBIAL THERAPY

Because *Clostridium* spp. are suspected as a causative agent of DPJ, penicillin often is administered to affected horses. However, one should consider broad-spectrum antimicrobial coverage for horses with DPJ. One can add an aminoglycoside (gentamicin, amikacin) or third-generation cephalosporin (ceftiofur [Naxcel], Upjohn Co., Kalamazoo, Michigan) to the penicillin therapy, keeping in mind the potential adverse effects of these drugs on renal function.

13.11.3.6 ANTISECRETORY THERAPY

Effective antisecretory medications targeting the equine small intestine have not been identified.

13.11.3.7 MANAGEMENT OF NUTRITION

One should consider the nutritional needs of horses with DPJ. Most horses have a total body protein loss from cachexia and a protein-losing enteropathy. Total parenteral nutrition may be indicated in horses that remain anorectic for more than 3 to 4 days. Parenterally administered solutions containing glucose, balanced amino acid solutions, lipid emulsions, balanced electrolyte and trace minerals, and vitamins have been administered to adult horses with small intestinal ileus or enterocolitis. Based on a small number of horses, this therapy has proved promising in terms of minimizing protein losses and decreasing the duration of illness. Providing for part of the nutritional requirements of the horse (8000 to 12,000 kcal/day) is possible with glucose–amino acid solutions, which are of moderate cost. One may suppose reasonably that providing nutritional support to an anorectic, severely ill horse will facilitate the healing process and even shorten the duration of illness. Thus the overall cost of providing parenteral nutritional supplementation to horses with DPJ may well be offset by quicker recovery and diminished requirements for other, expensive treatments.

13.11.3.8 INTESTINAL MOTILITY MODIFIERS

Normal (healthy) intestine is necessary for optimum performance of prokinetic agents in horses. Many motility-modifying agents likely are ineffective in cases of DPJ. However, some benefit may come of the judicious use of prokinetic agents in inflammatory conditions of the equine intestine, particularly if the agent provides additional effects such as analgesia. For example, lidocaine infusion has several actions that may be beneficial in the treatment of ileus, including suppression of primary afferent firing, antiinflammatory properties, an observed analgesic effect, and direct stimulation of smooth muscle.[9] An infusion dose of 15 to 20 mg/min over 5 to 6 hours has been recommended. The reader is referred to Chapters 13.6 and 13.15 for a complete description of motility modifying agents.

13.11.3.9 NONRESPONSIVE CASES

Medical therapy is sufficient in most cases of DPJ, but in those cases in which the horse continues to produce copious enterogastric reflux, one may consider surgery as an option. Refractory cases have been observed to improv e with surgical intervention; however, some horses with refractory DPJ have been observed to recover with supportive medical care alone even after 20 days of refluxing large amounts of fluid every 2 to 4 hours (personal observation). The decision of when to intervene surgically often is difficult. One may elect surgery to determine the extent of gross pathologic condition and intestinal distention and to perform intestinal bypass so as to direct enterogastric reflux toward the cecum and colon, where the fluid can be reabsorbed. Allen and Clark[5] have described two approaches for surgical therapy in such cases. A standing right flank laparotomy with resection of the last rib has been used to approach the duodenum and cecal base. Using this approach, one 877
makes a small stoma between the duodenum and cecum using a handsewn 1.0- to 1.5-cm side-to-side 878
anastomosis. The stoma may act as a shunt to decompress the proximal small intestine and deliver the small intestinal fluid to the cecum for reabsorption. Following recovery, the stoma likely will close.

When a veterinarian is confronted with a horse exhibiting abdominal discomfort, with small intestinal distention palpable per rectum, and greater than 2 L of gastric reflux, the veterinarian should recommend referral of the horse to a facility capable of performing abdominal surgery. The chance that such a horse has an intestinal obstruction is too great to decide to treat it as if it may have DPJ. Surgery on such horses is not unusual, even though DPJ is possible, to rule out an obstruction. At present, the survival of horses with DPJ that undergo surgery is much greater than previously described, and certainly greater than that of horses with

small intestinal obstruction that do not have surgery. Horses with DPJ that receive appropriate therapy have a reasonably good chance of making a full recovery. Horses that continue to have frequent episodes of voluminous nasogastric reflux and systemic signs of endotoxemia and septicemia have a poorer prognosis for recovery. Frequent complications of DPJ include laminitis, thrombophlebitis, and weight loss.

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13.12 13.12—Proliferative and Inflammatory Intestinal Diseases Associated With Malabsorption and Maldigestion

Malcolm C. Roberts

Malabsorption is associated with pathologic conditions of the small intestine characterized by substantial reduction of the available absorptive surface area. By virtue of the extent of the morphologic changes, interference with digestive processes occurs, although maldigestion may be difficult to confirm in the absence of assessment measures. The small bowel problem may alter the composition and availability of substrate presented for fermentation in the large intestine. Clinical signs may be modified or accentuated if involvement of the large intestine is concomitant. Diarrhea indicates significant alterations in bidirectional fluid and electrolyte fluxes and in large intestinal transit.

The clinical signs of chronic wasting and poor body condition, although nonspecific for a diagnosis of malabsorption antemortem, can be attributed to proliferative or inflammatory intestinal disorders, often collectively

referred to as chronic inflammatory bowel diseases.[1] Clinical signs include alimentary lymphosarcoma, granulomatous enteritis, multisystemic eosinophilic epitheliotropic disease (MEED), and lymphocytic-plasmacytic enterocolitis—conditions affecting young and adult horses. Proliferative enterocolitis,[2] a transmissible disease of foals 3 to 7 months of age characterized by significant small intestinal pathologic changes, will be included in this group. However, several other primarily small intestinal conditions described from a morphologic perspective, such as chronic postinfarctive inflammation and mycobacterial infections,[3] will not be discussed. In addition, a single case of AA amyloid-associated gastroenteropathy in an 18- year-old Morgan stallion that had evidence of severe malabsorption based on poor d-xylose absorption is included.[4]

For comparative purposes, Table 13.12-1 lists the clinical and clinicopathologic features of the diseases, and Tables 13.12-2 and 13.12-3 present the gross morphologic and histopathologic findings, respectively. The extent of small 878
intestinal disease is the key to determine whether one can demonstrate malabsorption based on abnormal 881
carbohydrate absorption. As described in Chapter 13.4, this is not an all-or-nothing situation. In the same animal the staging of the pathologic changes differs in different regions of the small and large intestines, thus influencing severity of clinical signs and absorption findings. Furthermore, the extent of pathologic changes in different animals with ultimately the same morphologic diagnosis affects absorption studies and progress of the disease. Early diagnosis remains a challenge, and even multiple intestinal biopsies taken at exploratory laparotomy may prove unhelpful. By contrast, intestinal infiltration with the predominant cell types can be found in grossly normal appearing intestinal tissue.

TABLE 13.12-1 Predominant Clinical and Clinicopathologic Features of Horses With a Primary Problem of Chronic Wasting (or a More Rapid Weight Loss) Attributable to Proliferative and Inflammatory Bowel Diseases

CONDITION	AGE RANGE/ BREED	OTHER PRESENTING SIGNS	DERMATITIS/CORONITIS	HEMATOLOGY	CHEMISTRY*	ABSORPTION TESTS
Alimentary lymphosarcoma	2 to aged Majority ≤4 years None*	Poor appetite, edema, depression, occasional fever, occasional diarrhea or colic, pain	+/+/− Scurfy skin	Anemia, neutrophilia; lymphocytosis is rare	Decreased albumin Total protein normal to increased Increased globulin	Reduced absorption; partial to complete malabsorption
Granulomatous enteritis	1–6 years Majority ≤3 years Standardbred	Severe wasting, edema, poor to ravenous appetite, depression, infrequent diarrhea, occasional slight fever	+/− Scurfy skin; severe lesions rare	Anemia Leucocytes normal to slightly decreased or increased	Decreased albumin Total protein normal to decreased GGT normal ALP normal to increased	Reduced absorption; partial to complete malabsorption
Multisystemic eosinophilic epitheliotropic disease	1–17+ years Majority ≤4 years Standardbred, Thoroughbred	Severe wasting, edema, appetite poor to often ravenous, slight fever, diarrhea or soft feces common; depression, colic rare, oral ulcers	++++/Severe skin lesions and ulcerative coronitis prominent	Anemia rare to slight; neutrophilia and eosinophilia rare	Decreased albumin Total protein normal to decreased GGT normal to increased ALP normal to increased	Slower absorption; reduced or normal peak concentration delayed (shifted to right)
Lymphocytic-plasmacytic enterocolitis	3–26 years None	Inappetance, depression diarrhea, colic, edema	—	Normal	Decreased albumin Decreased total protein Increased fibrinogen	Inadequate absorption or delayed peak

Proliferative enteropathy	3–7 months Arabian	Depression, colic, diarrhea, edema, appetite often normal, concurrent infection	+/– Scurfy skin	Anemia Leukocytosis	Decreased albumin Decreased total protein Increased CPK	Normal absorption in 3 of 3 foals tested

* *GGT,* γ-Glutamyltransferase; *ALP,* alkaline phosphatase; *CPK,* creatine phosphokinase; *none,* no predominant breed.

TABLE 13.12-2 Gross Morphologic Features of Proliferative and Inflammatory Bowel Diseases of Horses

CONDITION	SMALL INTESTINE	LARGE INTESTINE	OTHER ORGANS/ SYSTEMS
Alimentary lymphosarcoma	**Constant;** extensive thickening, thickened mucosa, fissures, serosal plaques, nodules, congestion	**Infrequent;** unremarkable to thickened segments	Mesenteric lymph nodes (MLNs) massively enlarged Other lymph nodes involved in liver, spleen, stomach (rare)
Granulomatous enteritis	**Constant;** thickened wall, thickened mucosa, fissures, widespread ulceration (tiny ulcers)	**Common;** generally discrete	MLNs enlarged, edematous Stomach commonly affected (generally discrete) Liver, pancreas rare
Multisystemic eosinophilic epitheliotropic disease	**Common;** diffusely thickened, especially proximal duodenum and distal ileum; serosal nodules or granularity; ulceration	**Constant;** severe; segmental, or multifocal granulomata; mucosal (predominantly) and transmural thickening; extensive ulcers	MLNs (and other nodes) enlarged Stomach and esophagus commonly affected Liver/pancreas commonly affected; may be hyperkeratotic Skin: exudative dermatitis, ulcerative coronitis
Lymphocytic-plasmacytic enterocolitis	**Constant;** mucosal/ submucosal edema, prominent folds	**Common;** edema, congestion, areas of mucosal ulceration	MLNs enlarged
Proliferative enteropathy	**Constant;** significant mucosal thickening, corrugated appearance from proximal jejunum to distal ileum	**Uncommon;** submucosal edema	MLNs unremarkable

TABLE 13.12-3 Significant Histopathologic Features of Proliferative and Inflammatory Diseases of Horses

CONDITION	SMALL INTESTINE	LARGE INTESTINE	OTHER ORGANS/ SYSTEMS
Alimentary lymphosarcoma	Villous atrophy (partial to total); crypts disappear with hyperplasia; infiltrate of pleomorphic lymphoid cells, plasma cells; transmural	Nothing evident to diffuse mucosal infiltration	Mesenteric lymph nodes (MLNs): extensive infiltration; more moderate in other lymph nodes (hepatic, abdominal, inguinal, superficial)
Granulomatous enteritis	Villous atrophy (partial to total), crypt hyperplasia and abscesses, diffuse granulomatous inflammation; mononuclear cells (lymphoid), giant cells, epithelioid foci; lymphangiectasia	Similar infiltrate usually discrete; mucosa, submucosa	Similar infiltrate; stomach discrete MLNs: discrete to florid macrophage infiltration Diffuse cortical hyperplasia
Multisystemic eosinophilic epitheliotropic disease	Villous atrophy rare; lymphocytic and eosinophilic infiltration most severe in cranial duodenum, ileum, ileocecal junction; infiltrate more widespread than gross lesions	Segmental/multifocal lesions, severe infiltration, reactive fibrosis, tissue eosinophilia, walled off granulomata, central necrotic core of eosinophilic material	Similar infiltration with fibrosis of MLNs, liver, pancreas Skin: acanthosis, hyperkeratosis, diffuse infiltrate of eosinophils; lymphocytes in dermis; focal eosinophilic accumulations
Lymphocytic-plasmacytic enterocolitis	Villous blunting to atrophy; moderate to severe infiltration of lymphocytes, plasma cells; edema, dilated lymphatics	Similar infiltrate, less remarkable	Minimal evidence
Proliferative enteropathy	Villous shortening, severe hyperplasia of crypt epithelium, small curved bacteria in apical cytoplasm, mononuclear infiltrate	No evidence	No evidence

13.12.1 Alimentary Lymphosarcoma

Alimentary lymphosarcoma of the horse may represent a primary neoplasia of the gut associated lymphoid tissue with significant cellular infiltration of the small intestine and associated lymph nodes with minimal large

intestinal or systemic involvement. Case series and pathology reports indicate that young horses 2 to 4 years of age primarily are affected, although the age range can be broad.[5–7] No breed or sex predilection exists. Prevalence is unknown. Despite the progressive nature of lymphomata, onset of clinical signs can be rapid and the animal may become acutely ill. As with all adult cases of chronic inflammatory bowel disease, antemortem diagnosis is by a process of exclusion and usually is confirmed post mortem. Frequently, the horse has anemia, thrombocytopenia, neutrophilia or neutropenia, hypoalbuminemia, normal serum protein or hyperproteinemia, and hypergammaglobulinemia. Lymphocytosis is rare. One may palpate intraabdominal masses, mainly enlarged mesenteric lymph nodes, rectally. Abdominocentesis has been of diagnostic value. Carbohydrate absorption tests usually reveal partial to total malabsorption indicative of the severely reduced surface area resulting from significant villous atrophy and the extensive mucosal or transmural infiltration. Rectal biopsy has aided diagnosis. Early confirmation of a suspected diagnosis necessitates exploratory laparotomy to obtain multiple intestinal and lymph node biopsies. In the future, markers of cancer cells may become available and may be cost-effective to aid diagnosis. Prognosis is poor. Natural progress of the disease is unknown. Most horses are presented in an advanced state of disease. Immunosuppressive drugs or chemotherapy may afford temporary improvement. However, outcome is unaffected.

13.12.2 Granulomatous Enteritis

The chronic wasting condition granulomatous enteritis was first described in 1974[8]; 9 of 10 horses were young Standardbreds. Most affected horses are 2 to 3 years of age. Case reports from many countries revealed a predominance of Standardbred over Thoroughbred horses by three to one.[9,10] Some of the Standardbreds were related, implicating a genetic predisposition. Prevalence is low. The condition is sporadic and has an insidious onset, and the course can be protracted. Significant diagnostic features include anemia, slight increases or decreases in white blood cell counts, hypoalbuminemia, normal serum protein or hypoproteinemia, occasional increases in serum alkaline phosphatase activity, normal serum γ-glutamyltransferase activity, and enlarged mesenteric lymph nodes on rectal palpation. Reduced carbohydrate absorption to the level of partial to total malabsorption is reported frequently, consistent with the severe morphologic changes throughout the small intestine. One can attribute the low proportion of horses exhibiting diarrhea[9,10] to the preferential distribution of inflammatory infiltration in the small intestine,[11] with lesser involvement of the large intestine. Rectal biopsy can be a useful aid to diagnosis.[12]

Treatment of horses with granulomatous enteritis with a variety of drugs, particularly corticosteroids, has not affected the outcome except in the short term.[13] One successful response has been reported. Prolonged corticosteroid administration produced clinical remission in a 6-year-old Standardbred gelding based on improvement in clinical signs and in d-xylose absorption.[14] Five months after cessation of approximately 5 months therapy, d-xylose absorption was normal and the horse was bright, alert, and resumed a level of athletic performance. Parenteral administration of dexamethasone sodium phosphate was tapered to achieve a minimal effective dose to reduce intestinal inflammation and abolish clinical signs. Adverse effects were not reported. The outcome of this single case is encouraging. Surgery may be indicated if the disease is localized. Two young horses underwent resection of the thickened terminal small intestine to confirm a diagnosis and provide a means of treatment; one horse died 4 months after surgery, and the other has remained clinically normal for at least 10 years.[10]

The cause of granulomatous enteritis is unknown. Several infectious agents have been implicated, including *Mycobacterium avium*.[15] The condition may represent a granulomatous hypersensitivity reaction. Immune-mediated responses to dietary, parasitic, or bacterial antigens may be important initiating factors.[1] Recently, six

cases purported to represent granulomatous enteritis were linked to environmental contamination with aluminum.[16] Although the case definition was flawed and problems existed with the data and interpretation,[17] the report nevertheless raised the possibility that a toxicologic basis may exist for some equine inflammatory bowel disorders.

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13.12.3 Multisystemic Eosinophilic Epitheliotropic Disease

MEED encompasses disorders characterized by a predominant eosinophilic infiltrate in the gastrointestinal tract, associated lymph nodes, liver, pancreas, skin, and other structures and accompanied by some degree of malabsorption and enteric protein loss. The disorders include chronic eosinophilic gastroenteritis,[18] eosinophilic granulomatosis,[9] chronic eosinophilic dermatitis,[19] and probably basophilic enterocolitis.[20] The condition differs from idiopathic eosinophilic enterocolitis,[10] in which segmental lesions in the small or large intestine induce signs of colic requiring surgical intervention[21,22] without evidence of malabsorption or multisystem involvement.

Although prevalence is low, MEED appears to be more common than granulomatous enteritis based on the accumulated published reports and personal experience in Australia and the United States. Most affected horses are 2 to 4 years of age, and Standardbreds and Thoroughbred are reported to predominate. The condition is sporadic and has an insidious onset, and the course is protracted with a duration of 1 to 10 months. Diarrhea is common in contrast to granulomatous enteritis. Severe skin lesions with exudative dermatitis and ulcerative coronitis are prominent and frequently are the principal reason for veterinary attention being sought. Despite extensive tissue eosinophilia, systemic eosinophilia is rare. Hematologic values are usually unremarkable. Notable features include hypoalbuminemia and normal serum protein or hypoproteinemia, and because of liver involvement, serum γ-glutamyltransferase and alkaline phosphatase activities may be increased. Most reports of carbohydrate absorption test findings (glucose or d-xylose) indicate retarded absorption and a reduced or normal peak concentration delayed to at least 180 minutes. One can interpret this pattern as the existence of sufficient small intestinal absorptive capacity to enable moderate absorption with possibly delayed gastric emptying or ileocecal ejection. Morphologic changes are less pronounced in the small intestine than in the large intestine,[9] and small intestinal lesions predominate segmentally in the proximal duodenum and distal ileum. Furthermore, significant hyperkeratosis of the fundic region may contribute to gastric muscle contractile disruption. Diarrhea can be a consequence of the severe segmental or multifocal granulomatous lesions in the large intestine with mucosal and transmural thickening and extensive ulceration. Abundant fibrosis is a feature of all affected tissues (see Table 13.12-3).

The cause of MEED is unknown and could represent a chronic ongoing immediate hypersensitivity reaction against undefined antigens ingested or excreted into the lumen from parasitic, bacterial, or dietary sources. Infectious agents have not been identified.[18,19] Widespread use of the avermectins has tended to reduce parasite loads and composition to favor small strongyles (cyathostomes). Eosinophilia is a feature of parasitism in the equine intestinal tract, although nematodes rarely have been identified in any lesions of MEED.[3,18] However, failure to detect larval structures in these lesions may be attributable to chronicity of the disease and destruction of the parasites in tissue.[10]

Biopsies of the rectal mucosa[12] or of the skin, liver, intestinal tract, and lymph nodes may assist in diagnosis. Treatment has been attempted with a variety of drugs, including antibiotics, corticosteroids, and anthelmintics with larvicidal activity. Immediate improvement has not been borne out in the long term. Prognosis is poor. The clinical objective is to reach a tentative diagnosis early in the course of the disease for intervention to be more

than transient. Unlike the other conditions (see Table 13.12-1), MEED has definitive liver and pancreatic involvement, and thus maldigestion may make a significant contribution to the wasting disease. For example, the lowered albumin and protein could result in part from impaired pancreatic enzyme digestion, and the effects of inflammatory lesions in the liver and ileum may decrease bile salt concentrations.

13.12.4 Lymphocytic-Plasmacytic Enterocolitis

The morphologic findings in lymphocytic-plasmacytic enterocolitis reflect the predominant infiltrative cellular elements of this rarely encountered condition. A retrospective study of 14 horses[23] provided the information presented in the tables. No specific clinical or clinicopathologic features differentiate this condition antemortem from other inflammatory diseases of adult horses. Carbohydrate absorption was abnormal or delayed in 9 of 12 horses, consistent with the predominance of small intestinal pathologic changes. Rectal biopsies were abnormal in 3 of 7 horses, two of which were reported as having lymphocytic-plasmacytic proctitis. Prognosis is poor. Treatment has been unsuccessful, probably because of the advanced nature of the condition at the beginning of treatment.

13.12.5 Proliferative Enteropathy

Proliferative enteropathy has not been associated with abnormal carbohydrate absorption based on three horses subjected to carbohydrate absorption tests. However, the florid mucosal lesions in the jejunum and ileum undoubtedly contribute to impaired digestive function and potential malabsorption of vitamins, minerals, and 882
amino acids in the distal small intestine. The condition affects foals 3 to 7 months of age, particularly those that 883
have been weaned recently. The disease is caused by *Lawsonia intracellulare,* an obligate intracellular bacterium found in the cytoplasm of proliferative crypt epithelial cells of the intestine. The condition in a foal was described first as intestinal adenomatosis,[24] because of similarity to the swine disorder of the same name. Later, molecular studies showed that intestines from an affected foal contained *L. intracellulare* sequences as determined by polymerase chain reaction analysis and confirmed by Southern blot hybridization.[25] Recently, studies of a cluster of affected foals on three breeding farms in Canada provided much information on the clinical syndrome, laboratory investigations, and response to treatment.[2]

Two of the three farms bred Arabians, hence a demographic predominance of Arabian foals exists. Clinical signs included depression, rapid and significant weight loss, edema, diarrhea, and colic. Poor body condition, a rough hair coat, and potbelly appearance were common findings. Other problems often were concurrent, including respiratory tract infection, dermatitis, intestinal parasitism, and gastric ulceration. Significant laboratory findings were anemia, transient leucocytosis, hypoalbuminemia, hypoproteinemia, and elevated serum creatine kinase concentrations.

Diagnosis was confirmed by identifying characteristic intracellular bacteria within the apical cytoplasm of proliferating crypt epithelial cells using silver stains and by results of polymerase chain reaction analysis and immunohistochemical testing. Antemortem diagnosis relied on clinical signs, hypoproteinemia, and exclusion of common enteric infections. One can confirm diagnosis in live animals by fecal polymerase chain reaction analysis (positive in 6 of 18 foals tested) and serologic testing; 7 foals with proliferative enteropathy were evaluated serologically and had antibodies against *Lawsonia intracellulare*.[2]

Treatment is effective. Most foals received erythromycin estolate (15 to 25 mg/kg per os every 6 to 8 hours), alone or with rifampin (7 to 10 mg/kg per os every 12 hours) for 2 to 4 weeks. Foals frequently needed

supportive therapy at the outset for stabilization. Response to therapy has been excellent.[2] Rapid improvement in clinical signs even within 24 hours preceded the rise in plasma protein concentration. The source of the infection was undetermined. No apparent link existed between the three farms and a swine operation or solid and liquid waste disposal on pasture. However, one cannot exclude airborne spread of dried fecal material over distances. Comparisons of epidemiologic findings from the swine disease indicated that overcrowding, feed changes, antibiotic usage, and mixing and transportation were potential risk factors at two of the farms. Recent weaning appeared to be a key element in the pathogenesis.

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13.13 13.13—Inflammatory Diseases of the Gastrointestinal Tract Causing Diarrhea

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13.13.1 Equine Acute Diarrhea

Acute diarrhea caused by colitis in adult or young horses is a potentially life-threatening disorder of a variety of causes (Table 13.13-1) characterized by hypersecretion of fluid, motility disturbances, altered microbial flora in the colon, and an impaired mucosal barrier caused by direct injury or inflammation. Many of the clinical and clinicopathologic features are similar regardless of the cause. Severe dehydration with profound electrolyte abnormalities is common, as is systemic inflammation from absorption of endotoxin or other bacterial products through the compromised mucosa. Gastrointestinal protein loss may result in reduced colloid oncotic pressure from hypoproteinemia, leading to tissue edema. Colitis is a highly catabolic disorder, and weight loss may be rapid and severe. Some cases of colitis may be complicated by extensive mucosal ulceration, serosal inflammation, or mural ischemia/infarction extending from the inflammation or resulting from coagulopathies. Thus diagnostic measures aimed at determining the cause necessarily must be accompanied by clinical and laboratory assessment of hydration, electrolyte and acid-base balance, plasma protein concentration and colloid oncotic pressure, organ function, and evaluation of the degree of systemic inflammation and of the integrity of the intestinal wall. Although therapeutic strategies are similar for many causes of colitis, consisting primarily of control of local and systemic inflammation, maintenance of fluid and electrolyte balance, promotion of tissue perfusion, replacement of plasma protein, preservation of colloid oncotic pressure, promotion of mucosal repair, restoration of the microbial ecology of the colon, and nutritional management, some causes of acute colitis have specific therapies aimed at eliminating the cause.

13.13.2 Infectious Diseases

A variety of infectious organisms has been identified as causes of acute colitis in adult horses. The clinical syndromes associated with these infections are indistinguishable in most horses. However, appropriate diagnostic tests including fecal bacterial culture, fecal bacterial toxin analysis, PCR, and/or serology may identify specific infectious organisms.

13.13.2.1 SALMONELLOSIS

13.13.2.1.1 Pathogenesis

Salmonella is a genus of gram-negative facultatively anaerobic bacteria that are common gastrointestinal pathogens in horses. Many serotypes of *Salmonella* have been reported to infect horses, but those classified in group B appear to be associated more commonly with disease than those in other groups. Group B includes *S. typhimurium* and *S. agona,* two of the species most frequently isolated from horses.[1–3] *S. typhimurium* is the most pathogenic serotype in horses and is associated with a higher case fatality rate than other species of *Salmonella*.[1] The number of horses that are infected inapparently with and actively shed *Salmonella* in their feces has been reported to be as high as 10% to 20%, but actual prevalence of *Salmonella*-shedding in the general horse population is likely to be much lower, less than 1% to 2%.[4] Horses shedding salmonellae are a potential source of infection to susceptible horses,[1,5] as are environmental reservoirs.[6–8] For these reasons, salmonellosis is one of the most common nosocomial diseases in horses. Nosocomial salmonellosis significantly affects morbidity and mortality in hospitalized horses.[9] The emergence of multidrug resistance in equine *Salmonella* isolates has been a cause of concern because of the importance of salmonellosis as a nosocomial disease and because *Salmonella* represents a significant zoonotic pathogen.[7,10–12]

The virulence of the bacteria varies tremendously with serotype and even among strains of the same serotype in part because of the important role of host susceptibility in the pathogenicity of particular organisms. The infective dose is generally millions of organisms inoculated orally, but various 884
environmental and host factors can reduce the infective dose to a few thousand or even hundreds of 885
organisms.[13–15] Environmental factors or stresses that increase susceptibility to *Salmonella* infection are not well defined, but high ambient temperature, for example, is known to increase the prevalence of salmonellosis in horses greatly. Indeed, the peak incidence of salmonellosis in horses occurs in late summer and fall.[6,14,15] Other environmental and host factors that increase the risk of *Salmonella* infection include transportation, antibiotic administration, gastrointestinal surgery, general anesthesia, preexisting gastrointestinal disease, change in diet, and immunosuppression.[1,8,15]

TABLE 13.13-1 Differentials and Diagnosis of Some Causes of Acute Diarrhea in Adult Horses

CATEGORY	DIFFERENTIALS	DIAGNOSIS
Infectious	Salmonellosis	Fecal culture (five consecutive)
		Fecal polymerase chain reaction (PCR)
	Clostridium perfringens	Quantitative fecal culture
		Fecal toxin immunoassay or PCR
	Clostridium difficile	Fecal culture
		Fecal toxin immunoassay or PCR
	Lawsonia intracellulare	Serologic testing
		Fecal PCR
		Small intestinal ultrasound
	Ehrlichia risticii	Fecal or blood PCR
		Serologic testing
Parasitic	Strongylosis	Fecal egg counts
		Cranial mesenteric artery palpation
		Serum immunoglobulin G(T)
	Cyathostomiasis	Fecal egg count
		Rectal biopsy
		Cecal or colonic biopsy
Toxic	Nonsteroidal antiinflammatory drugs	History and clinical signs
		Right dorsal colon ultrasonography
		Laparoscopy or laparotomy
	Cantharidin	History of exposure
		Fecal or urine cantharidin concentrations
	Arsenic	History of exposure
		Fecal, blood, urine, or tissue arsenic concentrations

Miscellaneous	Carbohydrate overload	History of inappropriate ingestion of carbohydrates
		Blood lactate concentration
	Sand enteropathy	Auscultation of ventral colon
		Fecal sand content
		Abdominal radiography

Host factors that restrict gastrointestinal colonization and invasion by pathogens include gastric pH, commensal gastrointestinal flora, gastrointestinal motility, the mucosal barrier and mucosal immunity.[1,16] Gastric acidity is an important defense mechanism preventing live organisms from reaching the intestine. Altering the gastric pH with histamine$_2$ receptor antagonists, for example, may increase susceptibility to infection. Gastrointestinal flora inhibits the proliferation and colonization of *Salmonella* by secreting bacteriocins, short-chain fatty acids, and other substances that are toxic to *Salmonella*. In addition, elements of the normal flora compete for nutrients and space, especially on the mucosa.[16] Being predominantly anaerobic, the normal flora maintain a low oxidation-reduction potential in the environment of the large intestine, which inhibits the growth of many bacterial pathogens.[17] The importance of normal host gastrointestinal ecology is illustrated by the fact that disturbances of the colonic flora with antibiotics, changes in feed, ileus, or other underlying gastrointestinal disease greatly increase the susceptibility of the host to infection by *Salmonella,* often resulting in serious disease.

The immune status of the host may be one of the most important factors determining not only the susceptibility to *Salmonella* infections but also the degree of invasion and subsequent outcome of the infection. Local immunity, such as mucosal antibody secretion and enterocyte-derived cationic peptides, prevents colonization of the mucosa.[16,18,19] Opsonizing antibodies and activation of the complement cascade are important in fighting systemic invasion by *Salmonella* by increasing the efficiency of phagocytosis and by direct bactericidal activity. Humoral immunity, however, is often ineffective in 885
preventing disease and dissemination once invasion occurs and *Salmonella* has established in its 886
intracellular niche. Following invasion, *Salmonella* is capable of surviving and multiplying within macrophages, rendering humoral (noncellular) immune systems ineffective.[20,21] Specific cellular immunity may be the most effective defense mechanism in the host arsenal against dissemination and systemic infection by *Salmonella*.[21,22] Oral inoculation with small numbers of virulent organisms may induce protective immunity in horses and calves, but the duration of the immunity is not known.[23,24] Oral and parenteral vaccines using killed or attenuated organisms and bacterial products have been promising but are effective only against homologous organisms and are usually not cross-protective among different serogroups.[23–25]

In adult horses, *Salmonella* primarily infects the cecum and proximal colon, causing enterocolitis, and the ability to disseminate beyond the intestine and cause enteric fever is limited. In foals, however, salmonellosis often is associated with septicemia. The ability of *Salmonella* to cause enterocolitis depends on the ability of the bacteria to invade the gastrointestinal mucosa.[16,20] Invasion of the gastrointestinal mucosa occurs preferentially through specialized enterocytes called M cells that overlay intestinal lymphoid tissues such as Peyer's patches in nonequine species. A variety of enteric pathogens exploit M cells during infection of intestinal tissue.[26] Invasion of the epithelium occurs by self-induced uptake via the apical membrane of the M cell, often killing the cell in the process.[20] Salmonellae then invade neighboring cells

via the basolateral membrane, eventually spreading the destruction of the epithelium beyond the principle area of attack. Virulent salmonellae have a well-developed invasion mechanism involving generation of an apparatus called a type III secretory system that enables virulence gene products to be injected directly into enterocytes.[27] Virulence proteins injected by salmonellae into enterocytes engage the cellular machinery and induce the cell to engulf the bacteria by macropinocytosis. *Salmonella* virulence gene products also induce enterocyte chloride and fluid secretion and upregulate enterocyte transcription of inflammatory cytokines (tumor necrosis factor α and interleukin-1β) and chemokines that trigger a mucosal inflammatory response.[20,27,28]

After salmonellae invade the mucosa, they are phagocytosed quickly by macrophages and dendritic cells in the lamina propria and lymphoid tissues. The ability of salmonellae to disseminate systemically and cause enteric fever is associated with the ability to survive and proliferate in macrophages. Indeed, phagocytes have an important role in dissemination of the pathogen to blood, lymph nodes, liver, and spleen. Most salmonellae in the blood and tissues of infected animals that are competent to cause enteric fever are within phagocytic cells.[29] In adult horses with salmonellosis, dissemination appears to be limited to the intestine and mesenteric lymph nodes, and *Salmonella* rarely is cultured from blood. However, in foals and in some adults, *Salmonella* causes an enteric feverlike disease with dissemination to mesenteric lymph nodes, liver, spleen, and blood.

Salmonella organisms require specific virulence gene clusters encoded on the chromosome or on plasmids for intracellular survival in macrophages.[20] Some of these genes are sensors that signal the bacteria that it has entered an intracellular environment and turn on genes required for intracellular survival. Others, like invasion genes, are transported from the bacteria and injected into macrophage cytosol by a type III secretory system to prevent phagosome/lysosome fusion and subvert other essential macrophage killing mechanisms. Salmonellae also possess multiple genes that confer resistance to reactive oxygen and nitrogen metabolites, perhaps the most lethal antimicrobial mechanisms of macrophages.[30]

Diarrhea associated with salmonellosis has multiple causes. A *Salmonella* cytotoxin inhibits protein synthesis in mucosal cells, causing morphologic damage and altered permeability.[31] Virulent salmonellae also produce an enterotoxin similar to the heat-labile toxin (LT) produced by *Escherichia coli*.[32,33] The enterotoxin contributes to but is not required in the pathogenesis of diarrhea.[34,35] *Salmonella* enterotoxin increases secretion of chloride and water by colonic mucosal cells in many species, including horses, by increasing intracellular cyclic adenosine monophosphate concentrations.

The ability of virulent salmonellae to cause diarrhea appears to be associated most closely with the ability to invade enterocytes and to trigger an inflammatory reaction in the intestinal tissue.[20,36] Gene products injected into enterocyte cytosol by the type III secretory system of invading salmonellae stimulate chloride and fluid secretion.[27] *Salmonella* invasion of enterocytes is also a potent activator of inflammatory chemokine and cytokine production, resulting in the recruitment of leukocytes, particularly neutrophils, and activation of resident macrophages and mast cells. Products of these activated leukocytes, including prostaglandins, leukotrienes, reactive oxygen metabolites, and histamine, are potent stimulators of chloride secretion in the colon of many species.[16,37–39] The enteric nervous system integrates the diverse processes of pathogen recognition, triggering of the inflammatory response, and induction of enterocyte fluid secretion.[39]

Many of the inflammatory mediators studied stimulate colonic secretion by prostaglandin-dependent mechanisms, resulting in increased intracellular cyclic adenosine monophosphate or calcium concentrations or both in mucosal cells.[37] In addition, these mediators and the enteric nervous system may stimulate secretion by prostaglandin-independent mechanisms, inhibit sodium and water absorption, cause motility disturbances, and potentiate tissue injury, all of which enhance the pathogenicity and dissemination of *Salmonella* and contribute to the pathogenesis of diarrhea.[37,39] Neutrophils recruited to the mucosa by signals generated by the infected enterocytes physically contribute to mucosal injury by producing a variety of products that are lethal to pathogens but are also toxic to host cells.[40,41] Moreover, neutrophils attracted to infected epithelial cells accumulate beneath the monolayer, lifting it off the basement membrane in sheets. Neutrophils also migrate across the epithelial monolayer in potentially massive numbers. Transepithelial migration of neutrophils increases the permeability to macromolecules, bacterial products, and even bacteria.[41] Potentially massive losses of electrolytes, water, and protein can occur depending on bacterial and host factors. Perhaps most devastatingly, mucosal injury and altered permeability allow systemic absorption of bacterial products and dissemination of bacteria, resulting in systemic inflammatory responses such as occur with endotoxemia and septicemia.

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13.13.2.1.2

Clinical Signs and Diagnosis

Four syndromes of *Salmonella* infection have been described clinically and reproduced experimentally in horses: (1) inapparent infections with latent or active carrier states; (2) depression, fever, anorexia, and neutropenia without diarrhea or colic; (3) fulminant or peracute enterocolitis with diarrhea; and (4) septicemia (enteric fever) with or without diarrhea. Inapparent infections can be activated to clinical disease in compromised horses, such as horses with colic or horses being treated with antibiotics, causing mild to severe enterocolitis. In addition, latent infections (nonshedding) can become active infections (shedding) under certain conditions, such as transportation stress and antibiotic treatment. Horses with depression, anorexia, fever, and neutropenia without diarrhea generally have a good prognosis and recover in several days without specific treatment.[42] The septicemic form is restricted mostly to neonatal foals and is uncommon in adult horses. This discussion focuses on acute enterocolitis.

Acute enterocolitis is characterized by severe fibrinonecrotic typhlocolitis, with interstitial edema and variable degrees of intramural vascular thrombosis that may progress to infarction.[1] Severe ulceration of the large intestinal mucosa may occur with serosal ecchymoses and congestion. The earliest signs of enterocolitis are usually fever and anorexia.[1,15] Signs of colic may be apparent early in the course of the disease, especially if ileus is present. Clinical signs of endotoxemia are common and range from fever, elevated heart and respiratory rates, poor peripheral perfusion, and ileus to fulminant and rapidly progressive signs of endotoxemic shock. Oral mucous membranes are often pale with perigingival hyperemia (a toxic rim) but may be brick red or cyanotic, with prolonged capillary refill time. One may note weakness, muscle fasciculations, cold extremities, and other signs suggestive of hypotensive shock; synchronous diaphragmatic flutter; abdominal pain; and significant metabolic and electrolyte abnormalities in severe cases of enterocolitis. One also may note signs of mild dehydration before diarrhea is apparent. Once diarrhea is evident, dehydration may become severe rapidly. Occasionally, horses die peracutely, without developing diarrhea.

Diarrhea may not be apparent for several days but usually occurs by 24 to 48 hours after the fever begins.[1,15] The duration of the diarrhea may be days to weeks. The character of the first diarrheal feces is usually

watery with particles of roughage but may become fluid rapidly without solid material. Finding frank blood and fibrin in the feces is unusual. The volume of feces is often large, with frequent defecation. One may note straining or signs of colic when the patient is defecating, and rectal prolapse may occur occasionally. Persistent straining and rectal prolapse may be a sign of colonic infarction. Abdominal borborygmi are often absent early in the course of the disease because of ileus but become evident later, usually when diarrhea begins. Fluid and gas sounds are commonly audible, but normal progressive motility is less frequently audible than normally. Transrectal palpation may reveal edematous rectal mucosa and colon and fluid-filled colon and cecum. One may obtain gastric reflux, especially early in the course when ileus is evident.

Hematologic abnormalities early in the course of the disease include moderate to severe neutropenia, lymphopenia, and leukopenia, a mild to moderate left shift, and toxic changes in the neutrophils.[1,15] Thrombocytopenia, moderate to severe hemoconcentration, and hyperfibrinogenemia are also common. Neutropenia is an early but nonspecific indicator of salmonellosis, often occurring concurrently with the onset of fever.[1] Later in the course of disease, one may see neutrophilic leukocytosis, indicating recovery. A degenerative left shift, with metamyelocytes and myelocytes in the peripheral blood, is a poor prognostic sign.

Serum biochemical analysis may reveal azotemia, elevations in serum sorbitol dehydrogenase and γ-glutamine aminotransferase activity, and elevated serum lactic acid concentration. Azotemia is often prerenal, but acute hemodynamic renal failure may occur in severely dehydrated, endotoxemic, or septicemic patients. Indeed, elevation of creatinine concentration is a poor prognostic indicator in horses with acute colitis.[43] Hemodynamic renal disease may be complicated by toxic injury caused by 887
administration of nephrotoxic drugs. Hyponatremia may also contribute to prerenal azotemia. Elevations in 888
hepatocellular enzymes are usually mild and reflect damage to the hepatocytes from absorbed toxins such as endotoxin and from poor perfusion caused by hypotensive shock or dehydration. Lactic acidemia may be present, reflecting poor tissue perfusion. Plasma protein rapidly drops as protein is lost in the gastrointestinal tract, causing moderate to severe hypoalbuminemia and hypoglobulinemia. Peripheral or organ edema (vascular leak syndrome) may occur if hypoproteinemia is severe, coupled with increases in endothelial permeability induced by systemic inflammation.

Hypokalemia, hyponatremia, hypochloridemia, and hypocalcemia are common electrolyte abnormalities in patients with enterocolitis. Metabolic acidosis also may be present. Coagulopathies, such as decreased antithrombin III activity and disseminated intravascular coagulation, may occur. Urinalysis may reveal isosthenuria, proteinuria, hematuria, cylindruria, and glucosuria if hemodynamic or toxic renal injury is present. The number of leukocytes in the feces usually is elevated, and occult blood may be detectable. Peritoneal fluid is usually normal except when severe mural inflammation or colonic infarction occurs.

Routine detection of salmonellae in feces involves five daily cultures of large samples (10 to 30 g) of feces using enrichment techniques.[1,44,45] However, the sensitivity of fecal culture can be as low as 30% to 50%, even if one cultures several fecal samples collected daily. Concurrent culture of rectal biopsy specimens and feces increases the sensitivity of culture techniques to 60% to 75%.[45] Currently, the polymerase chain reaction (PCR) is the most sensitive and rapid test for detecting *Salmonella* in feces. A single PCR test applied early in the course of disease is a more sensitive test for the presence of *Salmonella* than repeated fecal cultures.[46,47] Detection of salmonellae in feces does not prove a diagnosis of salmonellosis, but the positive predictive value of a positive PCR or culture results is high in horses with compatible clinical signs. Culture of peripheral blood may allow isolation of the organism if bacteremia or septicemia is present, but blood cultures are not a sensitive test for salmonellosis in adult horses. Although foals are more likely than

adults to become septicemic, culture of blood is recommended in all cases with signs suggestive of septicemia. Increased numbers of fecal leukocytes suggest an invasive process in the colon but are not specific for salmonellosis.

Early in the course of the disease, dehydration, electrolyte and acid-base imbalances, endotoxemia, and sepsis may be life threatening. Aggressive treatment during the acute stages to replace fluids lost in the diarrhea and to control sepsis and endotoxemia is often effective in controlling the primary disease. Weight loss and hypoproteinemia are often severe. Complications such as multiorgan dysfunction, vascular leak syndrome with peripheral and organ edema, laminitis, acute renal failure, venous thrombosis and septic phlebitis, irreversible protein-losing enteropathy or chronic malabsorption, pulmonary aspergillosis, and gastrointestinal infarction can occur. In many instances, horses recover from acute salmonellosis with aggressive treatment, only to succumb to complications of the disease, partially explaining the high fatality rate of equine salmonellosis compared with human salmonellosis. Chronic, mild to moderate diarrhea occasionally occurs in horses after a bout of severe salmonellosis, usually with protein-losing enteropathy. If the chronic diarrhea persists beyond 4 to 5 weeks after the onset of signs, the prognosis for recovery is poor.[15]

13.13.2.2 POTOMAC HORSE FEVER

13.13.2.2.1 Pathogenesis

Potomac horse fever (equine monocytic ehrlichiosis) is caused by the obligate intracellular rickettsial organism *Neorickettsia risticii*.[48–51] The disease is most common from late summer to early fall, with a peak incidence in July and August. Potomac horse fever was described first in the Northeast but since has been diagnosed in most areas of the continental United States, with a particularly high prevalence in the Northeast and Midwest. The geographic distribution is characterized by a significantly higher percentage of cases found along waterways and rivers.[48,49] The disease occurs sporadically, temporally and geographically, and can affect any age group of horses. The case fatality rate is 5% to 30%.[48]

Transmission of *N. risticii* has been reproduced experimentally by oral, intramuscular, intradermal, subcutaneous, and intravenous routes.[48,52] However, the natural route of infection has remained a mystery until recently. The epidemiologic data, the fact that many other rickettsial diseases are transmitted by insect vectors, and the finding that the disease can be transmitted via whole blood have implicated insect vectors in the natural transmission of the organism. Attempts to transmit the disease experimentally with ticks *(Dermacentor variablis)* or biting flies *(Stomoxys calcitrans)* have been unsuccessful.[53,54] Recently, *N. risticii* has been found to infect virgulate cercariae larval stages of trematodes that use operculate freshwater snails (*Juga* spp.) as part of their life cycle in northern California.[55] Infected virgulate cercariae have been identified in aquatic snails collected in other parts of the country as well. Virgulate cercariae are part of the life cycle of trematodes that are common parasites of many species and use freshwater snails and aquatic insects as intermediate hosts. Although the trematode species infected with *N. risticii* remains to be identified definitively, at least two species are considered potential vectors.[52] During the trematode life 888
cycle, aquatic snails release large numbers of infected cercariae into water, where they seek their next 889
intermediate host. Infected metacercaria have been identified in a variety of aquatic insects.[56] Preliminary studies suggest that *N. risticii* in fact may be transmitted via ingestion of mature caddis flies containing infected metacercariae.[57] Possibly horses are infected by drinking water containing infected cercaria

released from snails or by ingesting infected metacercariae in other aquatic insects.[52] The number of PCR-positive snails in endemic regions corresponds to the seasonal incidence of Potomac horse fever and may be as high as 26%.[58]

The pathogenesis of *N. risticii* is not understood completely. The organism infects and survives in monocytes and monocyte-derived leukocytes and can be found in blood monocytes during natural infections, but the sequence of events resulting in enterocolitis remains speculative. The organism appears first to infect blood monocytes in experimentally infected horses, which may be the vehicle of organ infection.[50,59] However, in naturally infected horses, whether leukocytes of monocytic lineage or epithelial cells are infected first is unclear. The target organ is the gastrointestinal mucosa, with the most severe lesions found in the large intestine.[59,60] Infection of human colonic cells in vitro does not cause major cytopathologic effects for several days. Disruption of the microvilli in the region of the plasma membrane where sodium chloride channels are located has been observed in human colonic cell cultures.[61] Infection in horses is associated with variable degrees of morphologic damage.[59,60] Mild morphologic damage and mononuclear cell infiltration of the lamina propria occur early during the infection, but fibrinous, necrotizing typhlocolitis with severe mucosal ulceration and inflammation of the lamina propria may occur later in the disease. Vasculitis and intravascular coagulation are consistent features in the large intestine, with perivascular edema.[60] One can observe *N. risticii* in mucosal cells and macrophages and mast cells of the lamina propria.[59,60] *N. risticii* can survive and multiply in macrophages by inhibiting the production of reactive oxygen intermediates and by avoiding lysosomal digestion by blocking phagosome-lysosome fusion.[62–64]

Evidence of impaired sodium chloride absorption in the colon has been suggested to contribute to diarrhea in infected horses and may be related to destruction of the enterocyte membrane structure in the region of sodium chloride channels.[61,65] Direct injury to the mucosa by *N. risticii* and colonic inflammation are likely to be prominent features leading to diarrhea, especially later in the disease.[60] Fluid, protein, and electrolyte loss likely is caused by mucosal injury and effects on enterocyte fluid secretion caused by the inflammatory response. Like other inflammatory conditions of the colon, systemic inflammation caused by absorption of bacteria and bacterial products is a potential complication of *N. risticii* infections if mucosal injury is severe, which contributes to the clinical signs seen during the disease.

13.13.2.2.2

Clinical Signs and Diagnosis

N. risticii infection is clinically similar to other forms of enterocolitis and is characterized by anorexia, depression, and fever.[48,60,66] Experimental infections produce a biphasic fever occurring 6 to 7 days apart. Decreased gastrointestinal motility, manifested as reduced borborygmi, occurs during the early stages before the onset of diarrhea. Diarrhea occurs in 75% of cases and occurs 2 days after the second fever episode during experimental infections.[66,67] The diarrhea can be moderate to severe and dehydrating. Ileus can develop at any stage of the disease and can cause signs of moderate to severe colic. Systemic signs of endotoxemia, shock, and peripheral edema may occur and are similar to those described for salmonellosis. Experimental and natural infection with *N. risticii* can cause abortion of infected fetuses in pregnant mares.[68,69] Laminitis is a complication in 20% to 30% of naturally occurring cases and is often severe. Other complications include protein-losing enteropathy, thrombosis, and renal failure, as described for salmonellosis.

Hematologic abnormalities reflect endotoxemia, dehydration, and sepsis and are essentially identical to those described for salmonellosis. Neutropenia with a left shift is a consistent feature and occurs concurrently with or soon after the onset of diarrhea. Thrombocytopenia is common and often severe.[67] Neutrophilic leukocytosis occurs later in the course of the disease. Hyperfibrinogenemia is usually more pronounced than that with salmonellosis. Serum electrolyte, acid-base, and biochemical abnormalities are also similar to those described for salmonellosis. Coagulopathies are common during *N. risticii* infection and reflect activation of coagulation pathways. Disseminated intravascular coagulation is common and may be related to the high frequency of laminitis associated with *N. risticii* infection.[70]

One cannot base diagnosis of *N. risticii* infection solely on clinical signs because the disease is clinically similar to other forms of enterocolitis. However, in endemic areas, acute colitis is likely to be caused by *N. risiticii,* and thus the clinical signs of acute inflammatory colitis in fact may have a high predictive value. Serologic evidence of infection, such as rising antibody titers to *N. risticii* detected by indirect immunofluorescence or enzyme-linked immunosorbent assay in paired serum samples may be helpful in establishing a diagnosis.[49,71] One should take care when interpreting the indirect immunofluorescence serologic test for *N. risticii* because the test appears to have a high false-positive rate.[72] Culture of the organism from blood is possible but is difficult and is generally useful only in the research laboratory. 889
Recently developed polymerase chain PCR tests for *N. risticii* DNA are rapid, highly sensitive (as sensitive 890
as culture), and specific for *N. risticii* infection and can be applied to blood or feces.[73–75]

13.13.2.2.3 Prevention

Prevention of the disease by reducing exposure to the causative organism is difficult because the mode of transmission is not known. A killed vaccine has been developed that is effective in preventing clinical illness other than fever in 80% of experimentally challenged horses using the vaccine strain. However, field studies suggest the vaccine has limited benefit for prevention or decreasing the severity of natural infection. Vaccine failures have been attributed to strain differences in antigenicity or to poor antibody responses to the vaccine.[76,77]

13.13.2.3 EQUINE INTESTINAL CLOSTRIDIOSIS

13.13.2.3.1 Pathogenesis

Clostridiosis is an important cause of acute enterocolitis in foals and adult horses. *Clostridium perfringens* and *C. difficile* are associated most commonly with intestinal clostridiosis in horses, but other clostridial species, including *C. septicum, C. cadaveris,* and *C. sordellii* also have been isolated from horses with enterocolitis.[78–83] In horses of all ages, clostridial enterocolitis appears to be a common antibiotic-associated and nosocomial cause of enterocolitis.[82,84,85] However, clostridiosis in neonatal foals is a distinct clinical entity and is discussed in more detail elsewhere. This chapter focuses on adult intestinal clostridiosis.

Clostridia are obligate anaerobic to aerotolerant spore-forming gram-positive rods that are ubiquitous in the environment in the spore form.[83] Clostridia are elements of the normal flora of horses of all ages and are among the first bacteria acquired after birth. However, clostridia inhabiting the gastrointestinal tract

normally are found in low numbers and do not produce enterotoxins. Clostridiosis is associated with an increase in the number of a particular species of clostridia in the gastrointestinal tract and, perhaps most importantly, exotoxin production. Although the conditions resulting in exotoxin production are not understood fully, several factors increase clostridial numbers in the gastrointestinal tract. Dietary factors are known to affect the numbers of *Clostridium* species shed in the horse feces.[78] Experimental induction of colic increases fecal shedding of *Clostridium* species in the absence of diarrhea.[86] Antibiotics, particularly administered orally or recycled via the enterohepatic system, are well documented to increase the recovery of clostridia colony-forming units (CFUs) in equine feces and may result in clinical clostridiosis.[79,81,87–90] Indeed, clostridiosis associated with *C. perfringens* or *C. difficile* is likely to be the most important cause of antibiotic-induced enterocolitis in the horse.

13.13.2.3.1.1

Clostridium perfringens

C. perfringens is made up of many genetically distinct strains of variable virulence that produce one or more of a large group of exotoxins. The pattern of exotoxin production is used to classify *C. perfringens* into five types, A, B, C, D, and E. *C. perfringens* type A is the most common clostridium isolate from healthy horses of all ages and adults and foals with diarrhea worldwide. *C. perfringens* types A, B, C, and D have been associated with hemorrhagic enteritis in foals less than 10 days of age, with type C being the most common cause in North America.

The primary toxin produced by *C. perfringens* type A is α-toxin (phospholipase C), which interferes with glucose uptake and energy production and activates arachidonic acid metabolism and signaling pathways in enterocytes.[83] Oral administration of α-toxin does not cause tissue necrosis but causes increased secretion by small intestinal mucosal cells in human beings.[91,92] The β-toxin of types B and C is a cytotoxin that causes enterocyte necrosis, ulceration, and ultimately severe intestinal inflammation and hemorrhage.[93] A novel toxin designated β2 may also have a role in *C. perfringens* enterocolitis.[94] The biologic activity of the β2-toxin is similar to β-toxin, but β2-toxin is not related to β-toxin in sequence. The β2-toxin was prevalent in two groups of horses with acute enterocolitis but not in healthy horses.[95] The β2-toxin appears to be associated predominantly with *C. perfringens* that would have been classified otherwise as type A but that in fact may represent a previously undescribed type.

Virulent strains of *C. perfringens* type A and to a lesser extent type C also may produce enterotoxin. Enterotoxin is a cytotoxin that inserts into cell membranes to form pores, which alter permeability to water and macromolecules and ultimately lead to cellular necrosis.[96] Massive desquamation of the intestinal mucosa resulting from enterotoxin cytotoxicity triggers an inflammatory response, intestinal edema, mural hemorrhage, and systemic inflammation.[97] Enterotoxin also alters tight junction integrity, resulting in increased paracellular permeability by a noncytotoxic mechanism.[98]

13.13.2.3.1.2

Clostridium difficile

C. difficile produces several toxins, but only two, toxin A and toxin B have been studied in detail. Toxin B is a potent cytotoxin in vitro, but its role in enterocolitis is less clear than the role of toxin A. Toxin B does not induce fluid secretion, inflammation, or characteristic alterations in intestinal morphology.*C. difficile* induces an inflammatory response with hypersecretory diarrhea that is induced in large part by the enterotoxin toxin A.[99] Toxin A induces neutrophil influx into intestinal tissue, mast cell

degranulation, and secretion of prostaglandins, histamine, cytokines, and 5-hydroxytryptamine by these activated leukocytes.[100,101] The products of neutrophils and mast cells have a prominent role in the vasodilatory and secretory responses in the intestine during *C. difficile* infection. 890 891

The enteric nervous system is central to the induction of intestinal inflammation and mucosal secretion by toxin A. A model for toxin A–induced secretory diarrhea has emerged in which toxin A stimulates substance P–containing afferent sensory nerve fibers, which in turn stimulate mast cell degranulation, recruitment and activation of polymorphonuclear leukocytes, and vasodilation.[102–104] Toxin A–induced stimulation of enterocyte secretion can occur via secretomotor neuronal stimulation by substance P–containing sensory neurons or products of mast cells and polymorphonuclear leukocytes. Neural blockade or depletion of substance P abolishes mast cell degranulation, polymorphonuclear leukocyte influx, and enterocyte secretion. How toxin A triggers the sensory component of the enteric nervous system is not known yet, but toxin A–induced necrosis of enterocytes likely exposes afferent neurons to the noxious milieu of the intestinal contents.

13.13.2.3.2 Clinical Signs and Diagnosis

Equine intestinal clostridiosis is clinically similar to other forms of acute enterocolitis in horses.[78,83] Although the clinical course is usually acute, peracute colitis with rapid death may occur. Occasionally, a milder, more prolonged clinical course occurs. One may note fever, anorexia, and depression before the onset of gastrointestinal signs, but more commonly no prodromal signs are apparent. Signs of endotoxemia and shock may accompany acute signs of colic and severe, dehydrating diarrhea. Diarrhea may not be profuse but is usually dark and foul smelling. Like the clinical signs, hematologic and serum biochemical abnormalities are similar to those associated with other forms of enterocolitis and reflect fluid, protein, and electrolyte loss and systemic inflammation from endotoxemia. Neutropenia, leukopenia, and hemoconcentration are common. Hypoproteinemia may be profound. One often may note hyponatremia, hypokalemia, hypochloremia, hypocalcemia, and a mixed prerenal/renal azotemia, as well as metabolic acidosis and coagulopathies. Serum concentrations of hepatocellular enzymes such as sorbitol dehydrogenase may be elevated, and liver function may be reduced.

Preliminary diagnosis of equine intestinal clostridiosis caused by *C. perfringens* is based on the isolation of greater than 100 CFUs of *C. perfringens* type A per gram of feces from patients with diarrhea and signs suggestive of toxemia. Similar criteria are used to screen human patients for *C. perfringens* type A infection. Normal horses shed fewer than 100 CFUs/g of feces, and usually horses with intestinal clostridiosis shed greater than 1×10^6 CFUs/g.[78,105] However, identification of increased numbers of *Clostridium* organisms in the feces does not prove infection. Detection of *C. perfringens* toxins in feces or intestinal contents in horses with increased numbers of fecal CFUs and clinical signs of enterocolitis is more conclusive evidence of an enterotoxigenic infection than culture alone. Immunoassays are available that are primarily designed to detect *C. perfringens* enterotoxin.[83] However, the reliability (specificity) of some immunoassays for diagnosis of *C. perfringens* infection has come into question. Recently, PCR multiplex and gene probe assays have been developed to detect the major lethal toxins in isolates or fecal samples to determine the pattern or toxin production and are currently the preferred methods of detection.[106–108]

As for *C. perfringens,* diagnosis of *C. difficile* infection consists of culture of the organism from feces and identification of toxins in the feces. Bacterial culture of *C. difficile* may be difficult and may be an insensitive test in horses.[109–110] Detection may require enrichment techniques and culture of multiple fecal

samples.[110,111] Detection of toxins A and B in feces is regarded as the preferred test for diagnosis of *C. difficile* infection in human beings.[83] Commercial enzyme-linked immunosorbent assays are available for toxins A and B that are specific and appear to be more sensitive than a single culture for identifying *C. difficile* infection in adult horses.[109,110] One also may induce toxin production by *C. difficile* isolates. Sensitive PCR methods also have been developed for application to fecal samples and isolates to detect the genes for toxins A and B.[83]

13.13.2.4 PROLIFERATIVE ENTEROPATHY

13.13.2.4.1 Pathogenesis

Proliferative enteropathy is a chronic hyperplastic disorder of the small intestine and has been described in a variety of mammalian and avian species.[112,113] The only causative agent identified to date that induces proliferative enteropathy is the obligate intracellular pathogen *Lawsonia intracellulare*.[113,114] The pig is the most frequently naturally affected species. However, the reports of equine proliferative enteropathy associated with *L. intracellulare* have increased in recent years.[115–118] The relatedness of the strains of *L. intracellulare* causing proliferative enteropathy in pigs and horses or even among other affected species is not known. No host restriction is apparent because hamsters and other rodents can be infected with porcine strains of *L. intracellulare*. Before the year 2000, reports of proliferative enteropathy in the literature describing isolated cases were sporadic.[116–118] However, since 2000, outbreaks on breeding farms have been documented on farms in Canada, suggesting that a new strain has emerged.[115]

The mode of infection is fecal-oral, and infected animals can shed large numbers of organisms in feces.[113] 891
892 Affected animals shedding the organism in the feces serve as a source of infection for herdmates. Possibly nonequine species serve as reservoirs contributing to outbreaks on horse farms. Factors that increase the risk of proliferative enteropathy in pigs include overcrowding, ration changes, transport, and weaning.[113,114] Like pigs, horses are affected as weanlings. Factors associated with weaning and other stresses may affect immunity and increase susceptibility to infection. The incubation period is 2 to 3 weeks in nonequine species and is presumed to be similar in horses.

Experimental *L. intracellulare* infection produces characteristic pathologic lesions in pigs and hamsters that are identical to lesions in horses with proliferative enteropathy.[113,114] A profound hyperplasia of the mucosa predominantly caused by proliferation of crypt epithelium and crypt hyperplasia is induced locally in infected islands of tissue and eventually extends to the entire distal jejunum and ileum. *L. intracellulare* preferentially infects proliferating cells, thus the tropism for the crypt epithelium. Infected cells proliferate far more rapidly than uninfected cells, suggesting that *L. intracellulare* directly induced the proliferative response. However, the molecular basis for the enhanced proliferation is not known. *L. intracellulare* penetrates epithelial cells in a membrane-bound vesicle but eventually escapes the vacuole and is found free in the cytoplasm concentrated at the apical pole of the cell.

The gross pathologic lesions found in equine proliferative enteropathy are characteristic.[115–118] Lesions may be segmental and typically are found in the ileum and terminal jejunum in horses. However, lesions also may affect the duodenum. Severe mucosal hypertrophy often occurs but may wane during the chronic stages of the disease. The mucosa may become corrugated with focal erosions or ulcers. One often can identify submucosal edema easily on cut sections of affected segments. Moderate to severe crypt

hyperplasia with atrophy of the intestinal villi is a consistent feature. The hyperplastic crypts are branched and may herniate into the submucosa. Necrosis, edema of the submucosal and lamina propria, hemorrhage, mononuclear inflammation and muscular hypertrophy have been reported in affected intestinal segments but are not consistent. Special stains such as silver stain are required to detect intracellular organisms. The organisms are curved or comma-shaped rods found clustered in the apical cytoplasm of hyperplastic crypt epithelium.

The proliferative response of the intestinal mucosa alters absorption of nutrients and fluid secretion by disrupting the architecture of the villi and by altering the maturation of epithelial cells into absorptive cells, accounting for the secretory diarrhea and often severe weight loss.[112,114] The combined effects of the inflammatory response and malabsorption may account for the protein-losing enteropathy.

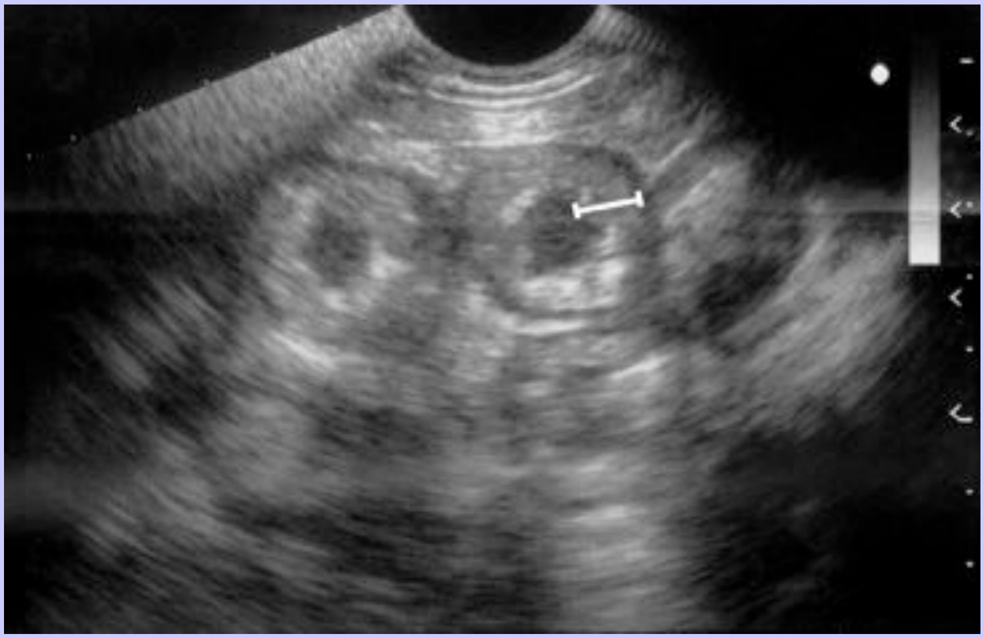
13.13.2.4.2 Clinical Signs and Diagnosis

Proliferative enteritis most commonly affects weanling foals ages 4 to 6 months. The clinical signs of proliferative enteropathy include ill thrift, weight loss, peripheral edema, diarrhea, and colic.[115–118] The diarrhea is usually in the form of soft feces but may be profuse and watery. Some foals with mild diarrhea have black, tarry feces. Secondary complications such as gastric ulceration, bronchopneumonia, or parasitism may occur concurrently with the proliferative enteropathy. Clinicopathologic features include mild to moderate anemia, moderate to severe hypoalbuminemia (often <2 g/dl), hypoglobulinemia, neutrophilic leukocytosis, and hyperfibrinogenemia. Creatine kinase activities may be elevated in affected foals. Prerenal azotemia and electrolyte imbalances such as hyponatremia may be associated with diarrhea. Peritoneal fluid analysis is usually unremarkable. Ultrasonographic examination of the small intestine often reveals significant thickening of the intestinal wall (Figure 13.13-1). Intestinal edema may be evident as a hypoechoic appearance to one or more layers of the intestinal wall.

Methods for antemortem diagnosis include serologic analysis of *L. intracellulare* antibodies and PCR analysis of feces.[115] Serologic analysis using an indirect immunofluorescent antibody test may be the most useful single test available. The PCR test is specific but the sensitivity may be low. By the time clinical signs appear, 90% of pigs are serologically positive for anti–*Lawsonia intracellulare* immunoglobulin G (IgG). In contrast, only 37% of pigs had positive fecal PCR tests.[119] Of the seven foals tested in an outbreak of equine proliferative enteropathy,[115] four foals with confirmed disease and three with suspected 892

893 proliferative enteropathy had serologic titers against *L. intracellulare* of 1:30 or greater. In contrast, serum samples collected from 72 foals before the outbreak were negative for *L. intracellulare* antibodies. Fecal PCR for *L. intracellulare* was positive in 6 of 18 foals tested, and half of the serologically positive foals had negative fecal PCR tests. Many clinicians combine serologic analysis with fecal PCR testing to increase the sensitivity and specificity of these diagnostic methods. Isolation and culture of the organism requires cell culture techniques that are not widely available. Thus no practical method exists for culturing the organism from feces or tissues that is available for clinical use.

Figure 13.13-1 Ultrasonogram of the small intestine in a weanling foal with weight loss, diarrhea, and hypoproteinemia. The extreme mural thickening of the small intestine *(bar)* is typical of proliferative enteropathy.



Definitive diagnosis requires histopathologic examination of affected tissues.[112] Diagnosis is based on typical histopathologic findings of mucosal hypertrophy and submucosal edema and identification of small, curved, rod-shaped intracellular bacteria at the apical pole of epithelial cells in affected segments of intestine. Special stains such as Warthin-Starry silver stain are required to detect the bacteria in histopathologic specimens. PCR analysis of affected intestinal tissue is a specific test for the presence of *L. intracellulare* and, unlike fecal PCR analysis, appears to be sensitive.[120]

13.13.2.5 STRONGYLOSIS

13.13.2.5.1 Pathogenesis

Strongyle infections in horses are caused by two groups of nematodes: large and small strongyles (see the section Cyathostomiasis). Large strongyles that are pathogenic in horses include *Strongylus vulgaris, S. edentatus,* and *S. equinus*. Of these species, *S. vulgaris* is by far the most important cause of disease in the large intestine and in fact is the most pathogenic parasitic infection in horses. *S. vulgaris* infection in horses is manifested clinically by two forms: acute and chronic disease.[121] The age and resistance of the host, the infective dose, and the size and function of the affected arteries influence the type and degree of disease that occurs. Sudden ingestion of large numbers of infective larvae by a naïve host causes acute strongylosis, whereas ingestion of fewer infective larvae over a long period of time by an older, more resistant host

causes chronic strongylosis. Acute strongylosis is more likely to cause colic than diarrhea and may be rapidly fata l. Chronic strongylosis tends to cause debilitation and signs of colic but may also cause diarrhea.

Diarrhea associated with acute strongylosis occurs within several days of infection and is likely to be caused by migration of the larvae through the intestinal wall. Fourth-stage larvae migrate through the mucosa and submucosa into the arterioles of the intestine, causing mural edema, hemorrhage, and infiltration with inflammatory cells into the intestinal wall.[121,122] Increased secretion and decreased absorption of fluid and electrolytes, stimulated by inflammatory mediators such as prostaglandins and histamine, may play a role in the diarrhea induced by *S. vulgaris*. Interstitial edema and damage to the interstitial matrix and mucosa may result from inflammation and migration of the parasites, causing increased secretion of fluid and albumin loss. Abnormal gastrointestinal motility also may play a role in the development of diarrhea. Migration of larvae through the intestinal wall early in the course of infection affects myoelectric activity and motility in the large intestine and may affect retention of ingesta and absorption of fluid.[123,124] The cause of death in acute strongylosis has not been addressed but may be related to massive migration through the vasculature, causing thrombosis with ischemia and infarction of the intestine.

Chronic strongylosis causes typical verminous arteritis and is associated more commonly with natural infections in horses than with acute strongylosis.[121] Lesions of the large intestinal vasculature caused by migration of larvae through the intima are characterized by thrombus formation, narrowing of the arterial lumen, fibrosis, and thickening of the arterial wall.[121,122] Embolization may occur, causing acute segmental infarction of the large intestine, but more commonly reduced blood flow without embolization causes ischemia and occasionally infarction.[122,125] Postmortem examination of horses with colonic infarction failed to reveal embolization as the cause in most cases.[125] Reduced blood flow in the tissues of the intestine usually results from narrowing of the arterial lumen by the thrombus and formation of microthrombi at sites independent of the parasites. Release of vasoconstrictive inflammatory mediators such as leukotrienes from platelets, neutrophils, and eosinophils and elaboration of parasitic antigens or toxins may cause vasoconstriction and ischemia.[126] Horses with experimental strongylosis were found to have a 50% reduction of blood flow in the colonic vasculature.[127]

Clearly, reduced blood flow is an important effect of chronic strongylosis, but the relationship between blood flow and diarrhea is unclear. Disrupted motility resulting from ischemia may lead to diarrhea by reducing the retention of ingesta and absorption of fluid. Acute infarction and mucosal ulceration have been found to cause severe chronic diarrhea in naturally infected horses.[128] Release of inflammatory mediators such as prostaglandins, histamine, and kinins from inflammatory cells associated with thrombi and inflamed intestine also may affect secretion, absorption, and motility, leading to diarrhea.

13.13.2.5.2 Clinical Signs and Diagnosis

The clinical signs of acute strongylosis caused by *S. vulgaris* infection are characterized by depression, moderate to severe colic, and fever.[129] Diarrhea is less often a feature of acute strongylosis than is colic.[121] Most cases of acute strongylosis occur in young, naïve horses that are introduced to an infested environment or are inoculated experimentally with infective larvae. This form of strongylosis often is not recognized naturally. Chronic strongylosis, however, occurs most commonly as a natural syndrome. Weight loss or poor weight gain; chronic, intermittent colic; fever; poor appetite; and diarrhea are frequent signs.[121,122] Diarrhea may be profuse and watery, or the feces may be soft but of normal volume. Transrectal palpation

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may reveal thickening and fremitus in the cranial mesenteric artery. Young horses are most commonly affected, but older horses also may be affected. Horses with acute infarction or large intestinal ulceration following chronic strongylosis may have signs of severe abdominal pain, sepsis, and endotoxemia, and profuse, watery diarrhea is common.

Hematologic abnormalities associated with strongylosis include neutrophilic leukocytosis and eosinophilia.[129–131] Neutrophilia appears to be an early event during the course of the disease, and eosinophilia tends to appear later.[129,131] Hyperfibrinogenemia also may occur, especially later in the course of the disease. Serum α- and β-globulin and IgG(T) concentrations are characteristically elevated.[130–132] Horses with chronic ulcerative colitis following strongylosis may exhibit severe hypoalbuminemia.[128] Peritoneal fluid analysis may reveal an elevated protein concentration and eosinophilia.[130,131] Tentative diagnosis is based on clinical signs, hematologic abnormalities, and peritoneal fluid analysis. Elevated serum α- and β-globulin concentrations and IgG(T) concentration support the diagnosis.[132] Fecal analysis may reveal strongyle eggs, but fecal egg counts are often unreliable because nonpatent larvae cause the disease.

13.13.2.5.3 Prevention

Appropriate preventive measures are important in controlling this disease, including management procedures such as preventing overcrowding, reducing exposure of susceptible individuals, and instituting proper deworming schedules. Ivermectin is the preferred anthelmintic used to control strongylosis in horses. Monitoring fecal egg counts as a means of evaluating the efficacy of parasite control measures is recommended.

13.13.2.6 CYATHOSTOMIASIS

13.13.2.6.1 Pathogenesis

Infection with small strongyles (cyathostomiasis) is well recognized as a cause of diarrhea and large intestinal disease in horses of all ages.[133–138] Clinical disease is caused by intramural larval stages. The cyathostome life cycle requires migration by fourth-stage larvae through the mucosa of the large intestine and may include a period of hypobiosis during which the larvae remain encysted within the mucosal layer of the large intestine. After a period of hypobiosis the larvae emerge in response to a largely unknown stimulus. Most cases occur when larval emergence takes place, classically in the late winter and spring in the northern temperate zones when larvae are expected to emerge and in the late fall or winter months in the southeastern United States and subtropical regions.[133] Sudden emergence of encysted larvae causes the mucosal injury, ulceration, and inflammatory reaction responsible in large part for the clinical disease.[133,139] However, migration of the larvae as they penetrate the mucosa affects motility patterns and can cause inflammation that may contribute to diarrhea.[139] Chronic, eosinophilic, granulomatous colitis and diarrhea with histopathologic evidence of hypobiotic cyathostome larvae in the large intestine have been reported in two horses during a period in which emergence of larvae would not be expected to occur (early winter).[133]

Natural emergence of cyathostome larvae causes fibrinous inflammation of the large intestine, focal necrosis, mural hemorrhage, and ulceration of the large intestinal mucosa, which even may result in bleeding into the lumen. Mild to moderate eosinophilic and mononuclear inflammation of the lamina

propria occurs, and moderate to severe interstitial edema frequently occurs.[122,139] Colonic inflammation and interstitial edema may contribute to the diarrhea, along with the loss of the mucosal barrier, by causing increased active and passive secretion of fluid, electrolytes, and protein. Protein loss is often significant, resulting in profound hypoalbuminemia and interstitial edema of skin and other organs. Chronic, granulomatous colitis has been reported to occur in response to encysted larvae and may cause diarrhea by increased secretion following granulomatous inflammation or disruption of the interstitium by granulomatous infiltration. Administration of an anthelmintic to horses with a heavy load of encysted larvae also may cause rapid larval death and acute and often severe inflammation similar to natural emergence.

13.13.2.6.2 Clinical Signs and Diagnosis

Cyathostomiasis may be the most commonly identified cause of chronic diarrhea in the horse.[140–142] However, an acute syndrome also has been associated with cyathostomiasis.[138] Clinical signs of cyathostomiasis are characterized by moderate to severe weight loss or poor weight gain, ill thrift, ventral edema, intermittent fever, and intermittent, mild colic. Acute onset of diarrhea is typically profuse and progresses to chronic diarrhea that is often mild, is the consistency of bovine feces, and may be intermittent.[133–138,142] Appetite is usually normal, but affected horses occasionally have a ravenous appetite. Transrectal palpation usually does not reveal any abnormalities. Cyathostomiasis may affect any age of 894
horse, and clinical signs are more common during periods of emergence of larvae, corresponding to late 895
winter and spring in northern temperate zones. The deworming history may appear to be adequate.

Neutrophilic leukocytosis is typically evident, but the white blood cell count may be normal.[133–138] Profound hypoalbuminemia is a characteristic feature of cyathostomiasis, manifested clinically by ventral edema. Plasma α- and β-globulin concentrations may be elevated, which can result in a normal total plasma protein concentration in spite of hypoalbuminemia.[134–136] The serum IgG(T) concentration, however, has been reported to be normal, which may help distinguish cyathostomiasis from *S. vulgaris* infection.[133,135,136] In addition, peritoneal fluid analysis does not usually reveal any abnormalities, in contrast to horses with *S. vulgaris* infection. Fecal analysis may be unrewarding because the infection is often not patent when clinical signs are apparent. Measurement of plasma fructosamine may provide a measure of protein catabolism or protein loss in the absence of hypoalbuminemia. Plasma fructosamine concentrations are significantly lower in horses with experimental cyathostomiasis than in normal controls,[142,143] suggesting that this test may be a useful diagnostic tool. However, the test has not yet been validated in naturally occurring cases, and neither the specificity nor the sensitivity is known. Rectal scrapings or rectal mucosal biopsies may reveal evidence of cyathostome larvae.[133,136] Definitive diagnosis usually requires microscopic examination of biopsy specimens of the cecum and ascending colon, collected by laparotomy. Examination of biopsy specimens collected from the small intestine is recommended to rule out other causes of weight loss and diarrhea. One should include appropriate diagnostic tests, such as culture of feces for pathogenic bacteria, in the workup to rule out other causes.

13.13.2.6.3 Prevention

Preventive measures are appropriate for other horses on premises known to have a problem with cyathostomiasis, particularly frequent deworming (every 6 weeks) during times of high infectivity (spring and summer in the north and fall, winter, and early spring in the south) to eliminate parasites before they become patent.[133] Because of high levels of resistance to benzimidazoles, avermectins (ivermectin or

moxidectin) are the drugs of choice.[144–146] Resistance to ivermectin has been demonstrated, but the prevalence of ivermectin resistance appears to remain low.[144] Although daily pyrantel pamoate administration also has been reported to reduce worm burdens and pasture infectivity in young and mature horses effectively,[147] cyathostome resistance has been reported and is a concern for the use of this drug as a routine preventive anthelmintic.[145,148]

13.13.3 Toxicologic Diseases

Diarrhea in adult horses may also occur secondary to administration of antimicrobial or antiinflammatory medications or after ingestion of toxic compounds. Affected horses exhibit clinical signs that may be indistinguishable from signs exhibited by horses with diarrhea of infectious etiology.

13.13.3.1 ANTIBIOTIC-ASSOCIATED DIARRHEA

13.13.3.1.1 Pathogenesis

Antibiotic-associated diarrhea has been reported in many species, including horses.[149] Certain antibiotics—such as trimethoprim-sulfonamide combinations, erythromycin, penicillins, tetracyclines, clindamycin, and lincomycin—are associated with naturally occurring and experimental enterocolitis syndromes in horses.[79,149–152] In some cases, such as with trimethoprim-sulfonamide combinations, the geographic incidence of antibiotic-associated diarrhea appears to differ considerably.

Clostridium perfringens, C. difficile, and *Salmonella* spp. are apparently the most common causes of antibiotic-associated diarrhea in horses. Outbreaks of *C. difficile* have been reported in hospitalized horses being treated with antibiotics.[81,85] In Sweden, accidental erythromycin ingestion has been associated with *C. difficile* enterocolitis in mares in which their foals were being treated for *Rhodococcus equi*.[89,151,153] Interestingly, this phenomenon has not been reported in other areas of the world. Foals being treated with erythromycin are at a higher risk for diarrhea than foals being treated with other antibiotics. Tetracycline administration has been shown to be associated with an increase in the numbers of gram-negative enteric bacteria and *C. perfringens* in the feces of horses and reactivation of salmonellosis and prolongation of fecal shedding of *Salmonella*.[78,154]

The most common mechanism by which antibiotics cause diarrhea is by disrupting the gastrointestinal flora. The normal large intestinal flora, comprised of mainly obligate anaerobes and streptococci, protects the host from pathogenic bacteria by colonization resistance.[17] Ecologic factors play an important role in colonization resistance. For example, surface bacteria in the large intestine interact with receptors on the mucosal cells, facilitating adherence to the mucosa.[17,155] In doing so, the normal organisms compete more successfully for this important niche. Competition for space and nutrients is an important means of preventing colonization and proliferation of pathogenic bacteria. In addition, anaerobic bacteria produce short-chain fatty acids (SCFAs) and other metabolites that are toxic to facultative anaerobic bacteria, especially in the conditions of the large intestine.[16,17,155] Organisms of the normal flora also produce bacteriocins that inhibit growth of potential pathogens.[16] 895

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Antibiotics that deplete the population of obligate anaerobes and streptococci efficiently decrease colonization resistance.[16] Production of fatty acids diminishes, thus reducing competition for space and

nutrients. As a result, gram-negative enteric bacteria such as *Salmonella* are able to proliferate. In addition, pathogenic anaerobes normally found in low numbers can proliferate. Antibiotic-resistant strains of bacteria, especially gram-negative enteric bacteria and possibly clostridia, may be selected by antibiotic administration, allowing proliferation of pathogenic bacteria resistant to many antibiotics.[156] Obligate anaerobic commensal organisms, perhaps the most critical group of microbes for maintaining colonization resistance, are usually susceptible to macrolides, tetracyclines, β-lactams, and lincosamides, perhaps explaining the high incidence of diarrhea associated with the administration of these antibiotics.[83]

In addition to reduction of colonization resistance, depletion of the normal anaerobic microbial population in the intestine decreases carbohydrate fermentation and production of SCFAs, which contributes to the pathogenesis of antibiotic-associated diarrhea by decreasing absorption of sodium and water by the colonic mucosa.[157] Ampicillin decreases colonic fermentation of carbohydrates in human beings.[158] Human patients with antibiotic-associated diarrhea have greatly impaired colonic fermentation and low production of SCFAs. Erythromycin, ampicillin, or metronidazole treatment is associated with decreased production of SCFAs in patients with and without diarrhea.[88] Absorption of sodium and water is stimulated by absorption of SCFAs in the equine colon, suggesting that reduction of colonic SCFA content by antibiotic-induced depletion of anaerobic flora has similar effects in horses as in human beings.[157]

Broad-spectrum antibiotics exert a more profound effect on the gastrointestinal flora than narrow-spectrum antibiotics. Antibiotics administered orally, especially those that are poorly absorbed, are more likely to cause diarrhea than are parenterally administered antibiotics. For instance, clindamycin is less likely to cause diarrhea in human beings when administered intravenously than when administered orally. Antibiotics with extensive enterohepatic circulation, such as tetracyclines and erythromycin, are excreted in high concentrations in the bile and are associated more commonly with diarrhea than antibiotics that do not undergo enterohepatic circulation.[159]

Antibiotics may cause diarrhea by means other than by disrupting the normal flora. Direct toxic effects may play a role in producing irritation, increasing secretion, and disrupting motility patterns. Tetracyclines are irritating to the gastrointestinal mucosa and may cause inflammation and increase secretion.[159] Erythromycin has been shown to interact with smooth muscle cells, stimulating gastrointestinal motility.[159,160] Normal peristalsis plays an important role in suppressing the population size of potentially pathogenic bacteria. Normally, bacteria that are prevented from adhering to the mucosa by colonization resistance are swept aborally by peristalsis and are excreted in the feces. Disruption of normal motility patterns may prevent clearance of pathogenic bacteria, contributing to the colonization of mucosal surfaces.

13.13.3.1.2 Clinical Signs and Diagnosis

Diarrhea induced by antibiotics usually occurs within 7 days of antibiotic administration or can occur several days after cessation of antibiotic treatment. The clinical syndrome of antibiotic-associated diarrhea can vary from mild diarrhea to fulminant enterocolitis with severe diarrhea. Mild cases of diarrhea are common, especially in foals receiving erythromycin, trimethoprim-sulfonamide combinations, or rifampin.[151,161] Mild cases of diarrhea are usually not clinically significant. However, acute, severe enterocolitis can occur in all ages of horses receiving antibiotics and can be life threatening. Clinical signs are identical to other causes of acute enterocolitis. Severe, dehydrating diarrhea, endotoxemia, sepsis, and shock may occur. Hemoconcentration, neutropenia, hypoproteinemia, and electrolyte and acid-base imbalances are common. Severe hyponatremia may occur in foals with antibiotic-associated diarrhea, especially if trimethoprim-

sulfonamide and rifampin combinations are the cause.[161] More detailed descriptions of the clinical and laboratory findings were given previously. Diagnosis is presumptive, because definitive diagnosis of antibiotic-associated diarrhea is impossible. Fecal culture and PCR testing may reveal *Salmonella* or *Clostridium* infection.

13.13.3.2 NONSTEROIDAL ANTIINFLAMMATORY DRUGS

13.13.3.2.1 Pathogenesis

Toxicity resulting from nonsteroidal antiinflammatory drug (NSAID) administration has been well documented in several species, including horses.[162–168] In horses and human beings, NSAID toxicity is manifested by renal and gastrointestinal disease. Elderly human patients are more susceptible to NSAID toxicity, but the effects of age on NSAID toxicity in horses are less well defined. Foals are considered to be more susceptible than adult horses to gastrointestinal disease following NSAID administration, and ponies may be more susceptible than horses. All NSAIDs are capable of inducing gastrointestinal and renal damage at toxic concentrations, and the toxicity is not significantly different among products. Aspirin is potentially more toxic than other NSAIDs because it irreversibly inactivates cyclooxygenase by acetylation,
whereas other NSAIDs reversibly inhibit cyclooxygenase.[162] However, phenylbutazone is the drug most 896
commonly reported to cause toxicity in horses, perhaps because of its widespread use by veterinarians and 897
horse owners. Phenylbutazone toxicity in horses is characterized by mucosal ulceration throughout the gastrointestinal tract, oral ulceration, renal papillary necrosis, vasculopathy, thrombosis, and protein-losing enteropathy with hypoalbuminemia.[164–166] This discussion focuses on the toxic effects of NSAIDs on the large intestine but necessarily includes elements of upper gastrointestinal and renal disease.

Horses with large intestinal disease resulting from NSAID toxicity generally are receiving inappropriately large doses. The dosage regimen recommended for phenylbutazone (4.4 mg/kg twice in 1 day and then 2.2 mg/kg twice daily) is considered to be safe. Experimental studies in horses, however, have shown toxicity to occur when greater than the recommended dosage (6.6 mg/kg/day) is administered for several days.[164,165] In most reported cases of phenylbutazone toxicosis horses were receiving higher than recommended dosages.[166,168,169] Regardless, administration of phenylbutazone at the recommended dosage has been reported to cause a significant decrease in plasma protein concentration and gastrointestinal disease.[165,170] Moreover, signs of NSAID toxicity have been reported in normovolemic horses treated with appropriate doses of phenylbutazone.[170,171] Dehydration, sepsis, and endotoxemia exacerbate the renal and gastrointestinal toxicity of NSAIDs.[162] Clearly, the margin of safety is narrow for phenylbutazone and probably for other NSAIDs used in horses as well.

Gastrointestinal disease induced by NSAIDs is manifested by mucosal ulceration, inflammation, bleeding, and protein-losing enteropathy.[164,165,168,170] In addition to direct effects on the mucosal barrier, NSAID administration has been shown to cause an acute relapse of preexisting colonic inflammatory disease and worsen colonic inflammation in human beings.[164,165,170] Whether this occurs in horses is not clear. The mechanism by which NSAIDs induce mucosal damage is probably multifactorial. Direct irritation may play a role in oral and gastric irritation and ulceration; however, parenteral administration of NSAIDs produces oral and gastric ulceration as well. Inhibition of prostaglandin synthesis by inhibition of cyclooxygenase 1 (the constitutive COX) and cyclooxygenase 2 (the inducible COX) appears to be the most important mechanism of mucosal injury. Prostaglandins, particularly PGE_2 and PGI_2, are critical for mucosal health.

[172,173] PGE_2 has been shown to increase mucosal blood flow; increase secretion of mucus, water, and bicarbonate; increase mucosal cell turnover rate and migration; stimulate adenyl cyclase activity; and exert other protective effects in the gastric mucosa of several species.[162,172,173] Perhaps most importantly, PGE_2 and PGI_2 have a role in maintaining epithelial tight junction integrity, which is indispensable for mucosal barrier function and repair after mucosal injury.[172]

In spite of the overwhelming amount of information about the role of prostaglandins in maintaining the mucosal barrier in other species and clear clinical and experimental evidence that NSAIDs injure the equine colonic mucosa, the role of prostaglandins in mucosal protection in the equine colon is not yet well defined. Inhibition of COX-1 and COX-2 in equine colonic mucosa with flunixin meglumine resulted in reduced electric resistance of the mucosa and increased permeability to macromolecules in vitro (A.T. Blikslager and S.L. Jones, 2002), suggesting that flunixin treatment disrupts the epithelial tight junctions in the equine colon. Mucosal changes were correlated with a profound inhibition of PGE_2 and PGI_2 concentrations in the treated tissues. In other studies, administration of a PGE_2 analog prevented the gastrointestinal manifestations of phenylbutazone toxicosis in ponies.[165]

Recent development of NSAIDs specific for COX-2 have greatly reduced the frequency and severity of gastrointestinal side effects in human beings taking NSAIDs for chronic musculoskeletal conditions.[174] Thus COX-2–specific NSAIDs hold promise for use in horses to treat arthritis and reduce the incidence of toxicity. For example, the COX-2–specific inhibitor etodolac was less harmful to equine colonic mucosa than flunixin meglumine in vitro (A.T. Blikslager and S.L. Jones, 2002). Moreover, etodolac was significantly more permissive than flunixin for recovery of the mucosa in equine ischemic-injured intestinal tissues, and in fact, recovery was no different than control tissues.[175] However, their use is at present limited because the specificity of the so-called COX-2– selective inhibitors and their efficacy as analgesics have not been demonstrated in the horse.

NSAID-induced mucosal injury is associated with a significant inflammatory response to microbial products exposed to the lamina propria.[176] This inflammation exacerbates mucosal dysfunction and injury associated with NSAID toxicity. For example, depletion of neutrophils or blockade of neutrophil influx into gastrointestinal tissues or inhibition of neutrophil activation and release of toxic products prevents many of the pathophysiologic effects of NSAID toxicity in the gastrointestinal tract.[177–180] The inflammatory response alone may result in moderate to severe gastrointestinal ulceration, mural vascular thrombosis and edema, fluid secretion, protein-losing enteropathy, and mucosal hemorrhage.

13.13.3.2.2 Clinical Signs and Diagnosis

NSAID colitis manifests as two clinical syndromes: right dorsal colitis (RDC) and generalized NSAID toxicity. RDC is an ulcerative disorder isolated to the right dorsal segment of the large intestine.[167,168,171] The most prominent clinical signs of RDC are anorexia, lethargy, and colic. Anorexia, depression, diarrhea, 897
fever, and signs of endotoxemia also may be features. If the RDC is chronic, weight loss, intermittent colic, 898
lethargy, anorexia, and ventral edema are common clinical signs, along with soft and unformed feces. Severe ulceration of the right dorsal colonic mucosa results in protein-losing enteropathy and significant hypoproteinemia attributable mainly to hypoalbuminemia. Hypoproteinemia may be severe enough to cause peripheral (usually ventral) edema. In some cases, one may note dehydration, electrolyte abnormalities,

neutropenia or anemia, azotemia, and biochemical abnormalities if the ulceration and diarrhea are severe or if systemic inflammation is present.

Clinical signs of generalized NSAID toxicity may vary from mild diarrhea with no systemic signs to severe dehydrating diarrhea with anorexia, fever, depression, peripheral edema, oral ulceration, and colic.[165,166,169] Clinical signs of systemic inflammation caused by endotoxemia may occur, manifested as poor peripheral perfusion, tachycardia, tachypnea, weakness, trembling, and cyanotic or hyperemic oral mucous membranes. Hematuria or oliguria may be present if renal involvement is present. Complications associated with other forms of severe enterocolitis, such as laminitis, thrombophlebitis, and severe weight loss, may occur.

Hematologic abnormalities of generalized NSAID toxicity are nonspecific and include neutropenia with a left shift or leukocytosis and hemoconcentration. Serum biochemical analysis is characterized by profound hypoproteinemia, hyponatremia, and metabolic acidosis.[169,170] Hypocalcemia, hypokalemia, hypochloremia, and elevated hepatocellular enzyme activities also may occur. Hypoproteinemia may occur without signs of diarrhea. Azotemia may be prerenal from dehydration but frequently is caused by renal failure resulting from a combination of hemodynamic and toxic renal injury. Urinalysis frequently reveals hematuria, proteinuria, cylindruria, and isosthenuria. Fecal occult blood is frequently detectable.

Diagnosis of either form of NSAID colitis is often presumptive, with a history of overdose of NSAIDs being strong evidence of NSAID toxicity. But as discussed earlier, toxicity may occur with dosage regimens that are not considered inappropriate, particularly if the horse experiences a concurrent period of dehydration. One can use ultrasonographic examination of the right dorsal colon to confirm a diagnosis of RDC, but the sensitivity of this method is questionable. Ultrasonography (3.5- to 5-MHz transducer at the right twelfth to fifteenth intercostal spaces below the margin of the lung axial to the liver) may reveal a thickened right dorsal colon (>0.5 cm) and evidence of colonic edema in horses with RDC.[181] However, the sensitivity of this method of diagnosis is questionable. One can use nuclear scintigraphy of horses after infusion with technetium 99–labeled white blood cells to document inflammation of the right dorsal colon.[182] Diagnosis of RDC may require one to perform laparotomy or laparoscopic examination of the right dorsal colon. One must rule out other causes of enterocolitis, such as salmonellosis, Potomac horse fever, clostridiosis, and antibiotic-associated diarrhea.

13.13.3.3 CANTHARIDIN TOXICITY

13.13.3.3.1 Pathogenesis

Cantharidin is the toxic principle found in beetles of the genus *Epicauta,* commonly known as blister beetles.[183–185] Ingestion of the beetles in contaminated alfalfa hay causes release of the toxin from the tissues of the beetle and absorption through the gastrointestinal tract. Transcutaneous absorption may occur but appears to be rare in horses.[184] Blister beetles feed on the flowers of alfalfa and may be incorporated into processed alfalfa hay if the hay is cut and processed simultaneously, as by crimping.[183–185] The beetles often swarm, and one may find large numbers of beetles in small portions of hay. The lethal dose of cantharidin is less than 1 mg/kg, but the concentration of cantharidin varies from species to species of blister beetles and between sexes.[183,184] As many as 100 to as few as 6 to 8 beetles may be lethal. Usually, only one or a few horses fed contaminated hay ingest beetles because the beetles are concentrated in a small portion of the hay. However, outbreaks involving many horses on a farm have occurred. Most cases occur

in Texas and Oklahoma, but horses in other states may be affected as well, especially if hay is imported from states where blister beetles are common. Peak incidence is in late summer and fall.[186] The fatality rate may be 50% or greater,[183,187] but if the patient survives several days, recovery is probable.

Cantharidin is absorbed from the gastrointestinal tract and excreted via the kidney. Cantharidin is a potent irritant, causing acantholysis and vesicle formation when applied topically.[183,185,187] The chemical is thought to disrupt oxidative metabolism in the mitochondria, causing mitochondrial swelling, plasma membrane damage, and changes in membrane permeability.[183] The mucosa of the gastrointestinal tract is affected most commonly in horses because they ingest the toxin. Cell swelling and necrosis occur, resulting in mucosal ulceration. Oral, esophageal, gastric, and small and large intestinal ulceration have been observed in natural and experimental canthariasis.[183,185,187] Severe fibrinous to pseudomembranous inflammation and submucosal edema of the intestine also have been reported. Diarrhea probably results from the severe ulceration and inflammation of the large intestine, causing increased secretion of water, electrolytes, and protein and decreased absorption of fluid. Large volumes of fluid and protein are lost in the 898
gastrointestinal tract, causing hemoconcentration and profound hypoalbuminemia in some cases.[183,184,187] 899

Cystitis and myocarditis occur in natural and experimentally produced cases of cantharidin toxicity.[183,185,187] Cystitis occurs because renal excretion of cantharidin results in high concentrations in urine. Occasionally, hemorrhagic cystitis may occur, with hematuria or frank hemorrhage into the bladder.[183] The cause of the myocarditis and myocardial necrosis is unknown but also may be a direct effect of the toxin on the myocardium. Elevated plasma creatine kinase activity often occurs and has been postulated to arise from the damaged myocardium.[183,184] Horses have a characteristically stiff gait, but histopathologic evidence of skeletal muscle injury that explains the elevated plasma creatine kinase activity has not been observed.[184] The kidneys are often pale, swollen, and moist, with occasional infarcts.[185]

Hypocalcemia and hypomagnesemia are biochemical features of cantharidin toxicity in horses that have not been explained.[183,184,187] Hypocalcemia may occur from hypoalbuminemia, but the ionized calcium concentration often is decreased along with the total calcium concentration, indicating that hypoalbuminemia is not responsible for the hypocalcemia.[184] In addition, clinical signs of hypocalcemia, such as synchronous diaphragmatic flutter, are often associated with hypocalcemia from cantharidin toxicity. Hypocalcemia associated with hypoalbuminemia alone does not produce clinical signs.

13.13.3.3.2 Clinical Signs and Diagnosis

Cantharidin toxicity can cause a range of clinical signs from mild depression and abdominal discomfort to fulminant signs of toxemia and rapid death, depending on the ingested dose of toxin. Most commonly, clinical signs include depression, sweating, irritability, abdominal pain, elevated heart and respiratory rates, fever, polyuria, polydypsia, and profuse diarrhea.[183,184,187] Blood is rarely visible in the feces. Affected horses frequently posture to urinate; indeed, stranguria and pollakiuria are characteristic of cantharidin toxicity.[183] Signs of hypocalcemia include synchronous diaphragmatic flutter and tremors. A stiff and stilted gait may be evident. One may note neurologic signs such as head pressing, swaying, and disorientation.[187] Signs of systemic inflammation from endotoxemia may be apparent in severe cases. Some horses develop severe depression and toxemia and may die within hours after ingestion of cantharidin without developing diarrhea.[183,187]

Hematologic abnormalities include hemoconcentration and neutrophilic leukocytosis. Occasionally, neutropenia and leukopenia may accompany endotoxemia. Serum biochemical analysis usually reveals elevated creatine kinase activity, hypocalcemia, and hypoalbuminemia.[183,184] Biochemical abnormalities include hypocalcemia (ionized and total calcium concentrations), hypomagnesemia, and azotemia.[183,184,187] Urine specific gravity is characteristically in the hyposthenuric range.[183,184] Microscopic hematuria and mild proteinuria may be evident. Fecal occult blood is often present, but hematochezia is unusual.

One can make a tentative diagnosis based on clinical signs and the finding of blister beetles in the hay. Determining the species of the insects may be necessary to estimate the amount of cantharidin ingested. All species of *Epicauta* contain cantharidin, but some have small amounts. Definitive diagnosis requires the measurement of the cantharidin concentration in gastric or intestinal contents and urine.[183,186] Measurement of cantharidin concentration in the beetles is often done but is not necessary.

13.13.3.4 ARSENIC TOXICOSIS

13.13.3.4.1 Pathogenesis

Arsenic toxicosis is an unusual cause of diarrhea in horses, resulting from ingestion of arsenic-containing herbicides, insecticides, and other pest control products contaminating water or roughage used as a food source.[188] The toxicity of arsenic depends on the valence of the element.[188,189] Arsenate may be reduced to arsenite in mammalian systems, and arsenite is thought to be more toxic than arsenate and less rapidly excreted in urine. Arsenate and arsenite uncouple oxidative phosphorylation, leading to breakdown of energy metabolism in the cells of many tissues.[189] Widespread cellular injury and death occur rapidly during acute arsenic toxicosis. Multiorgan failure usually results. In fact, cardiomyopathy and pulmonary disease are common causes of death in human beings.[190] Damage to the large intestine is probably caused in part by direct cellular toxicity and corrosion by the compound. However, vasculitis is a hallmark of the disease in human beings and horses and is thought to be the most important mechanism of large intestinal disease in human beings.[181,191] Acute hemorrhagic colitis is a feature of arsenic toxicosis, with severe mural edema and mucosal ulceration.[188] Profuse, hemorrhagic diarrhea and abdominal pain result. Chronic arsenic toxicity can occur but appears to be rare in horses.

13.13.3.4.2 Clinical Signs and Diagnosis

Acute depression, weakness, abdominal pain, hemorrhagic diarrhea, and shock are characteristic of acute arsenic toxicosis in horses. Death may occur before diarrhea is evident. Initial clinical signs may be difficult to distinguish from other peracute forms of colitis and are related to endotoxic shock, metabolic disturbances, and dehydration. Later, cardiac arrhythmias, pulmonary edema, acute renal failure, and
neurologic deficits (ataxia and stupor) may develop.[188] One may observe anuria or polyuria. Hemolytic 899
anemia caused by preferential binding of arsenic compounds to red blood cells is a feature of arsenic 900
poisoning in human beings. Hematologic abnormalities may be apparent after the peracute stages from injury to bone marrow cells and ongoing hemolysis. Leukopenia and thrombocytopenia have been described in human patients.[190] Serum biochemical analysis may reveal azotemia, hepatocellular enzyme activities higher than generally attributed to endotoxemia, and elevated creatine kinase activity.[188] Urine specific gravity may be in the isosthenuric range, with hematuria, cylindruria, and proteinuria evident by urinalysis.

Diagnosis may be possible by measuring blood and urine arsenic concentration, but these tests may not be diagnostic. Postmortem diagnosis is by measuring the arsenic concentration in liver and kidney samples.[188] History of exposure and clinical signs remain the primary means of diagnosis.

13.13.4 Miscellaneous Disorders of the Large Intestine

Other disorders associated with diarrhea in adult horses include anaphylaxis, carbohydrate overload, and sand enteropathy. Careful evaluation of history, environment, and management will assist the clinician in arriving at an accurate diagnosis.

13.13.4.1 INTESTINAL ANAPHYLAXIS

13.13.4.1.1 Pathogenesis

Severe intestinal anaphylaxis is a syndrome in horses characterized by peracute, rapidly fatal colitis.[192] The severe syndrome is clinically and pathologically similar to other known causes of peracute colitis, such as salmonellosis, clostridiosis, and antibiotic-associated diarrhea. Some cases are less severe and manifest as mild to moderate diarrhea or colic. An IgE-mediated type I hypersensitivity or an IgE-independent anaphylactoid reaction can produce the syndrome of intestinal anaphylaxis.[193,194] Local gastrointestinal exposure to a food, environmental contaminant, drug, or other allergen usually induces intestinal anaphylaxis,[193,195] but anaphylaxis also may occur with systemic exposure to an allergen.[196–199] Massive mast cell degranulation, secretion of inflammatory mediators, and activation of enteric neural reflexes in the intestine causes profound alterations in blood flow, increased vascular permeability and interstitial edema, recruitment of neutrophils, altered motility, mucosal injury, absorption of microbial products, and mucosal hypersecretion.[200–204] Systemic signs may be caused by the anaphylactic reaction or may be associated with systemic inflammation triggered by microbial products (endotoxin) absorbed through the injured and hyperpermeable mucosa.

Intestinal anaphylaxis in horses may be a peracute, fulminant enterocolitis with endotoxemia that may be fatal.[192,205] This form is characterized by severe intramural edema and hemorrhagic inflammation of the large intestine, often producing submucosal thickening on the order of many centimeters. Vascular thrombosis may be widespread with mucosal and serosal petechia and ecchymoses. Less severe forms of intestinal anaphylaxis may manifest as patchy areas of intestinal edema and congestion.[196] Diarrhea results from intestinal inflammation initiated by the type I hypersensitivity response. Many of the mediators of type I hypersensitivity, such as histamine and 5-hydroxytryptamine, have well-documented stimulatory effects on mucosal secretory activity, vascular and epithelial permeability, and motility[200–202] in the intestine. Systemic inflammation from endotoxemia may be overwhelming once the mucosal barrier breaks down. Infarction of intestinal segments and other organs may occur from intravascular coagulation. Ileus, abdominal distention, and moderate to severe abdominal pain may result from motility disturbances and infarction of the large intestine.

13.13.4.1.2 Clinical Signs and Diagnosis

The clinical signs are similar to those described for other forms of peracute colitis. However, the severity may vary, manifesting as colic or moderate diarrhea. Characteristically, severe shock, signs of systemic inflammation from endotoxemia, and severe metabolic disturbances are observable.[192,205] Heart and respiratory rates may be elevated greatly, with other signs of cardiovascular collapse such as weak and thready peripheral pulses and peripheral vasoconstriction. However, peripheral vasodilation may occur later in the course of disease. Dark red, muddy, or cyanotic mucous membranes with a prolonged capillary refill time signify sepsis. Borborygmi are usually absent, and abdominal tympany may be heard on percussion, following ileus. Moderate to severe colic may accompany ileus. Severe diarrhea may occur, but death may occur before diarrhea is evident. Multiorgan failure from disseminated intravascular coagulation is not unusual. Rapid onset of weakness, staggering, and trembling commonly precedes death. The syndrome may cause death in 4 to 24 hours.

Hematologic abnormalities include severe neutropenia and leukopenia, thrombocytopenia, and hemoconcentration.[192] Serum biochemical alterations include hyponatremia, hypokalemia, hypocalcemia, and severe metabolic acidosis. Blood urea nitrogen and creatinine may be elevated from prerenal or renal azotemia. If acute renal failure accompanies the colitis, hyperkalemia may result. Hepatocellular enzyme activity may be elevated in the serum from endotoxemia. Severe coagulopathies are common, resulting in 900
prolonged coagulation times, elevated fibrinogen, decreased antithrombin III activity, and elevated plasma 901
concentration of fibrin degradation products. Analysis of peritoneal fluid may be valuable because infarction of the large intestine is not unusual. Protein concentration and the white blood cell count may be elevated. Red blood cell counts are less likely to be elevated, because infarction and not strangulation of the intestine occurs.

Diagnosis is based on clinical signs, postmortem findings, and exclusion of other causes. Cultures and toxicologic analysis of fecal samples and gastrointestinal tissues fail to demonstrate a clear cause. Other diagnostic tests are also inconclusive. If an antigen is suspected as the trigger of the anaphylaxis, a Prausnitz-Küstner passive cutaneous anaphylaxis sensitization test can confirm the presence of antigen-specific IgE in the patient serum.[196]

13.13.4.2 CARBOHYDRATE OVERLOAD

13.13.4.2.1 Pathogenesis

Overeating of soluble carbohydrates, especially so-called hot grains such as corn, overwhelms the digestive capability of the small intestine, resulting in a high percentage of the soluble carbohydrates entering the large intestine. The amount of soluble carbohydrates that produce diarrhea varies according to the previous dietary history of the individual. Horses fed diets higher in soluble carbohydrates are more resistant to the deleterious effects of carbohydrate overload. Gradual accommodation to a diet high in carbohydrates can be accomplished over several weeks. However, horses fed an unusually large amount of grains or other form of soluble carbohydrates often develop diarrhea and may, depending on the amount ingested, develop severe colitis, systemic inflammation from endotoxemia, metabolic acidosis, and laminitis.[206–209]

The pathogenesis of colitis from carbohydrate overload is caused primarily by the toxic effects on the microbial flora in the large intestine.[207] A sudden delivery of soluble carbohydrates to the large intestine causes rapid fermentation by gram-positive lactic acid–producing bacteria and a sudden increase in organic acid production. The cecal pH rapidly decreases, and the lactic acid concentration rapidly increases. Rapid organic acid production overwhelms the buffering capacity of the large intestine not only by directly depleting the buffers found in the contents but also by reducing the efficiency of buffer secretion. Bicarbonate secretion is linked to absorption of volatile fatty acids, which are produced in low amounts by fermentation of soluble carbohydrates. The contents of the large intestine become profoundly acidic, resulting in unfavorable conditions for the microbial flora. Lactic acid–producing bacteria flourish, while the gram-negative bacteria, especially the Enterobacteriaceae, are killed in large numbers by the acids. Large quantities of endotoxin are released from the dying bacteria.[208]

The osmotic load from the lactic acid produced in the large intestine is an important factor in the development of diarrhea because organic acids such as lactic acid are absorbed poorly. Mild cases of carbohydrate overload may result purely from osmotic diarrhea. In more severe cases, the acidic contents of the large intestine are toxic to the mucosa, causing necrosis of the mucosal tissues, similar to that occurring in ruminal acidosis. Mucosal ulceration allows absorption of large quantities of endotoxin and lactic acid produced by the massive die-off of acid-intolerant microbes and fermentation of soluble carbohydrates, normally poorly absorbed by intact mucosa.[209] Systemic inflammation from endotoxemia may be overwhelming, and profound metabolic acidosis may occur. Secretory diarrhea caused by the direct effects of acid luminal contents on the mucosa, as well as the effects of inflammatory mediators on enterocyte secretion, worsens the acidosis and dehydration. Systemic inflammation from endotoxemia, along with intestinal inflammation, adversely affects intestinal motility, and ileus develops. Ileus and gas production from fermentation of the carbohydrates may cause severe distention of the large intestine and signs of abdominal pain. Laminitis is a frequent complication of endotoxemia and lactic acidosis. In fact, carbohydrate overload is used to induce laminitis as an experimental model because of the consistency of the laminitis produced.[207–209]

13.13.4.2.2

Clinical Signs and Diagnosis

Clinical signs of colitis from carbohydrate overload can vary according to the amount of carbohydrates ingested and accommodation of the flora to a high-carbohydrate diet. Mild cases may result in a transient osmotic diarrhea with no systemic effects. More severe cases are characterized by signs similar to those described for other forms of colitis, including abdominal pain, moderate to severe diarrhea, and dehydration. Signs of endotoxemia and sepsis are frequently present in severe cases. Elevated heart and respiratory rates are common, with peripheral vasoconstriction early in the disease, followed by peripheral vasodilation as the disease progresses. Depression may be profound from metabolic acidosis and endotoxemia. Abdominal auscultation and percussion may reveal ileus and intestinal tympany. Nasogastric intubation may yield significant gastric acidic reflux. One may note particles of grain in the gastric reflux and the feces, if grain overload is the source of the carbohydrate overload. Laminitis may complicate mild and severe cases of carbohydrate overload, especially if the animal has had previous bouts of laminitis.

Hematologic abnormalities include neutropenia and leukopenia. Severe dehydration may result in profound hemoconcentration. Protein loss later in the course of disease may result in hypoproteinemia. Serum 901
biochemical abnormalities include azotemia, elevated hepatocellular enzyme activity, hyponatremia, and 902
hypokalemia. Severe hypocalcemia and metabolic acidosis are characteristic of the disease. Serum lactate

concentrations are elevated in the absence of evidence of intestinal strangulation or infarction. Peritoneal fluid analysis often reveals no abnormalities.

13.13.4.3 SAND ENTEROPATHY

Sand enteropathy is described in more detail under the heading of obstructive diseases, because acute obstruction is often associated with abnormally large amounts of sand in the large intestine.[210] However, chronic sand-induced diarrhea is a distinct syndrome that can occur at any age from abnormal accumulation of sand in the large intestine.[211,212] Chronic diarrhea and signs of colic may occur without obstruction. The pathogenesis of sand accumulation in individual horses, other than simple ingestion of large quantities, is unclear. Presumably the sand causes irritation and may disrupt motility, leading to diarrhea. The diarrhea is usually not severe and dehydrating and may be intermittent. Weight loss is characteristic and can be severe in some cases. Complications may occur such as peritonitis and acute obstruction.[211]

Diagnosis usually is based on finding abnormal amounts of sand in the feces. Because sand-induced chronic diarrhea is associated primarily with sand accumulation in the ventral colon, auscultation of the ventral abdomen immediately behind the xiphoid process may reveal characteristic sand sounds.[213] This technique is only sensitive if peristalsis is present. Ultrasonography also may be useful to identify sand in the ventral colon but is not useful to quantitate the amount of sand. Occasionally, radiography may be required to detect sand in the colon.[211]

13.13.5 Principles of Therapy for Acute Diarrhea

The principles of therapy of acute diarrhea from colitis are similar regardless of the cause and include replacement of fluid and electrolyte losses, control of colonic inflammation and reduction of fluid secretion, promotion of mucosal repair, control of endotoxemia and sepsis, and reestablishment of normal flora. This section focuses on a review the principles of therapy with references to specific therapies for particular causes as they arise.

13.13.5.1 FLUID REPLACEMENT AND CIRCULATORY SUPPORT

Replacement of fluid and electrolyte losses is of primary concern in treating horses with salmonellosis. Depending on the severity of the disease, fluid losses may be minimal or massive. One can administer fluid and electrolytes orally or intravenously. Some horses with mild to moderate diarrhea may maintain hydration and electrolyte balance by consuming water and electrolytes voluntarily. Freshwater and water containing electrolytes should be available in all cases. In many instances, periodic nasogastric intubation and administration of water and electrolytes via the tube may be sufficient to maintain hydration.[214] In more severe cases, one can maintain indwelling nasogastric tubes and can administer up to 4 to 8 L of fluid by the tube every 20 to 30 minutes, if ileus is not evident. However, intravenous administration of fluids is preferred in most cases, requiring significant quantities of fluid to replace and maintain hydration and electrolyte balance.[215] For patients with severe diarrhea to require large volumes (50 to 100 L/day) of intravenous fluids to maintain hydration is not unusual. Frequent monitoring of packed cell volume, serum electrolyte concentration, venous blood gases or total serum carbon dioxide, blood urea nitrogen and creatinine, urine protein and cytologic findings, and body weight is important to monitor hydration, electrolyte and acid-base balance, and renal function.

Isotonic sodium chloride or lactated Ringer's solution frequently is used to restore and maintain fluid and electrolyte balance. One can add potassium chloride to the fluids and administer it at a rate up to 0.5 to 1.0 mEq/kg/hr. Generally, a rate of less than 0.5 mEq/kg/hr is used. Hypertonic NaCl solutions (1 to 2 L of 3% to 5% NaCl) have been used in horses that are severely hyponatremic ($<$120 mEq/dl). One should not administer hypertonic solutions to severely dehydrated horses, but such solutions have been used clinically without complication and with considerable beneficial effect in patients with endotoxemia. The beneficial effects of hypertonic NaCl are short-lived (30 to 60 minutes). One should administer isotonic solutions concurrently or immediately following administration of hypertonic NaCl solutions. Isotonic (1.3%) or hypertonic (5.0%) sodium bicarbonate solutions are used to correct metabolic acidosis. Prolonged administration of sodium-containing fluids may promote diuresis and renal water loss or accumulation of peripheral edema and should be used conservatively when one notes a free water loss. Administration of isotonic dextrose (5%) or 2.5% dextrose/0.45% NaCl solutions may be beneficial when free water loss (sodium excess) is evident.

Many horses with acute colitis are concurrently hypoproteinemic because of gastrointestinal losses and are absorbing bacterial products that induce a systemic inflammatory response. Thus plasma oncotic pressures are abnormally low in the face of increased vascular permeability. Interstitial edema formation is a clinical problem in these patients and contributes to organ dysfunction. Crystalloid fluids, although critical for 902
replacing water and electrolyte losses from diarrhea, actually may contribute to a drop in plasma oncotic 903
pressure because of hemodilution.[216,217] Administration of colloid solutions are important for volume expansion and to maintain plasma oncotic pressures, which improve tissue perfusion and oxygenation and organ function in hypovolemic, hypotensive, and hypoproteinemic patients with or without systemic inflammatory response syndrome.[218] Colloids are more effective than crystalloid fluids at expanding plasma volume and thus require smaller volumes. Moreover, the effect of colloid volume expansion is longer lasting than crystalloid fluid volume expansion, because colloids are retained in the vasculature better.[217,218] Natural colloids, such as plasma and purified albumin are used commonly. In addition to its beneficial colloidal properties, plasma harvested from donor horses immunized with rough mutants of *Escherichia coli* (J5) or *Salmonella typhimurium* may have other benefits for treatment of endotoxemia from gastrointestinal disease.[219,220] The horse may require large volumes (6 to 8 L/day) to increase and maintain plasma protein concentration significantly. Synthetic colloids such as dextrans, starches, or polymerized hemoglobin are also available for use in the horse. Hetastarch (5 to 10 ml/kg of a 6% solution) increases colloidal oncotic pressures for up to 24 hours in hypoproteinemic horses and has beneficial effects on cardiac output and other cardiorespiratory parameters, vascular permeability, interstitial fluid content, and tissue perfusion in models of hypoproteinemia and systemic inflammatory response syndrome. When one administers synthetic or even natural colloids, monitoring plasma oncotic pressure may be more relevant than monitoring plasma protein concentrations as a means of assessing the need for plasma or other colloid administration.[216] Hetastarch may prolong bleeding times by altering von Willebrand's factor function; thus one should use this synthetic colloid cautiously in horses with suspected coagulopathies, active hemorrhage, or other bleeding problems.[217]

13.13.5.2 INFLAMMATION

Control of colonic inflammation and secretion is a difficult and poorly studied aspect of equine acute colitis. The role of inflammation and mediators such as prostaglandins as causes of fluid loss is well known for *Salmonella* and *Clostridium* infections. COX inhibitors (NSAIDs) have antisecretory effects in the equine colon and in models of salmonellosis that appear to extend to clinical management of salmonellosis.[16,36,221–223] Indeed, NSAIDs commonly are administered to horses with salmonellosis. However, prostaglandins such

as PGE_2 and PGI_2 are also cytoprotective to gastrointestinal mucosa and critical for mucosal repair.[172] The doses of NSAIDs used pharmacologically to inhibit colonic inflammation and secretion in fact may be detrimental to the mucosa if not used judiciously. NSAIDs have been shown to exacerbate colonic inflammation in human beings with inflammatory colitis, impede mucosal healing in several models of mucosal injury, and have well-documented detrimental effects on colonic mucosa in horses.[164,172,224] In addition to toxicity to the colonic mucosa, gastric ulceration is not unusual in horses with enterocolitis and may be related to treatment with NSAIDs.

In addition to NSAIDs, other drugs occasionally are used as antiinflammatory or antisecretory therapy. Metronidazole has beneficial effects in experimental models of gastrointestinal inflammation, including NSAID toxicity[176] and may be useful for treating horses with colitis, but evidence supporting its use is lacking. Bismuth subsalicylate solutions administered orally often are used to decrease inflammation and secretion in the colon. In adult horses the volume of solution necessary to be beneficial is large (3 to 4 L every 4 to 6 hours). Often the solution is administered twice daily instead of 4 to 6 times daily. If one does not achieve a beneficial effect within 3 to 4 days of treatment, one should discontinue administration of bismuth subsalicylate solution. One can administer the treatment more frequently in foals, and clinical improvement occurs more often in foals than in adult horses.

In light of the role of reactive oxygen metabolites in colonic inflammation, free radical scavengers have been advocated to reduce the effects of these molecules. Sulfasalazine metabolites have been shown to reduce reactive oxygen metabolite–induced colonic inflammation in other species,[176] and sulfasalazine has been used to treat chronic inflammatory disease in horses but has not been used to treat acute colitis. The only free radical scavenger used commonly in horses with colitis is dimethyl sulfoxide, which at a dosage of 0.1 to 1.0 g/kg intravenously every 12 to 24 hours in a 10% solution has been used in clinical cases of colitis, but evidence of efficacy has not been established.

Systemic inflammatory response syndrome associated with endotoxemia frequently occurs in patients with salmonellosis. The principles of therapy for endotoxemia are covered in detail elsewhere in this chapter. Oral administration of activated charcoal and mineral oil is used commonly to reduce absorption of endotoxin in horses with colitis. Low doses of NSAIDs (such as flunixi n meglumine at 0.1 to 0.25 mg/kg intravenously every 6 to 8 hours) inhibit eicosanoid synthesis induced by endotoxin. In addition, administration of NSAIDs prevents laminitis from endotoxemia, a devastating complication of salmonellosis. One must remember that prostaglandins are important for mucosal healing and may worsen mucosal injury in colitis. Although the 903
benefits of low doses of NSAIDs administered to horses with systemic inflammatory response syndrome are 904
believed to outweigh the risks of worsening gastrointestinal damage, judicious use is recommended.

13.13.5.3 MUCOSAL REPAIR AND PROTECTION

Sucralfate (20 mg/kg orally every 6 hours) has been advocated to aid in healing the colonic mucosa, but the efficacy in the large intestine is questionable.[94] Misoprostol (2 μg/kg orally 3 to 4 times daily) and other synthetic PGE analogs have been shown in several species including horses to enhance mucosal healing in the intestine and promote recovery in experimental models of colitis.[225] Misoprostol may be particularly useful for treating NSAID toxicity, the generalized form or RDC. However, the efficacy of misoprostil for hastening mucosal healing is clinically unproven in equine colitis. The primary drawbacks of prostaglandin analogs such as misoprostol are the side effects of the drug, including abdominal cramping, diarrhea, sweating, and abortion in pregnant mares.

One can add psyllium mucilloid to the diet (5 tablespoons once or twice daily) to increase the production of SCFAs in the colon. Amylase-resistant fermentable fiber such as psyllium is hydrolyzed by colonic bacteria to SCFAs such as butyrate, which represent a major energy source for colonocytes. Butyrate and other SCFAs hasten epithelial maturation and stimulate salt (and thus fluid) absorption in the colon, improve the clinical course of ulcerative colitis, and hasten colon healing.[226] Psyllium is itself a source of butyrate in the colon and also promotes the movement of amylase sensitive carbohydrates into the distal colon, which then are fermented to SCFAs. Thus psyllium is thought to be clinically useful for promoting mucosal healing in colitis.

13.13.5.4 PAIN CONTROL

Many horses with salmonellosis or other forms of colitis have mild to severe signs of abdominal pain from gas and fluid distention of the colon, colonic ischemia, or infarction. One can accomplish analgesia with NSAIDs such as flunixin, but the potential for worsening mucosal injury or nephrotoxicity may prevent the use of analgesic doses, especially in horses with suspected NSAID toxicity. Newer NSAIDs that specifically target COX-2 (the inducible COX) but have little activity against COX-1 (the constitutive COX) may be useful analgesics that spare the gastrointestinal mucosa. For example, etodolac (10 to 15 mg/kg intravenously or orally once daily) has analgesic properties in horses and may spare the intestinal mucosa from the detrimental effects associated with nonselective COX inhibitors (A.T. Blikslager, personal communication, 2002). However, the specificity for COX-2 in horses is unproven. Thus avoiding the use of any NSAIDs in horses with RDC or other forms of NSAID toxicity is advisable.

Xylazine or detomidine may provide temporary relief of pain. Butorphanol is a useful analgesic that one can administer intramuscularly (0.1 mg/kg every 6 hours) or as a continuous infusion. An infusion of 13.2 μg/kg/hr in isotonic crystalloid fluid such as lactated Ringer's solution has been suggested.[227] Continuous lidocaine infusions (1.3 mg/kg intravenous loading dose administered slowly over 5 minutes and followed by 3 mg/kg/hr infusion in isotonic crystalloid fluids) can provide profound visceral analgesia and may have added prokinetic benefits if ileus is present.

13.13.5.5 ANTIBIOTICS

13.13.5.5.1 Neutropenia

Broad-spectrum antibiotic treatment often is recommended in neutropenic horses or horses with signs of septicemia. Neutropenia is associated with an increased risk of septicemia and septic complications such as septic phlebitis and infection of surgical site.[1] Septicemia is a potentially life-threatening complication of enterocolitis and may be caused directly by *Salmonella, Clostridium,* other invasive enteric bacteria, or indirectly by toxic injury to the colonic mucosa that breaks down the barrier to luminal microbes. Neutropenia possibly may weaken host defenses enough to render horses susceptible to organisms that breach the mucosal barrier. Although most attempts to culture bacteria from the blood of adult horses with colitis fail to isolate organisms, no detailed studies have been undertaken to determine the prevalence of bacteremia or septicemia in these patients. Disseminated aspergillosis has been reported in horses as a complication of acute colitis, demonstrating the potential for systemic infections with rarely pathogenic organisms stemming from colonic mucosal injury in the face of potential immunosuppression from neutropenia.[228,229] Broad-spectrum antibiotics lessen septic complications in human patients. However, evidence supporting this principle in horses with colitis is lacking.

13.13.5.5.2
Salmonellosis

Treatment with antibiotics is controversial in horses with salmonellosis and is not thought to alter the course of the enterocolitis. Antibiotics directly targeted at the *Salmonella* are reserved for patients with the enteric fever (septicemia) form of salmonellosis, documented with positive blood cultures. Lipid-soluble antibiotics are suited ideally for *Salmonella* infections, because the bacteria persist intracellularly. Trimethoprim-sulfadiazine or other potentiated sulfa drugs, enrofloxacin, and chloramphenicol are preferred antibiotics for the enteric fever form of salmonellosis for this reason.

13.13.5.5.3
Equine Monocytic Ehrlichiosis

As with other causes of enterocolitis, the use of antibiotics for equine monocytic ehrlichiosis is controversial. Fear of inducing salmonellosis or other forms of antibiotic-induced diarrhea and the difficulty of diagnosing the disease early have caused most authors to recommend judicious use of antibiotics.[49] However, in patients with a high suspicion of *Neorickettsia risticii* infection, treatment with antibiotics often is indicated before definitive diagnosis. Lipid-soluble drugs are desirable because the organism can live within cells. Oxytetracycline (6.6 mg/kg intravenously every 24 hours), doxycycline (10 mg/kg orally every 12 hours), trimethoprim-sulfadiazine (5 mg/kg trimethoprim orally or intravenously every 8 to 12 hours and 25 mg/kg sulfadiazine every 8 to 12 hours), or erythromycin-rifampin (30 mg/kg and 5 mg/kg, respectively, orally every 12 hours) have been used effectively to treat clinical cases.[49,230–232] The tetracyclines appear to be the most effective antibiotics for treatment of Potomac horse fever. Treatment is most successful if initiated before the onset of diarrhea.[49,231]

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13.13.5.5.4
Clostridiosis

If one has administered antibiotics since the onset of enterocolitis, one should discontinue administration as soon as possible. Specific treatment with metronidazole (15 to 25 mg/kg orally every 8 hours) is effective for treating clostridiosis in human beings and appears to be effective in horses.[83,233] Metronidazole resistance in clinical isolates of *Clostridium difficile* has been reported in one outbreak but appears to be rare in most human and equine cases.[234] Metronidazole-resistant isolates we re sensitive to vancomycin, which may be effective for treating clinical cases if one suspects metronidazole resistance. However, metronidazole remains the treatment of choice. Some authors describe the off-label use of *C. perfringens* type C antitoxin in cases of neonatal clostridiosis, described in more detail elsewhere.[235] Antitoxin preparations generally are not advocated for use in adult horses with clostridiosis.

13.13.5.5.5
Proliferative Enteropathy

Lawsonia intracellulare is susceptible to a variety of antibiotics in vitro, including chlortetracycline, erythromycin, penicillin, difloxacin, and ampicillin.[236] Lipid-soluble antibiotics with a large volume of distribution usually are chosen to treat proliferative enteropathy because *L. intracellulare* is an intracellular organism. Erythromycin estolate (15 to 25 mg/kg orally every 6 to 8 hours) alone or with rifampin (5 mg/kg orally every 12 hours) is the most commonly reported efficacious treatment for proliferative enteropathy. Chloramphenicol (50 mg/kg orally every 6 hours) has also been reported to be effective if erythromycin worsens the diarrhea.[115] Anecdotal reports suggest that oxytetracycline and doxycycline also may be

effective. Supportive care including maintenance of hydration and electrolyte balance and plasma or colloid administration to increase colloid oncotic pressure in hypoalbuminemic patients is also indicated. One should treat affected foals until clinical signs, hypoproteinemia, and ultrasonographic evidence of intestinal thickening resolve. The prognosis depends on the duration of the disease and the degree of fibrosis and destruction of the intestinal architecture.

13.13.5.6 ANTICOAGULATION

Hypercoagulability is a common complication of enterocolitis, associated with systemic inflammation from endotoxemia. Administration of heparin (20 to 80 IU/kg subcutaneously or intravenously every 6 to 12 hours) may prevent thrombosis in these patients, provided antithrombin III concentrations are adequate in the plasma. Concentrated sources of antithrombin III are not available for use in horses, but whole plasma may provide an important source. Treatment with heparin is thought to decrease thrombosis, especially of the jugular vein, a serious complication of salmonellosis. Low-dose aspirin treatment (15 mg/kg orally every 24 to 48 hours) along with heparin treatment may provide added benefit by irreversibly inhibiting platelet function.[237] Heparin and aspirin may have protective effects on the digital lamina.[237,238] Heparin also may enhance the phagocytic activity of the reticuloendothelial system by enhancing the efficiency of opsonins such as fibronectin and immunoglobulin, thereby stimulating phagocytosis of products of coagulation and possibly other particles, including bacteria.[239,240]

13.13.5.7 PROBIOTICS

13.13.5.7.1 Salmonellosis

Maintenance of the bacterial flora and antagonism of pathogenic bacteria such as *Salmonella* in the gastrointestinal tract are important defense mechanisms preventing colonization by pathogenic bacteria. The use of probiotic preparations containing beneficial bacteria has been shown to prevent colonization of pathogenic bacteria, including *Salmonella,* in poultry.[241] Little work has been done to investigate the efficacy of these products in preventing salmonellosis in horses, but ongoing studies may provide important information. Probiotic and other preparations designed to restore normal flora to the gastrointestinal tract, such as fecal suspensions, sour milk, and yogurt, have been used clinically to shorten the course of salmonellosis, with variable results. Therefore prevention of infection by using probiotic agents and other means is important. Exposure of susceptible horses to *Salmonella* should be avoided, but the task is difficult, especially because asymptomatic infections are common and the bacteria are ubiquitous in the environment. Prophylactic use of probiotic preparations, judicious use of antibiotics in susceptible horses, 905
control of environmental conditions such as temperature, and restricted exposure to pathogenic bacteria are 906
important for control of salmonellosis.

13.13.5.7.2 Clostridiosis

Because altered large intestinal flora appears to play an important role in the pathogenesis of equine intestinal clostridiosis or any antibiotic-associated diarrhea, probiotic preparations have been advocated to treat affected horses. Sour milk, a product containing lactose-producing *Streptococcus* species, appears to improve the clinical course greatly in horses suspected of having *Clostridium perfringens* type A infection. Sour milk may benefit the patient by altering the flora and antagonizing enterotoxigenic *C. perfringens* type

A but also is reported to be bactericidal against *C. perfringens* type A.[78] Preparations of *Saccharomyces boulardi* are effective for reducing diarrhea and the frequency of *C. difficile* recurrence in human beings.[83] However, whether relapse is a problem in horses with *C. difficile* colitis is not clear. *Lactobacillus* preparations have a protective effect in human beings and decrease the severity and duration of antibiotic-associated diarrhea.[242,243] However, evidence of their clinical usefulness in horses is lacking.

13.13.5.8 NUTRITION

Good nursing care and adequate nutrition are vital to the treatment of horses with salmonellosis. Salmonellosis is a severely catabolic disease, increasing caloric requirements greatly. Normal intake of roughage to provide energy may be inadequate; however, one should avoid feeding of grains to prevent carbohydrate overload. Dietary management usually consists of restricting or eliminating long-stem roughage (hay) from the diet and feeding exclusively a complete pelleted diet (at least 30% dietary fiber). The rationale behind this recommendation is to reduce the mechanical load on the colon. Frequent meals (4 to 6 times a day) are recommended. One can add corn oil (1 cup every 12 to 24 hours) to the pellets to increase the caloric intake without adding roughage or grain. One should note that if a horse with colitis refuses to eat pelleted feed, then one should feed good-quality grass hay. In anorectic or severely catabolic patients, enteral and parenteral nutrition (total and partial) has been used successfully to provide calories and nutritional support.

13.13.5.9 SPECIFIC THERAPIES

13.13.5.9.1 Strongylosis

Strongylus vulgaris infection requires treatment of the migrating parasite larvae and the lesions produced by the parasite. Fenbendazole (10 mg/kg orally every 24 hours for 3 days or 10 mg/kg orally every 24 hours for 5 days) and ivermectin (200 mg/kg orally) are effective in killing fourth-stage larvae.[121] Other anthelmintics also may be effective when given at higher doses than those required to kill adult worms. The efficacy of these anthelmintics against larvae within thrombi is not known.

Thrombolytic and antithrombotic therapy has been advocated in horses with suspected strongylosis.[121,128] Heparin (20 to 80 IU intravenously or subcutaneously every 6 to 12 hours) is often administered as an anticoagulant. Aspirin (10 to 30 mg/kg orally every 12 to 48 hours) is usually combined with heparin to inhibit platelet adhesion. Aspirin also may inhibit release of platelet products such as thromboxane that affect the motility of the large intestine. Low-molecular-weight dextrans have been advocated as antithrombotics that act by inhibiting platelet function and coagulation.[128,218] The clinical efficacy of dextran administration appears to be good, but no controlled studies have been performed.

13.13.5.9.2 Cyathostomiasis

Anthelmintic administration is usually the only treatment necessary for mild to moderate cases of cyathostomiasis treated early in the course of the disease (within 1 to 3 weeks of onset). Fenbendazole is effective against many larval stages, but resistance is increasing. Although the reported efficacy of ivermectin varies against certain stages,[244] one study reported an overall efficacy of 75%.[245] Currently, fenbendazole (7.5 to 10 mg/kg orally every 24 hours for 5 days) followed on day 6 by ivermectin (200 mg/kg orally) is the most commonly advocated treatment regimen.[133,246] Moxidectin (400 μg/kg orally once

daily) also may be effective against adults and L_3 and L_4 larval stages[247] and may be useful for treating cyathostomiasis. Antiinflammatory therapy also may be beneficial, especially in severe or refractory cases. NSAID administration may have limited value, but dexamethasone appears to be efficacious in refractory cases when used with larvicidal anthelmintics.[133,136] Pretreatment with dexamethasone or prednisolone is indicated before anthelmintic administration if heavy larval loads are suspected to prevent an acute exacerbation of the disease by rapid death of encysted larvae. Bismuth subsalicylate often is administered orally as an antisecretory agent in young animals. Supportive care may be necessary in severe cases, particularly if hypoproteinemia is severe. Horses occasionally require administration of intravenous crystalloid fluids and plasma or other colloids. Proper nutritional support is also important.

13.13.5.9.3 Cantharidin Toxicity

Supportive care is the most important principle of therapy for cantharidin toxicity. Intravenous fluid administration; maintenance of electrolyte balance, especially calcium; and prevention of further renal and urinary tract damage is important.[183,187] Diuresis by intravenous fluid administration is often sufficient to 906
prevent renal failure. Furosemide often is administered after rehydration of the patient to further promote 907
diuresis and to decrease the concentration of the toxin in the urine, which may ameliorate some of the effects on the urinary tract mucosa. Diuresis also has been suggested to increase clearance of the toxin, but no evidence for this has been found. Judicious use of NSAIDs may be necessary to control abdominal pain but should be reserved until the patient is rehydrated and renal failure has been ruled out. Cantharidin is lipid-soluble; therefore oral administration of mineral oil may prevent further absorption of the toxin.[183] Activated charcoal often is administered with the mineral oil.

13.13.5.9.4 Arsenic Toxicity

To reduce arsenic absorption, one should initiate administration of cathartics such as mineral oil and magnesium sulfate slurries and activated charcoal by nasogastric tube immediately. Chelation therapy with sodium thiosulfate 20 to 30 g in 300 ml of water orally and dimercaprol (BAL) 3 mg/kg intramuscularly every 4 hours is indicated.[188] Dimercaprol is a specific antidote for trivalent arsenicals, but its efficacy in horses is questionable. Intravenous fluid administration may help treat shock, replace fluid lost in feces, and promote diuresis but should be monitored carefully because pulmonary edema is a frequent complication. The horse may require more specific treatment of renal, cardiac, pulmonary, or neurologic disease.

13.13.5.9.5 Intestinal Anaphylaxis

Treatment of intestinal anaphylaxis is in principle similar to treatment of other forms of colitis but is often unsuccessful because of the rapidly progressive nature of the syndrome. Inclusion of heparin in intravenous fluids (20 to 80 IU/kg intravenously every 8 to 12 hours) may help prevent vascular thrombosis. Administration of hypertonic saline solutions or colloids may prove to be useful during initial periods of shock. Early treatment with prednisolone succinate (10 to 20 mg/kg intravenously) or dexamethasone (0.1 to 0.2 mg/kg intravenously) may be essential for successful treatment.[192]

13.13.5.9.6 Carbohydrate Overload

Mild cases of carbohydrate overload may not require treatment other than exclusion of grains from the diet for several days to weeks and gradual reintroduction of grain into the diet later if the horse needs the extra energy. Patients showing signs of colic or diarrhea without other systemic signs may benefit from administration of mineral oil, charcoal, and fluids via nasogastric tube. One also may lavage residual carbohydrates from the stomach with the nasogastric tube. NSAIDs such as phenylbutazone (2.2 to 4.4 mg/kg/day intravenously) or flunixin meglumine (1 mg/kg intravenously every 12 hours) often are administered to prevent laminitis. Phenoxybenzamine and heparin given before the onset of laminitis may prevent or decrease the severity of laminitis.[238,248]

More severe cases with dehydrating diarrhea, systemic signs of endotoxemia, or metabolic acidosis require intravenous fluid support to maintain water, electrolyte, and acid-base balance in addition to the previously mentioned treatments. Large amounts of bicarbonate-containing solutions may be required. One should take care when administering hypertonic bicarbonate solutions, because many patients already may be hyperosmotic from lactic acidemia. Isotonic sodium bicarbonate 1.3% may be useful in the hyperosmotic patient. Careful attention to calcium balance is also important, because severe hypocalcemia may occur. One should institute aggressive therapy for systemic inflammation from endotoxemia. One should administer broad-spectrum antibiotics intravenously to combat bacteremia and septicemia, which frequently complicate colitis induced by carbohydrate overload.

In extreme cases, especially if the patient has ingested a large quantity of grain, surgical removal of the grain from the large intestine may be indicated, especially if one can accomplish surgery before the onset of severe clinical signs. However, administration of oral cathartics, such as magnesium sulfate slurries or mineral oil, or a combination of these, is often sufficient to clear the carbohydrates from the large intestine before fermentation, mucosal damage, and absorption of endotoxin and lactic acid occur. Oral administration of activated charcoal may prevent absorption of endotoxin by binding the molecules in the lumen of the bowel. In any case, one should discontinue feeding of the source of the soluble carbohydrates, such as grains. One should feed the horse low-carbohydrate and low-protein roughage such as grass or oat hays until the microbial flora recovers. Oral administration of probiotic preparations containing *Lactobacillus* is contraindicated; however, other sources of normal equine large intestinal microbial flora, such as fecal extracts from normal feces, may be useful to reintroduce appropriate microorganisms. Complications from laminitis and sepsis are common and often cause death.

13.13.5.9.7 Sand Enteropathy

Treatment of sand enteropathy requires removal of the sand from the gastrointestinal tract using psyllium products and magnesium sulfate slurries administered orally. Analgesics may be required initially to relieve pain and stimulate appetite. A diet high in roughage often stimulates further passage of sand. Treatment 907
may require several weeks to remove as much sand as possible. Prevention of the disease is important, and 908
recurrence is not unusual.

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13.14

13.14—Ischemic Disorders of the Intestinal Tract

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13.14.1

Strangulating Obstruction

Strangulating obstruction of the intestine is characterized by simultaneous occlusion of the intestinal lumen and its blood supply. Although strangulation of the intestinal lumen results in clinical signs similar to those of simple obstruction, occlusion of the blood supply results in a more rapid deterioration of the intestinal mucosa and subsequent onset of endotoxemic shock. Although a great deal of interest in the relevance and treatment of intestinal reperfusion injury has arisen recently,[1–3] the lesion that develops during strangulation is often severe, leaving little viable bowel for further injury during reperfusion.[2] Although extensive lengths of strangulated small intestine may be resected, strangulation of the large colon presents a much greater treatment dilemma because strangulated intestine usually extends beyond the limits of surgical resection.[4] Therefore horses with large intestinal strangulation often recover with extensive intestinal injury left in place. Thus subtle degrees of reperfusion injury may be important in horses with large colon disease, warranting further work in this area in an attempt to reduce mortality.[3]

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Strangulating obstruction may be divided into hemorrhagic and ischemic forms.[5,6] Hemorrhagic strangulating obstruction, which is most common, involves initial occlusion of veins before occlusion of arteries because of the greater stiffness of arterial walls. This lesion is characterized by a darkened appearance to affected bowel and increased thickness as blood is pumped into the lesion. Ischemic strangulating obstruction occurs if the intestine

is twisted tightly enough to occlude arteries and veins simultaneously. In the case of the colon, such strangulation has been suggested to be determined by how much ingesta is in the colon, because intestinal contents may prevent the intestine from twisting tightly.[7] Tissue involved in ischemic strangulating obstruction appears pale and of normal or reduced thickness because of a complete lack of blood flow (Figure 13.14-1). Bowel peripheral to strangulating lesions also may become injured because of distention, which reduces mural blood flow once it reaches critical levels. Furthermore, as this intestine is decompressed, it also may undergo reperfusion injury.[8–10]

Figure 13.14-1 Ischemic strangulating obstruction of the small colon by a mesenteric lipoma. **A,** The lipoma *(arrow)* has encircled a segment of small colon tightly. **B,** Following resection of the lipoma, a pale area of strangulated small colon clearly is demarcated *(arrows),* the appearance of which is consistent with ischemic strangulating obstruction.

13.14.2 Small Intestinal Strangulation

13.14.2.1 CLINICAL SIGNS

Horses with small intestinal strangulating obstruction typically have moderate to severe signs of abdominal pain that are only intermittently responsive to analgesic medications. During the latter stages of the disease process, horses may become profoundly depressed rather than painful as affected intestine necroses. Horses have progressive signs of endotoxemia, including congested mucous membranes, delayed capillary refill time, and an elevated heart rate (>60 beats/min in most cases). In addition, one typically obtains reflux following passage of a stomach tube, and one usually can detect loops of distended small intestine on rectal palpation of the abdomen.[11] However, these latter findings vary depending on the duration and location of the obstruction. For example, horses with ileal obstructions tend to reflux later in the course of the disease process than horses with a jejunal obstruction. Furthermore, a horse that has an entrapment of small intestine in the epiploic foramen may not have palpable loops of small intestine because of the cranial location of these structures.[12] Abdominocentesis can provide critical information on the integrity of the intestine and is indicated in horses in which one suspects strangulation of the small intestine.[13] A horse that has signs compatible with a small intestinal obstruction and additionally has serosanguinous abdominal fluid with an elevated protein level (>2.5 914

mg/dl) is likely to require surgery, although one must differentiate these cases from proximal enteritis. In general, horses with small intestinal strangulation show continued signs of abdominal pain, whereas horses with proximal enteritis tend to be depressed after initial episodes of mild abdominal pain. In addition, horses with small intestinal strangulation continue to deteriorate clinically despite appropriate medical therapy and will likely begin to show an increased white blood cell count (>10,000 cells/μl) in the abdominal fluid as the duration of strangulation increases. However, cases occur in which the differentiation between small intestinal strangulation and proximal enteritis is not clear, at which point one may elect surgery rather than risking delay of abdominal exploration of a horse with a potential strangulating lesion.[14]

13.14.2.2 PROGNOSIS

The prognosis for survival in horses with small intestinal strangulating lesions is generally lower than for most forms of colic.[15] However, recent studies indicate that in excess of 80% of horses with small intestinal strangulating lesions are discharged from the hospital.[16] Nonetheless, veterinarians should warn owners that the long-term survival rates are reduced substantially to below 70%,[17] in part because of long-term complications such as adhesions.[18,19] In addition, the prognosis is particularly low for some forms of strangulation, including entrapment of small intestine within a mesenteric rent.[20]

13.14.2.3 EPIPLOIC FORAMEN ENTRAPMENT

The epiploic foramen is a potential opening (because the walls of the foramen are usually in contact) to the omental bursa located within the right cranial quadrant of the abdomen. The foramen thus is bounded dorsally by the caudate process of the liver and caudal vena cava and ventrally by the pancreas, hepatoduodenal ligament, and portal vein. Intestine may enter the foramen from the visceral surface of the liver toward the right body wall or the opposite direction. Studies differ as to which is the most common form.[12,21] In the case of entrapments that enter the foramen in a left-to-right direction, the omental bursa ruptures as the intestine migrates through the epiploic foramen, which may contribute to intraabdominal hemorrhage often seen with this condition. Clinical signs include acute onset of severe colic with examination findings compatible with small intestinal obstruction. The condition tends to be more prevalent in older horses,[12] possibly because of enlargement of the epiploic foramen as the right lobe of the liver undergoes age-associated atrophy.[22] However, the disease also has been recognized in foals as young as 4 months of age.[23] One makes a definitive diagnosis at surgery, although ultrasonographic findings of distended loops of edematous small intestine adjacent to the right middle body wall suggest epiploic foramen entrapment.[12] In general, thickened, amotile intestine on ultrasonographic examination is highly predictive for small intestinal strangulating obstruction.[24] Small intestine entrapped in the epiploic foramen may be limited to a portion of the intestinal wall (parietal hernia),[25] and the large colon may become entrapped within the epiploic foramen.[26] In treating epiploic foramen entrapment, one must not enlarge the epiploic foramen by blunt force or with a sharp instrument, because rupture of the vena cava or portal vein and fatal hemorrhage may occur. Prognosis has improved substantially over the last decade, with current short-term survival rates (discharge from the hospital) ranging from 74%[27] to 79%.[12] Preoperative abdominocentesis has been found consistently to be the most predictive test of postoperative survival.[12,27]

13.14.2.4 STRANGULATION BY PEDUNCULATED MESENTERIC LIPOMA

Lipomata form between the leaves of the mesentery as horses age and develop mesenteric stalks as the weight of the lipoma tugs on the mesentery. The stalk of the lipoma subsequently may wrap around a loop of small intestine or small colon causing strangulation. One should suspect strangulating lipomata in aged (>15 years old) geldings with acute colic referable to the small intestinal tract.[28,29] Ponies also appear to be at risk of developing disease,[29] suggesting alterations in fat metabolism may predispose certain horses to development of mesenteric lipomata. One usually makes the diagnosis at surgery, although on rare occasions one can palpate a lipoma per rectum.[30] Treatment involves surgical resection of the lipoma and strangulated bowel, although strangulated intestine is not always nonviable.[28] Studies indicate that approximately 50%[29] to 80%[28] of horses are discharged from the hospital following surgical treatment.

13.14.2.5 SMALL INTESTINAL VOLVULUS

A volvulus is a twist along the axis of the mesentery, whereas torsion is a twist along the longitudinal axis of the intestine. Small intestinal volvulus theoretically is initiated by a change in local peristalsis or the occurrence of a lesion around which the intestine and its mesentery may twist (such as an ascarid impaction).[11] Volvulus is reportedly one of the most commonly diagnosed causes of small intestinal obstruction in foals.[31,32] The theory is that young foals may be at risk of small intestinal volvulus because of changing feed habits and adaptation to a bulkier adult diet. Onset of acute, severe colic, a distended abdomen, and radiographic evidence of multiple loops of distended small intestine in a young foal suggest small intestinal volvulus. However, one cannot differentiate volvulus from other causes of small intestinal obstruction preoperatively. In 915
adult horses, volvulus frequently occurs in association with another disease process, during which small 916
intestinal obstruction results in distention and subsequent rotation of the small intestine around the root of the mesentery. Although any segment of the small intestine may be involved, the distal jejunum and ileum are affected most frequently because of their longer mesenteries.[11] One makes the diagnosis at surgery by palpating a twist at the origin of the cranial mesenteric artery. Treatment includes resection of devitalized bowel, which may not be an option because of the extent of small intestinal involvement (similar to large colon volvulus). Prognosis is based on the extent of small intestine involved and its appearance following surgical correction of the lesion. In general, horses with greater than 50% of the small intestine devitalized are considered to have a grave prognosis.[33]

13.14.2.6 STRANGULATION VIA MESENTERIC OR LIGAMENTOUS RENTS

A number of structures, when torn, may incarcerate a segment of intestine (typically the small intestine), including intestinal mesentery,[20] the gastrosplenic ligament,[34] the broad ligament,[35] and the cecocolic ligament.[36] Horses with such incarcerations have signs typical of a horse with strangulating small intestine, including moderate to severe signs of abdominal pain, endotoxemia, absent gastrointestinal sounds, distended small intestine on per rectal palpation, nasogastric reflux, and serosanguinous abdominal fluid. However, the prognosis for many of these horses appears to be lower than for horses with other types of small intestinal strangulations. For example, in horses with small intestine entrapped in a mesenteric rent, only 7 of 15 horses were discharged from the hospital, and only 2 of 5 horses for which follow-up information was available survived long term (>5 months).[20] Poor outcome may result from the difficulty in unentrapping incarcerated intestine, the degree of hemorrhage, and the length of intestine affected.

13.14.2.7

INGUINAL HERNIA

Inguinal herniae are more common in Standardbred and Tennessee Walking horses that tend to have congenitally large inguinal canals.[11] Inguinal herniae also may occur in neonatal foals but differ from herniae in mature horses in that they are typically nonstrangulating. The nature of the hernia (direct versus indirect) is based on the integrity of the parietal vaginal tunic. In horses in which the bowel remains within the parietal vaginal tunic, the hernia is referred to as indirect, because strictly speaking the bowel remains within the peritoneal cavity. Direct herniae are those in which strangulated bowel ruptures through the parietal vaginal tunic and occupies a subcutaneous location. These direct herniae most commonly occur in foals and should be suspected when a congenital inguinal hernia is associated with colic, swelling that extends from the inguinal region or the prepuce, and intestine that may be palpated subcutaneously.[37,38] Although most congenital indirect inguinal herniae resolve with repeated manual reduction or application of a diaper, surgical intervention is recommended for congenial direct herniae.[37]

Figure 13.14-2 Inguinal hernia in a horse with colic. The enlarged testicle has compromised venous drainage because of herniated small intestine within the inguinal canal.

Historical findings in horses with strangulating inguinal herniae include acute onset of colic in a stallion that recently had been used for breeding. A cardinal sign of inguinal herniation is a cool, enlarged testicle on one side of the scrotum (Figure 13.14-2).[39,40] However, inguinal herniae also have been reported in geldings.[41] One also can detect inguinal herniae on rectal palpation, and one can use manipulation of herniated bowel per rectum to reduce a hernia, but this is generally not recommended because of the risk of rectal tears. In many cases, the short segment of herniated intestine greatly improves in appearance after reduction and in some

cases can be left unresected. The affected testicle will be congested because of vascular compromise within the spermatic cord, and although the testicle may remain viable, resection generally is recommended.[42] The prognosis in adult horses is good, with up to 75% of horses surviving to 6 months.[40] Horses that have been treated for inguinal herniae may be used for breeding. In these horses, the remaining testicle will have increased sperm production, although an increased number of sperm abnormalities will be noticeable following surgery because of edema and increased temperature of the scrotum.

13.14.2.8 STRANGULATING UMBILICAL HERNIAE

Although umbilical herniae are common in foals, strangulation of herniated bowel is rare. In one study, 6 of 916 147 (4%) horses with umbilical herniae had incarcerated intestine.[43] Clinical signs include a warm, swollen, 917 firm, and painful hernia sac associated with signs of colic. The affected segment of bowel is usually small intestine, but herniation of cecum or large colon also has been reported. In rare cases, one may find a hernia that involves only part of the intestinal wall, called a Richter's hernia. In foals that have a Richter's hernia, an enterocutaneous fistula may develop. In one study, 13 of 13 foals with strangulating umbilical herniae survived to discharge, although at least 3 died of long-term complications.[44]

13.14.2.9 INTUSSUSCEPTIONS

An intussusception involves a segment of bowel (intussusceptum) that invaginates into an adjacent aboral segment of bowel (intussuscipiens). The reason for such invagination is not always clear but may involve a lesion at the leading edge of the intussusception, including small masses, foreign bodies, or parasites. In particular, tapeworms *(Anoplocephala perfoliata)* have been implicated.[45] Ileocecal intussusceptions are the most common intestinal intussusceptions in the horse and typically affect young animals. In one study evaluating 26 cases of ileocecal intussusception, the median age of the horses was 1 year old. Acute ileocecal intussusceptions are those in which the horses has a duration of colic of less than 24 hours and involve variable lengths of intestine that ranged in one study from 6 to 457 cm long. In acute cases the involved segment of ileum typically has a compromised blood supply. Chronic ileocecal intussusceptions typically involve short segments of ileum (up to 10 cm long), and the ileal blood supply is frequently intact.[46] Abdominocentesis results vary because strangulated bowel is contained within the adjacent bowel. Obstruction of the small intestine often is evident, including nasogastric reflux and multiple distended loops of small intestine on rectal palpation. Horses with chronic ileocecal intussusceptions have mild, intermittent colic, often without evidence of small intestinal obstruction. In one study, a mass was palpated in the region of the cecal base in approximately 50% of cases.[45] Transabdominal ultrasound may be helpful in discerning the nature of the mass. The intussusception has a characteristic target appearance on cross section.[47] Other segments of the small intestine also may be intussuscepted, including the jejunum (Figure 13.14-3). In one study of 11 jejunojejunal intussusceptions, the length of bowel involved ranged from 0.4 to 9.1 m.[48] Attempts to reduce intussusceptions at surgery are usually futile because of intramural swelling of affected bowel. One should resect jejunojejunal intussusceptions.

For acute ileocecal intussusceptions, one should transect the small intestine as far distally as possible and perform a jejunocecal anastomosis. In horses with particularly long intussusceptions (up to 10 m has been reported), one may attempt an intracecal resection.[49] For horses with chronic ileocecal intussusceptions, one should perform a jejunocecal bypass without small intestinal transection. The prognosis is good for horses

with chronic ileocecal intussusceptions and guarded to poor for horses with acute ileocecal intussusceptions, depending on the length of bowel involved.[46]

Figure 13.14-3 Jejunojejunal intussusception in a horse presented for colic. The intussusceptum has become ischemic because of invagination of intestine and its mesenteric blood supply into the intussuscipiens.

13.14.2.10 DIAPHRAGMATIC HERNIAE

Herniation of intestine through a rent in the diaphragm is rare in the horse and may involve any segment of bowel, although small intestine is herniated most frequently. Diaphragmatic rents may be congenital or acquired, but acquired herniae are more common. Congenital rents may result from incomplete fusion of any of the four embryonic components of the diaphragm: pleuroperitoneal membranes, transverse septum, and esophageal mesentery. In addition, abdominal compression of the foal at parturition may result in a congenital hernia.[50] Acquired herniae are presumed to result from trauma to the chest or a sudden increase in intraabdominal pressure, such as might occur during parturition, distention of the abdomen, a sudden fall, or strenuous exercise.[51] Herniae have been found in a number of different locations, although large congenital herniae are typically present at the ventral most aspect of the diaphragm, and most acquired herniae are located at the junction of the muscular and tendinous portions of the diaphragm.[50] A peritoneopericardial hernia has been documented in at least one horse.[52]

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Clinical signs usually are associated with intestinal obstruction rather than respiratory embarrassment.[51] However, careful auscultation may reveal an area of decreased lung sounds associated with obstructed intestine and increased fluid within the chest cavity.[53] Such signs may prompt thoracic radiography or ultrasound, both of which one can use to make a diagnosis. Auscultation also may reveal thoracic intestinal sounds, but differentiating these from sounds referred from the abdomen typically is not possible. In one report, two of three horses diagnosed with small intestinal strangulation by diaphragmatic hernia had respiratory acidemia attributable to decreased ventilation.[54] Treatment of horses with diaphragmatic hernia is fraught with compli cations because of the need to reduce and resect strangulated bowel and the need to repair the defect in the diaphragm.[55,56] Because dorsal defects in the diaphragm are among the common forms of diaphragmatic defect, closing the diaphragmatic hernia via the approach used for abdominal exploration may not be possible. However, because herniation is likely to recur,[55] scheduling a second surgery using an appropriate approach to resolve the diaphragmatic defect is appropriate.

13.14.3 Large Colon Volvulus

13.14.3.1 CLINICAL SIGNS

Horses with large colon volvulus have rapid onset of severe, unrelenting abdominal pain, most often in postpartum broodmares.[4] Once the large colon strangulates (≥270-degree volvulus), gas distention is significant, leading to gross distention of the abdomen, compromised respiration as the distended bowel presses up against the diaphragm, and visceral pooling of blood as the caudal vena cava is compressed. Horses with this condition are frequently refractory even to the most potent of analgesics. These horses may prefer to lie in dorsal recumbency, presumably to take weight off the strangulated colon. An abbreviated physical examination is warranted in these cases, because the time elapsed from the onset of strangulation to surgical correction is critical. Under experimental conditions, the colon is irreversibly damaged within 3 to 4 hours of a 360-degree volvulus of the entire colon.[57] Despite severe pain and hypovolemia, horses may have a paradoxically low heart rate, possibly related to increased vagal tone. In addition, results of abdominocentesis often do not indicate the degree of colonic compromise[4,58] and in many cases are not worth attempting because of extreme colonic distention.[59] Palpation per rectum reveals severe gas distention of the large colon, often restricting access to the abdomen beyond the pelvic brim. One may make the diagnosis tentatively based on signalment, severity of pain, and degree of distention.

13.14.3.2 SURGICAL FINDINGS

At surgery, the volvulus typically is located at the mesenteric attachment of the colon to the dorsal body wall and the most common direction of the twist is dorsomedial using the right ventral colon as a reference point. However, the colon may twist in the opposite direction, twist greater than 360 degrees (up to 720 degrees has been reported) or twist at the level of the diaphragmatic and sternal flexures.[4] In all cases, one should decompress the colon as much as possible, and in many cases a colonic evacuation via a pelvic flexure enterotomy greatly aids correction of the volvulus. One must determine after correction of the volvulus whether the colon has been injured irreversibly and should base the determination on mucosal color and bleeding (if an enterotomy has been performed), palpation of a pulse in the colonic arteries, serosal color, and appearance of muscular motility. If one judges the colon to be damaged irreversibly, one can consider the feasibility of a large colon resection. Although 95% of the colon can be resected (that part of the colon distal

to the level of the cecocolic fold), damage from the volvulus usually exceeds that which can be resected. In these cases, surgeons may elect to resect as much damaged bowel as possible or may advise euthanasia.[7]

13.14.3.3 PROGNOSIS

The prognosis is guarded to poor because of the rapid onset of this disease. In one study the survival rate was 35%.[58] In a more recent report the survival rate was 36% for horses with 360-degree volvulus of the large colon compared with 71% for horses with 270-degree volvulus.[4] However, one study in central Kentucky documented a high success rate, possibly because of early recognition of the disease and the proximity of the hospital to the surgical caseload.[60] Postoperative complications include hypovolemic and endotoxic shock, extensive loss of circulating protein, disseminated intravascular coagulation, and laminitis. In addition, large colon volvulus has a propensity to recur. Although one study documented a recurrence rate of less than 5%,[58] some authors believe recurrence may be as high as 50%.[7] Therefore one should consider methods to prevent recurrence in patients at risk of recurrence, particularly broodmares that tend to suffer from the disease recurrently during the foaling season.[61,62]

13.14.4 Other Causes of Large Intestinal Ischemia

The most common intussusceptions of the large intestine are cecocecal and cecocolic intussusceptions.[63,64] Both are likely attributable to the same disease process, with variable inversion of the cecum. These conditions tend to occur in young horses (63% were less than 3 years old in one study) and may be associated with intestinal tapeworms. Horses show highly variable clinical signs, including acute severe colic, intermittent pain over a number of days, or chronic weight loss.[64] These variable presentations likely relate to the degree to which the cecum has intussuscepted. Initially, the cecal tip inverts, creating a cecocecal intussusception, which does not obstruct flow of ingesta. As the intussusception progresses, the cecum inverts into the right ventral colon (cecocolic intussusception), obstructs flow of ingesta, and often causes severe colic. The cause of abdominal pain is often difficult to differentiate in these cases, although detecting a mass on the right side of the abdomen by per rectal palpation or ultrasound examination sometimes is possible.[63,64] Treatment involves manual surgical reduction by retracting the intussusceptum directly[63] or via an enterotomy in the right ventral colon.[65] However, a number of cases occur in which one cannot reduce the cecum readily because of severe thickening or in which surgical procedures result in fatal contamination. For example, one report stated that 8 of 11 horses were euthanized in the perioperative period because of complications,[63] and another report stated that 12 of 30 horses were euthanized before or during surgery. The latter included all of the horses with chronic disease because of irreversible changes to the cecum.[64] However, one recent report on cecocolic intussusceptions indicated that seven of eight horses that underwent right ventral colon enterotomy and cecal resection survived long-term,[65] suggesting that continued improvements in surgical techniques may improve the prognosis.

Colocolic intussusceptions are rare but have been reported to affect the pelvic flexure and the left colons.[66–69] Although the condition is reported to be more common in young horses,[67–69] the condition may affect older horses.[66] Clinical findings may include a palpable mass on the left side of the abdomen.[67] Ultrasonography also may be useful. Treatment requires manual reduction of the intussusception at surgery,[67,69] or resection of affected bowel.[66] Because the left colons may be exteriorized extensively and manipulated at surgery,[66–69] the prognosis is fair.

13.14.5 Rectal Prolapse

Rectal prolapse may occur following any disease that causes tenesmus, including diarrhea, rectal neoplasia, and parasitism,[70] or prolapse can occur following elevations in intraabdominal pressure during parturition or episodes of coughing.[71,72] Rectal prolapses are classified into four categories (Table 13.14-1) based on the extent of tissue prolapsed and the severity. Type I rectal prolapse is most common and is characterized by a doughnut-shaped prolapse of rectal mucosa and submucosa. Type II prolapses involve full-thickness rectal tissue, whereas type III prolapses additionally have invagination of small colon into the rectum. Type IV prolapses involve intussusception of proximal rectum or small colon through the anus in the absence of prolapse of tissue at the mucocutaneous junction at the anus.[73] One can differentiate type IV from other forms of prolapse by their appearance and a palpable trench between prolapsed tissue and the anus.

TABLE 13.14-1 Classification of Rectal Prolapse

GRADE	DESCRIPTION	PROGNOSIS
I	Prolapse of rectal mucosa	Good
II	Prolapse of full-thickness rectum	Fair
III	Grade II prolapse with additional protrusion of small colon	Guarded
IV	Intussusception of rectum and small colon through the anus	Poor

Type I prolapses occur most frequently in horses with diarrhea, in which the rectal mucosa becomes irritated and protrudes intermittently during episodes of tenesmus. If tenesmus persists, rectal mucosa can remain prolapsed. Rectal mucosa rapidly becomes congested and edematous under these conditions, which one should treat with osmotic agents such as glycerin or magnesium sulfate and by massaging and reducing the prolapse.[74] A purse-string suture may be required to keep the mucosa inside the rectum. Topical application of lidocaine solution or jelly, epidural anesthesia, and sedation may help reduce tenesmus that incites and exacerbates rectal prolapse. One can apply similar treatments to type II rectal prolapses. However, these more severe prolapses may not be reducible without surgical resection of mucosa and submucosa from the prolapsed bowel.[70,74]

Type III and IV rectal prolapses are more serious injuries because of involvement of small colon.[75] In horses with type III prolapses, one should perform an abdominocentesis to determine if injured small colon has resulted in peritonitis. One should reduce the small colon component manually if possible, although prolapsed rectal tissue typically requires mucosal/submucosal resection. One should perform surgical exploration of the abdomen to determine the status of the small colon, although one can use serial abdominocenteses in lieu of surgery to detect progressive necrosis of bowel. Type IV prolapses occur most commonly in horses with dystocia.[73] These prolapses are almost always fatal because of stretching and tearing of mesenteric vasculature, with subsequent 919
infarction of affected bowel. Therefore euthanasia usually is warranted based on physical examination findings. 920
However, confirmation of severe small colonic injury requires abdominal exploration via a midline approach or laparoscopy.[76] A horse with compromised small colon conceivably could undergo a colostomy of the proximal small colon, but the compromised small colon typically necroses beyond that which can be resected via a midline abdominal approach.[74]

13.14.6 Nonstrangulating Infarction

Nonstrangulating infarction occurs following cranial mesenteric arteritis caused by migration of *Strongylus vulgaris* and has become a rare disorder since the advent of broad-spectrum anthelmintics. Although thromboemboli have been implicated in the pathogenesis of this disease, careful dissection of naturally occurring lesions has not revealed the presence of thrombi at the site of intestinal infarctions in most cases.[77] These findings suggest that vasospasm plays an important role in this disease.[78] Clinical signs vary greatly depending on the extent to which arterial flow is reduced and the segment of intestine affected. Any segment of intestine supplied by the cranial mesenteric artery or one of its major branches may be affected, but the distal small intestine and large colon are more commonly involved. No clinical variables exist that one can use to differentiate this disease from strangulating obstruction reliably. In some cases, massive infarction results in acute, severe colic.[77] Occasionally, one may detect an abnormal mass and fremitus on palpation of the root of the cranial mesenteric artery per rectum. One should consider this disease a differential diagnosis in horses with a history of inadequate anthelmintic treatment and the presence of intermittent colic that is difficult to localize. Although one should perform fecal parasite egg counts, they are not indicative of the degree of parasitic infestation.

In addition to routine treatment of colic, dehydration, and endotoxemia, medical treatment may include aspirin (20 mg/kg every 24 hours) to decrease thrombosis.[78] Definitive diagnosis requires surgical exploration. However, these cases are difficult to treat because of the patchy distribution of the lesions and the possibility of lesions extending beyond the limits of surgical resection. In addition, further infarction may occur following surgery. The prognosis is fair for horses with intermittent mild episodes of colic that may be amenable to medical therapy but is poor in horses that require surgical intervention.[77,78]

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13.15

13.15—Obstructive Disorders of the Gastrointestinal Tract

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13.15.1

Approach to the Horse With Colic

Clinical management of colic is distinctly different from management of many other clinical syndromes because the initial focus is often not on defining the definitive diagnosis but rather on deciding whether a horse requires surgical exploration. Therefore the clinician must collect historical, physical examination, and clinicopathologic information and make a decision whether these findings warrant medical management or whether to perform surgical exploration of the abdomen because of a suspected obstructive or ischemic lesion. For example, one may examine a horse with signs of severe abdominal pain, poor cardiovascular status, and abdominal distention that may be compatible with an extensive list of differential diagnoses but that more importantly indicate the need for abdominal exploration to minimize the extent of intestinal injury. The speed with which one can make this clinical decision has a tremendous effect on the well-being of the patient,[1,2] because delaying surgical exploration of a horse with on-going intestinal injury exacerbates shock induced largely by endotoxin traversing damaged mucosa, and this in turn correlates with mortality.[3]

13.15.1.1

HISTORY

The initial clinical step in the workup of horses with colic is taking a thorough history. However, one may have to delay taking a complete history until after the physical examination and initial treatment, because management of abdominal pain may take precedence. If possible, one should obtain the vital components of the history before examination and treatment: the duration and severity of colic symptoms, analgesics already administered, and a history of any adverse drug reactions. The two most critical factors from a history that would support a decision to explore a horse with colic surgically are the duration of signs and the extent of pain. One deduces the latter from asking the owner about the presence and frequency of pawing, looking at the flanks, rolling, repeatedly going down and getting back up, posturing as if to lie down or urinate, among other clinical evidence of pain.[4] Table 13.15-1 lists other important components of the history one should obtain to try to ascertain why colic has occurred.

13.15.1.2

PHYSICAL EXAMINATION FINDINGS

Just as the history necessarily may need to be brief to allow rapid treatment of colic, so the clinician must be able to alter the extent of the physical examination to treat the horse in a timely fashion. The most critical

examination finding is the heart rate of the horse, because it provides an excellent assessment of the cardiovascular status of the horse.[4] The heart rate is likely the single most reliable predictor of the need for surgery and survival.[4,5] Because analgesics can alter the heart rate dramatically, if possible, one should obtain the heart rate before administering analgesics. Other components of the examination are designed specifically to gather information about the cardiopulmonary status of the horse (quality of the pulse, mucous membrane color, capillary refill time, respiratory rate, and full auscultation of the chest), and the nature of the intestinal obstruction (auscultation of gastrointestinal sounds, per rectal palpation of the abdomen, and presence of nasogastric reflux). Although classic presentations exist for horses with obstructions of the small or large intestine (Table 13.15-2), clinical presentations of the various types of intestinal obstructions can vary. For example, a horse that has a small intestinal obstruction may have several loops of distended small intestine without any evidence of gasric fluid accumulation (assessed as nasogastric reflux), depending on the site (distal versus proximal), extent, and duration of the obstruction. Other examples include horses with large 922
colon obstruction that may have gastric fluid accumulation because of direct compression of the small 923
intestine by distended colon or via tension on the duodenocolic ligament. The most useful diagnostic test for determining the type of intestinal obstruction is rectal palpation of the abdomen.[4] However, one can reach only approximately one third of the abdomen via the rectum, and this percentage may be substantially less in large horses or heavily pregnant horses. Nonetheless, attempting to determine the type of obstruction present (small intestine versus large intestine, and simple obstruction versus strangulating obstruction) is worthwhile; this information directly affects prognosis. In one study, interns and residents at a veterinary teaching hospital were able to predict the type of lesion with a specificity exceeding 90%.[6] Findings from palpation are helpful in educating the client about the potential findings in surgery and the likelihood of survival for the horse.

TABLE 13.15-1 History Findings and Their Relevance to Colic and Its Prevention

COMPONENT OF HISTORY	RISK FOR COLIC	POTENTIAL MECHANISMS
Feeding	Recent change in feed	Alteration in fluid flux or fermentation in the large colon
	Coastal Bermuda hay with a high fiber content	Obstruction of ileum by fine, fibrous hay
	Feeding round bales	Poor-quality hay
	Feeding off the ground	Horses may ingest sand in some regions of the country
	Excessive concentrate	Alteration in fluid flux or fermentation in the large colon
	Large, infrequent meals	Alteration in fluid flux or fermentation in the large colon
	Bolting feed	Large boluses of feed entering the esophagus and stomach
Environment	Excessive time in stall	Insufficient intake of roughage
		Insufficient exercise
	Insufficient access to water	Dehydration
Exercise	Exercise-induced exhaustion	Dehydration
		Reduced gastrointestinal motility
Preventive care	Insufficient dental care	Poor mastication of feed
	Insufficient anthelmintic treatment	Large parasite burden
Medication	Excessive administration of nonsteroidal antiinflammatory drugs	Mucosal damage, particularly in the stomach and colon
Previous medical history	Colic surgery	Adhesions
		Anastomotic obstruction

TABLE 13.15-2 Indications for Surgery in Patients With Colic According to Their Clinical Signs

INDICATION	CLINICAL SIGNS
Refractory pain	Repeated episodes of pain despite treatment with analgesics
	Violent episodes of pain
	Persistently elevated heart rate (>48 beats/min)
Endotoxemia in the face of colic	Persistently elevated heart rate
	Weak peripheral pulse
	Abnormal mucous membrane color (pale, hyperemic, purple)
	Delayed capillary refill time (>2 seconds)
Evidence of a refractory small intestinal obstruction	Refractory pain
	Nasogastric reflux
	Distended loops of small intestine on per rectum palpation
Evidence of a refractory large intestinal obstruction	Refractory pain
	Abdominal distention
	Distended large colon on per rectum palpation
	Tight band(s) on per rectum palpation
Evidence of devitalized bowel	Endotoxemia
	Abnormal abdominocentesis (total protein >2.5 g/dl, total nucleated cell count >10,000/l)

13.15.1.3 MANAGEMENT OF ABDOMINAL PAIN

Before considering how to manage signs of colic, one should remember that such signs are poorly localized. Therefore although colic is most frequently associated with intestinal disease, one should consider dysfunction of other organ systems, including urinary obstruction,[7,8] biliary obstruction,[9] uterine torsion or tears,[10,11] ovarian artery hemorrhage,[10] and neurologic disease as differential diagnoses.[12] However, the duration and 923
severity of colic signs are excellent predictors of whether a horse requires surgical exploration of the 924
abdomen. In fact, refractory pain supersedes all other predictors of the need for surgery in the colic patient. Once signs of colic have been recognized and categorized as to their severity, rapidly and effectively relieving the pain is critical for the well-being of the horse and to reduce the owner's anxiety. In addition, pain is best managed before it becomes severe.[13] Several classes of analgesics are readily available to treat horses with colic (Table 13.15-3), including α_2-agonists (xylazine, detomidine), opiates (butorphanol), and nonsteroidal antiinflammatory drugs (NSAIDs, such as flunixin meglumine). Although much of this information is familiar to most practitioners, several principles deserve emphasis. The short-duration drugs xylazine and butorphanol,

which provide analgesia for 30 to 45 minutes, allow the veterinarian to determine if pain is recurrent within the time period of the typical examination. In contrast, flunixin meglumine is not as potent as an analgesic but has a much longer duration of action. To avoid deleterious effects on gastrointestinal mucosa and the kidneys, one should not administer flunixin meglumine more frequently than recommended.[14,15] The recent discovery of two isoforms of cyclooxygenase (COX), the enzyme inhibited by NSAIDs, has resulted in discovery of drugs that can more selectively inhibit proinflammatory COX-2 while permitting continued constitutive production of prostanoids. Such specificity may be advantageous in horses with colic, particularly when one considers recent evidence of reduced intestinal recovery from an ischemic event with flunixin compared with a drug that is more selective for COX-2.[16] One should reserve the α_2-agonist detomidine for horses with severe, unrelenting pain because of its tremendous potency.[17] In addition, one should remember that α_2-agonists reduce the heart rate associated with a transient increase in blood pressure,[18,19] thereby reducing the predictive value of the heart rate and pulse pressure.

TABLE 13.15-3 Analgesics Commonly Used to Treat Colic in Horses

DRUG	DOSAGE	AMOUNT FOR AN ADULT HORSE
Butorphanol	0.025–0.05 mg/kg as needed	5–10 mg
Detomidine	10–20 μg/kg as needed	5–10 mg
Flunixin	0.25–1.1 mg/kg every 8 to 12 hours[*]	125–500 mg
Xylazine	0.3–0.5 mg/kg as needed	150–250 mg

* The longer treatment interval corresponds to the higher dose, whereas lower doses may be given more frequently.

13.15.1.4 CLINICAL PATHOLOGY

The most immediately useful clinicopathologic information in horses with colic are the packed cell volume and total protein, because one can use them to substantiate clinical estimates of dehydration and they correlate strongly with prognosis.[20,21] A serum biochemical profile is useful for assessing electrolyte imbalances, tissue perfusion (anion gap or lactate), and kidney and liver function. One can use serum biochemical or blood gas analysis to assess acid-base status. Horses with colic most frequently show evidence of metabolic acidosis associated with poor tissue perfusion caused by hypovolemia or endotoxemia, but one may note other abnormalities such as metabolic alkalosis in association with extensive loss or sequestration of stomach chloride. Metabolic acidosis has been investigated further in horses with colic by measuring blood lactate, although this test is not offered routinely in many laboratories. Lactate levels also have been inferred from measurement of the anion gap, although one study noted that lactate in horses with colic did not account for the entire anion gap.[22] Lactate levels and anion gap closely correlate with prognosis for survival.[20,23,24]

Other key components of assessment of the horse with colic are abdominocentesis and complete blood count. The total white blood cell count and differential can provide crucial evidence of systemic inflammation associated with endotoxemia stemming from colic attributable to colitis (leukopenia, neutropenia, and a left shift) rather than an obstruction (highly variable complete blood count findings). Peritoneal fluid may be helpful in determining the integrity of the intestine. Specifically, as the intestine becomes progressively

devitalized, the peritoneal fluid becomes serosanguinous as red blood cells leak into the abdomen, followed by an elevation in the total protein (>2.5 g/dl) and progressive increases in total nucleated cell count (>10,000 cells/μl). However, these findings do not always correlate well with the condition of the intestine, particularly in horses with large colon volvulus. For example, in a study of 57 horses with large colon volvulus, the average total protein (2.5 g/dl) and total nucleated cell count (1000 cells/μl) were normal despite the fact that only 36% with a 360-degree volvulus survived.[4,25] These measures may appear normal because the development of severe mucosal injury following large colon volvulus is rapid and may not allow enough time for protein and leukocytes to equilibrate with the abdominal fluid.[26]

Investigators have taken all the variables routinely assessed during evaluation of horses for colic and have attempted to develop models to predict accurately the need for surgery and the prognosis for life.[27–30] None of these predictor models has taken the place of clinical decision making, although these studies have added 924
tremendously to understanding of the importance of some prognostic factors, particularly those reflecting 925
cardiovascular function.

13.15.2 Small Intestinal Simple Obstruction

Simple obstruction involves intestinal obstruction of the lumen without obstruction of vascular flow. However, because a tremendous volume of fluid enters the small intestinal lumen daily,[31,32] the obstructed intestine tends to become distended, which in turn may reduce mural blood flow.[33] Ultimately, such distention may result in necrosis of tissues, particularly in the immediate vicinity of the obstruction.[34] Few are the causes of simple obstruction in the small intestine, and the incidence of these obstructions is low (approximately 3% of all referred horses in one large hospital-based study).[5] However, in some geographic regions, this type of obstruction is prevalent. For example, in the southeastern United States, ileal impactions are common.[35,36]

13.15.2.1 ASCARID IMPACTIONS

Impactions caused by *Parascaris equorum* typically occur in foals under 6 months of age that have been on a poor deworming program and have a heavy parasite burden. Products that cause sudden ascarid death, including piperazine, organophosphates, and pyrantel pamoate, have been incriminated in triggering acute intestinal obstruction by dead parasites. Ascarids are particularly problematic because of the large size of the adult parasite (Figure 13.15-1). Clinical signs include acute onset of colic following administration of an anthelmintic and signs compatible with small intestinal obstruction, including nasogastric reflux. Occasionally, dead parasites are present in the reflux. The onset of the disease varies according to the degree of obstruction. One tentatively may base diagnosis on the history and signs referable to small intestinal obstruction. Abdominal radiographs may indicate the presence of multiple loops of distended small intestine but are not required if clinical signs indicate the immediate need for surgery. Initial medical treatment should include treatment of hypovolemic shock resulting from sequestration of fluid in the small intestine and systemic inflammation from absorption of endotoxin. Surgical treatment typically involves an enterotomy made over the intraluminal impaction and removal of ascarids. The prognosis is fair in horses that are rapidly and appropriately treated but poor in foals with evidence of hypovolemic and endotoxic shock.[37]

Figure 13.15-1 Appearance of roundworms that have been retrieved within the nasogastric reflux from a foal with an ascarid impaction. The large size of these ascarids (bar = 1 cm) contributes to the risk of impaction following sudden kills of these parasites by broad-spectrum anthelmintics.

13.15.2.2

ILEAL IMPACTION

Ileal impactions most commonly occur in adult horses in the southeastern United States. Although feeding of coastal Bermuda hay has been implicated in the regional distribution of the disease, separating geographic location from regional hay sources as risk factors has been difficult. Nonetheless, feeding coastal Bermuda hay likely places horses at risk of ileal impaction, particularly if the coarse fiber content of the hay is high. Furthermore, sudden changes in feed from an alternate type of hay to coastal Bermuda hay likely places a horse at risk of ileal impaction.[38] Studies in England have revealed tapeworm infection as another important risk factor for ileal impaction. Based on risk analysis, the data suggested that in excess of 80% of the ileal impaction cases studied were associated with serologic or fecal evidence of tapeworm infection.[39] Because of the poor sensitivity of fecal analysis for tapeworms, Proudman and Trees have developed a serologic test (enzyme-linked immunosorbent assay) with a sensitivity of approximately 70% and a specificity of 95%.[40]

Clinical signs of horses with ileal impaction are typical for a horse with small intestinal obstruction, including onset of moderate to severe colic and loops of distended small intestine palpable per rectum as the condition

progresses. Because the ileum is the distal most aspect of the small intestinal tract, nasogastric reflux may take a considerable time to develop and is found in approximately 50% of horses requiring surgical correction of impacted ileum.[35,41] One usually makes the diagnosis at surgery, although on occasion one may palpate an impacted ileum per rectum. Multiple loops of distended small intestine frequently make the impaction difficult to palpate. Ileal impactions may resolve with medical treatment[36] but frequently require surgical intervention (Figure 13.15-2). At surgery, one can infuse fluids directly into the mass, allowing the surgeon to breakdown the impaction. The surgeon may include dioctyl sodium sulfosuccinate in the infused fluid to aid in disruption of the mass. Extensive small intestinal distention and intraoperative manipulation of the ileum may lead to postoperative ileus,[42] but recent studies indicate that this complication is less frequent as the duration of disease before admission decreases.[35] Recent studies indicate that the prognosis for survival is good.[35,36] 925 926

Figure 13.15-2 Intraoperative view of an ileal impaction. The distended appearance of the ileum as it courses toward the cecal base is notable.

13.15.2.3 ILEAL HYPERTROPHY

Ileal hypertrophy is a disorder in which the muscular layers (circular and longitudinal) of the ileum hypertrophy for unknown reasons (idiopathic) or following an incomplete or functional obstruction. For idiopathic cases, proposed mechanisms include parasympathetic neural dysfunction resulting in chronically increased muscle tone and subsequent hypertrophy of the muscular layers of the ileal wall. Such neural dysfunction possibly could result from parasite migration. Alternative hypotheses include chronic increases in the muscular tone of the ileocecal valve, leading to muscular hypertrophy of the ileum as it contracts against a

partially occluded ileocecal valve. The jejunum also may be hypertrophied, alone or with the ileum. Clinical signs include chronic intermittent colic as the ileum hypertrophies and gradually narrows the lumen diameter. In one study, partial anorexia and chronic weight loss (1 to 6 months) were documented in 45% of the horses, most likely because of intermittent colic and reduced appetite. Because hypertrophy does not affect the ileal mucosa, no reason exists to believe that these horses experience malabsorption of nutrients. One usually makes the diagnosis at surgery, although one may palpate the hypertophied ileum per rectum in some cases. For treatment, one performs an ileocecal or jejunocecal anastomosis to bypass the hypertrophied ileum. Without surgical bypass, intermittent colic persists and the thickened ileum ultimately may rupture.[43] The prognosis is fair with surgical treatment.[44]

Secondary ileal hypertrophy is most commonly notable in horses that previously have had colic surgery and that may have a partial or functional obstruction at an anastomotic site. For example, in one case report, a horse developed ileal hypertrophy after surgical correction of an ileocecal intussusception.[45] Ileal hypertrophy also was noted in a horse with cecal impaction in which an ileocolic anastomosis was oriented incorrectly.[46] Horses are typically re-presented for recurrence of colic in these cases. Surgical therapy is directed at addressing the cause of small intestinal obstruction and resecting hypertrophied intestine.

13.15.2.4 MECKEL'S DIVERTICULUM

Meckel's diverticulum is an embryonic remnant of the vitelloumbilical duct, which fails to atrophy completely and becomes a blind pouch projecting from the antimesenteric border of the ileum.[47,48] However, similar diverticula also have been noted in the jejunum.[49] These diverticula may become impacted, resulting in partial luminal obstruction, or may wrap around an adjacent segment of intestine, causing strangulation.[47] Occasionally, an associated mesodiverticular band may course from the diverticulum to the umbilical remnant and serve as a point around which small intestine may become strangulated. Mesodiverticular bands also may originate from the embryonic ventral mesentery and attach to the antimesenteric surface of the bowel, thereby forming a potential space within which intestine may become entrapped. Clinical signs range from chronic colic for an impacted Meckel's diverticulum to acute severe colic for intestine strangulated by a mesodiverticular band. One makes the diagnosis at surgery, and treatment requires resection of the diverticulum and any associated bands. The prognosis is good for horses with simple impaction of a Meckel's diverticulum and is guarded for horses with an associated small intestinal strangulation.[50]

13.15.2.5 ADHESIONS

Adhesions of one segment of bowel to another or of a segment of intestine to other organs and the body wall most typically occur following abdominal surgery and may be clinically silent, cause chronic colic attributable to partial obstruction, or result in acute obstruction. These differing clinical syndromes are attributable to the type of adhesions that develop. For example, a fibrous adhesion that does not by itself obstruct the intestinal 926
lumen might serve as the pivot point for a volvulus, whereas an adhesion between adjacent segments of the 927
intestinal tract may create a hairpin turn that causes chronic partial obstruction.[51] The number of adhesions that develop also may vary greatly from horse to horse. Some horses may develop a single adhesion adjacent to an anastomotic site or a discrete segment of injured intestine, whereas other horses may develop diffuse adhesions involving multiple segments of intestine, likely because of widespread inflammation of the peritoneum at the time of the original surgery.

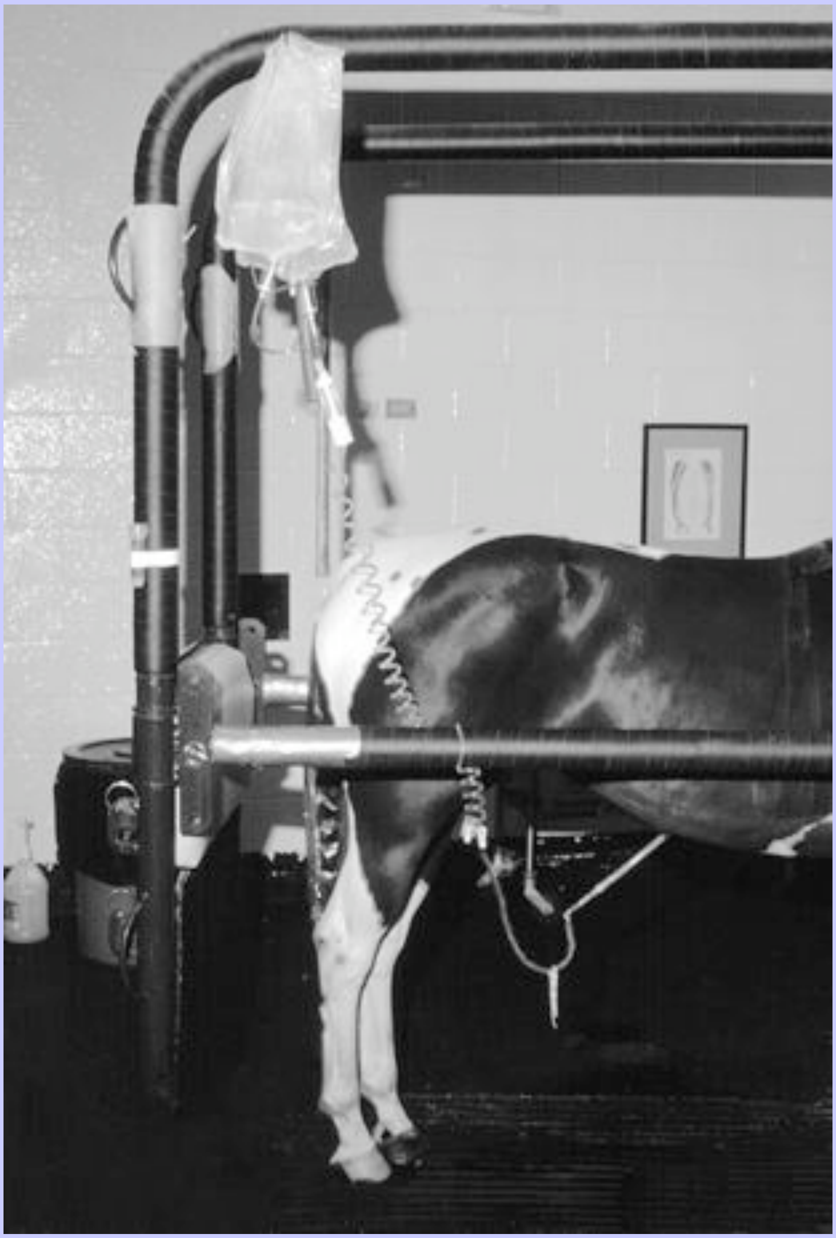
The mechanism whereby adhesions develop is complex but likely involves initial injury to the serosa initiated by intestinal ischemia, reperfusion injury, and luminal distention.[52] Importantly, such injury involves infiltration of neutrophils into the serosa accompanied by loss of mesothelial cells. In one study assessing the margins of resected small intestine, extensive neutrophil infiltration was documented in the serosa, particularly in the proximal resection margin that had been distended before correction of a variety of strangulating lesions.[53] Regions of serosal injury and inflammation subsequently undergo reparative events similar to any wound, including local production of fibrin, de novo synthesis of collagen by infiltrating fibroblasts, and ultimately maturation and remodeling of fibrous tissue. Unfortunately, during this process, fibrin may result in injured intestinal surfaces adhering to adjacent injured bowel or an adjacent organ. Once a fibrinous adhesion has developed, new collagen synthesis may result in a permanent fibrous adhesion. Alternatively, proteases released by local phagocytes may lyse fibrinous exudate, thereby reversing the adhesive process. Thus one can view formation of adhesions as an imbalance of fibrin deposition and fibrinolysis.[54]

Prevention of adhesions depends on inhibition of the mechanisms involved in adhesion formation, including reduction of serosal injury with early intervention and good surgical technique, reduction of inflammation by administration of antiinflammatory medications, physical separation of inflamed serosal surfaces (e.g., carboxymethylcellulose and hyaluronan),[55–57] and pharmacologic modulation of fibrinous adhesion formation (e.g., heparin).[58] In addition, early return of motility in the small intestine after surgery may reduce contact time between inflamed surfaces of intestine, thereby reducing the chances of adhesion formation.[54]

Horses at greatest risk of developing adhesions after colic surgery appear to be those that have small intestinal disease.[51,59] In one study of horses undergoing surgical correction of small intestinal obstruction, 22% developed a surgical lesion associated with adhesions. Foals appear to have an increased incidence of adhesions compared with mature horses regardless of the nature of the abdominal surgery.[51] One study indicated that 17% of foals developed lesions attributable to adhesions regardless of the type of initial surgery.[60] Studies conflict as to whether the degree of surgical intervention influences adhesion formation,[51] but in one study, horses that require enterotomy or resection and anastomosis were at greatest risk of developing adhesions.[59] As an indication of the importance of postoperative adhesion formation, adhesions were among the most common reasons for repeat laparotomy in postoperative colic patients.[59,61]

Clinical signs of horses with adhesions vary greatly depending on whether the adhesion is causing partial obstruction or complete luminal obstruction or involves intestinal vasculature. Adhesions would be an important differential diagnosis for intermittent colic in the postoperative period, particularly if such colic was not relieved by nasogastric decompression of the stomach. Continued intermittent colic should prompt abdominocentesis to determine if septic peritonitis is present, which may contribute to adhesion formation. Placement of a large bore drain and peritoneal lavage (Figure 13.15-3) aids resolution of peritonitis and may reduce adhesion formation by reducing intraabdominal inflammation. If postoperative colic persists, one may 927
elect repeat laparotomy or laparoscopy. In one study of adhesions, 70% of repeat laparotomies were performed 928
within 60 days, suggesting that surgical colic attributable to adhesions typically occurs within 2 months of an initial surgical procedure. Unfortunately, the prognosis for horses with colic attributable to adhesions is low, with only 16% of horses in one study surviving from adhesion-induced colic.[51]

Figure 13.15-3 Peritoneal lavage in a horse. The use of an intravenous administration set and a large-bore catheter placed in the dependent portion of the abdomen adjacent to the ventral midline incision is notable.



13.15.2.6

POSTOPERATIVE ILEUS

The definition of *ileus* is intestinal obstruction, including physical and functional obstructions. However, in veterinary medicine, the term typically is used to designate a lack of progressive aboral propulsion of ingesta resulting in functional obstruction.[62] One typically bases the diagnosis of postoperative ileus on the presence of excessive gastric fluid accumulation (reflected as excessive nasogastric reflux). Postoperative ileus may occur following any abdominal exploratory procedure. However, horses undergoing surgery for strangulating small intestinal lesions or small intestinal obstructive lesions such as an ileal impaction are at greatest risk.[42] Recently, the syndrome of postoperative ileus in horses has been broadened to include those horses that may have delayed transit of ingesta through the large intestine following surgery. This large intestinal ileus may follow any type of surgery, particularly horses that have had orthopedic surgery, and is characterized by reduced fecal output (fewer than three piles of manure per day) rather than excessive nasogastric reflux.[62] However, horses with excessive nasogastric reflux are unlikely to have normal fecal output, so the distinction between these two manifestations of ileus is not absolute.

Mechanisms involved in precipitating postoperative ileus characterized by small intestinal dysfunction likely involve local inflammation, reduced coordination of progressive motility, and increased sympathetic tone. A recent series of studies in the rat has shown that surgical manipulation of intestine results in delayed transit time associated with infiltration of neutrophils into intestinal longitudinal muscle[63–65] and upregulation of inducible nitric oxide synthase and COX-2. The mechanisms in the horse may be similar in that extensive manipulation of the intestine resulted in abnormal intestinal motility in ponies,[66] and prostanoids and nitric oxide alter or reduce intestinal motility in horses.[67–69]

Clinical signs of postoperative ileus following colic surgery include evidence of abdominal pain, increased heart rate, reduced gastrointestinal sounds, and reflux of gastric fluid via a nasogastric tube. Of these signs, heart rate is critical because it appears to be a more sensitive indicator of pain in the postoperative period than overt evidence of colic. Therefore a sudden increase in the heart rate of a postoperative patient following colic surgery should prompt immediate nasogastric intubation to decompress the stomach. Treatment should include attempts at obtaining reflux from the horse at frequent intervals rather than relying on passive flow of reflux. In addition, administration of intravenous fluids should account for the maintenance requirement (50 ml/kg/day, about 1 L/hr in the average horse) and fluid losses via reflux. In practice, this requires frequent monitoring of packed cell volume and total protein to ensure that the horse remains well hydrated. Although concerns have arisen that overhydrating horses may contribute to increased nasogastric reflux,[42] keeping horses well-hydrated to avoid hypovolemic shock is critical. Additionally, one should monitor electrolytes frequently, particularly considering their potential role in smooth muscle contraction and nerve excitability. Because of the important role of inflammation in postoperative ileus, including elaboration of COX-2–produced prostanoids,[70] administration of NSAIDs is indicated. NSAID administration is particularly necessary if postoperative ileus is associated with endotoxemia, because lipopolysaccharide-induced prostanoid production disrupts propulsive motility in horses.[71,72] Interestingly, phenylbutazone is more effective than flunixin meglumine at reducing the deleterious actions of lipopolysaccharide on intestinal motility.[73] However, one should use caution when administering NSAIDs to patients with postoperative ileus in light of research suggesting that complete inhibition of prostanoid production can alter motility patterns in normal equine intestine.[68] The advent of selective COX-2 inhibitors may provide optimal antiinflammatory treatment in the future.[74]

Other treatments aimed at specifically modulating intestinal motility include lidocaine (bolus of 1.3 mg/kg followed by 0.05 mg/kg/min for 24 hours), erythromycin (0.5 to 1.0 mg/kg slow intravenous infusion in 1 L saline every 6 hours), and metoclopramide (0.04 mg/kg/hr).[66,75,76] The mechanism of lidocaine is presumed to be inhibition of sensory nerve activity within the wall of the intestine, thereby reducing reflex sympathetic inhibitory activity. In addition, intravenously administered lidocaine appears to be an effective analgesic. Thus an important feature of intravenous lidocaine therapy may be to control postoperative pain-induced reduction of gastrointestinal motility and mucosal secretory activity.[77] Metoclopramide may stimulate intestinal motility by several mechanisms, including dopamine receptor blockade, cholinergic stimulation, and adrenergic blockade.[66] Although metoclopramide has been shown to be beneficial for reversing postoperative ileus in clinical patients and research animals, it has central nervous system excitatory side effects in the horse that make its use difficult. Nonetheless, administration of metoclopramide to horses with postoperative ileus 928
resulted in a significantly reduced duration of reflux and shorter postoperative hospital stays compared with 929
horses not receiving this drug.[76] In the same study, constant infusion of metoclopramide was superior to intermittent infusion. Recent in vitro studies indicate that metoclopramide effectively increases smooth muscle contractile activity throughout the small intestine. Similarly, the motilin agonist erythromycin had stimulatory effects on equine small intestine, although the results were not uniform throughout the small intestine. Erythromycin stimulates contractile activity in the longitudinal muscle of the pyloric antrum but inhibits contractile activity in circular smooth muscle in this segment of the gastrointestinal tract.[75] The latter may be attributable to activation of motilin receptors on inhibitory nerves and may result in enhanced gastric emptying. In vivo studies on erythromycin confirmed the stimulatory action of this drug on the distal small intestine and indicated this drug also stimulates contractile activity in the cecum and pelvic flexure. However, the stimulation depends on the temporal association with surgery. Erythromycin stimulated contractile activity in the postoperative period in the ileum and pelvic flexure but not the cecum,[78] suggesting this drug may be useful for treating select cases of postoperative ileus.

For horses with presumed ileus of the large colon, signs included reduced fecal output (fewer than three piles of manure per day), reduced gastrointestinal sounds, variable presence of colic, and on occasion a palpable impaction of the cecum or large colon. Risk factors for this syndrome include orthopedic surgery, length of the operative period, and most importantly inadequate treatment with phenylbutazone, presumably resulting from insufficient control of postoperative pain. Although treatment of large colon impaction in the postoperative period typically is uncomplicated, onset of cecal impaction is fatal in many cases because of the difficulty in recognizing horses that have cecal dysfunction. Therefore one should pay close attention to fecal production and optimal analgesic treatment in any horse following an orthopedic procedure.[62] Other painful procedures, including ophthalmologic procedures, also likely place horses at risk of developing ileus of the large intestine.

13.15.3 Large Intestinal Simple Obstruction

Simple obstructions of the large intestine such as impaction tend to have a more gradual onset than those of the small intestine, although horses may become acutely and severely painful with some forms of colon displacement. In fact, some of these cases mimic and may progress toward large colon volvulus. Medical therapy is frequently successful in correcting large colon impactions. However, cecal impactions present much more of a dilemma because of the greater propensity of this organ to rupture, the relative difficulty of surgically manipulating the cecum, and the onset of cecal dysfunction that may prevent the cecum from emptying following surgical resolution of impaction.

13.15.3.1 CECAL IMPACTION

Cecal impaction may be divided into two syndromes: primary cecal impactions that result from excessive accumulation of ingesta in the cecum and secondary cecal impactions that develop while a horse is being treated for a separate problem.[79,80] Although primary impactions typically consist of impacted, relatively dry fecal material and secondary cecal impactions tend to have fluid contents, considerable overlap exists between the two syndromes, and one must approach each case carefully. In horses with primary cecal impactions, onset of abdominal pain occurs over a number of days, reminiscent of the development of a large colon impaction. One should differentiate cecal impactions from large colon impactions on the basis of rectal palpation findings. Cecal impactions have a propensity to rupture before the development of severe abdominal pain or systemic deterioration and therefore must be monitored closely.[79] Secondary cecal impactions typically develop following unrelated surgical procedures that result in postoperative pain (particularly orthopedic surgeries). Secondary cecal impactions may be even more difficult to detect because one may attribute postoperative depression and decreased fecal output to the operative procedure rather than to colic. By the time horses with secondary cecal impactions show noticeable signs of colic, the cecum may be close to rupture. In many cases, no signs of impending rupture are evident.[80] Therefore all horses that undergo surgeries in which considerable postoperative pain may develop should have feed intake and manure production closely monitored. A recent study indicated that horses that produce fewer than three piles of manure daily in the postoperative period are at risk of developing a large intestinal impaction. Furthermore, horses that underwent prolonged (>1 hour) orthopedic surgery that received inadequate treatment with phenylbutazone were at considerable risk of reduced postoperative fecal output.[62] These results are in contrast to statements indicating that NSAIDs may place horses at risk of impaction, statements that appear to be based largely on clinical impressions rather than on risk analysis.[80]

The diagnosis of primary cecal impaction is based on palpation of a firm, impacted cecum per rectum. In some cases, cecal impactions may be difficult to differentiate from large colon impactions. However, careful palpation reveals the inability to move the hand completely dorsal to the impacted viscus because of the 929
attachment of the cecum to the dorsal body wall. Treatment for horses with primary cecal impactions may 930
include initial medical therapy, including aggressive administration of intravenous fluids and judicious use of analgesics.[80] However, if the cecum is distended grossly or if medical therapy hasno effect within a reasonable period of time, surgical evacuation of the cecum via a typhlotomy is indicated.[79] In addition, performing an ileocolostomy to bypass the cecum is advisable, because postoperative cecal motility dysfunction with recurrence of the impaction is common.[46,81]

In horses that develop secondary cecal impactions, diagnosis is based on palpation of a greatly distended cecum filled with semifluid intestinal contents. The nature of the contents likely is related to the more rapid progression of this disease compared with primary cecal impaction. One should not delay surgery because of the risk of cecal rupture.[82] However, if the cecum appears healthy following typhlotomy and evacuation, bypass of the cecum is not as critical as it is for primary impactions as long as one can control the inciting cause of the impaction (such as orthopedic pain).

The prognosis is guarded for surgical treatment of all cecal impactions because of the potential for the cecum to rupture during prolonged medical treatment or during surgical manipulation, the possibility of abdominal contamination during surgery, and the extensive surgical procedures required. In a recent report, seven of nine horses for which cecal impaction was treated by typhlotomy and ileocolostomy or jejunocolostomy lived long

term.[46] However, a separate report indicated that all horses with cecal impaction following another disease process had cecal rupture without any signs of impending rupture.[80]

13.15.3.2

LARGE COLON IMPACTION

Ingesta impactions of the large colon occur at sites of anatomic reductions in luminal diameter, particularly the pelvic flexure and the right dorsal colon.[83] Although a number of risk factors have been reported, most have not been proved. However, a sudden restriction in exercise associated with musculoskeletal injury appears frequently to be associated with onset of impaction.[84] Another consideration is equine feeding regimens, which usually entail twice daily feeding of concentrate. Such regimens are associated with large fluxes of fluid into and out of the colon, associated with readily fermentable carbohydrate in the colon and subsequent increases in serum aldosterone, respectively. One may prevent these fluid fluxes, which may cause dehydration of ingesta during aldosterone-stimulated net fluid flux out of the colon, with frequent small feedings.[32]

Amitraz, an acaricide associated with clinical cases of colon impaction, can induce impaction of the ascending colon.[85,86] This effect may provide some clues as to the pathogenesis of large colon impaction. In particular, amitraz appears to alter pelvic flexure pacemaker activity, resulting in uncoordinated motility patterns between the left ventral and left dorsal colon and excessive retention of ingesta. Absorption of water from the ingesta increases with retention time, dehydrates the contents of the colon, and results in impaction. Conceivably, parasite migration in the region of a pacemaker may have a similar action.[87] Other factors implicated in large colon impaction include limited exercise, poor dentition, coarse roughage, or dehydration.

Clinical signs of large colon impaction include slow onset of mild to moderate colic. Fecal production decreases, and the feces are often hard, dry, and mucus-covered because of delayed transit time. The heart rate may be elevated mildly during episodes of pain but is often normal. Signs of abdominal pain are typically well controlled with administration of analgesics but become increasingly more severe and refractory if the impaction does not resolve. The diagnosis is based on palpation of a firm mass in the large colon per rectum. However, one may underestimate the extent of the impaction by rectal palpation alone because much of the colon is out of reach. Adjacent colon may be distended if the impaction has resulted in complete obstruction. One should attempt initial medical treatment. Administration of analgesics (e.g., flunixin meglumine at 0.5 to 1.1 mg/kg intravenously every 8 to 12 hours; butorphanol at 0.04 to 0.1 mg/kg intramuscularly every 4 to 6 hours; or xylazine at 0.3 to 0.5 mg/kg intravenously as needed) controls intermittent abdominal pain. Administration of oral laxatives such as mineral oil (2 to 4 L by nasogastric tube every 12 to 24 hours) and the anionic surfactant dioctyl sodium sulfosuccinate (6 to 12 g/500 kg diluted in 2 to 4 L of water by nasogastric tube every 12 to 24 hours) are used commonly to soften the impaction. Saline cathartics such as magnesium sulfate (0.1 mg/kg in 2 to 4 L by nasogastric tube) also may be useful. One should not permit access to feed. For impactions that persist, one should institute aggressive oral and intravenous fluid therapy (2 to 4 times the maintenance fluid requirement). If the impaction remains unresolved, the horse becomes uncontrollably painful, or extensive gas distention of the colon occurs, surgery is indicated. In addition, one can monitor abdominal fluid serially to determine the onset of intestinal compromise.[83] At surgery, one evacuates the contents of the colon via a pelvic flexure enterotomy. The prognosis is good for those horses in which impactions resolve medically (95% long-term survival in one study) and fair in horses that require surgical intervention (58% long-term survival in the same study).[84]

13.15.3.3 ENTEROLITHS

Enteroliths are mineralized masses typically composed of magnesium ammonium phosphate (struvite).[88] 930
However, magnesium vivianite also has been identified in enteroliths, along with variable quantities of 931
sodium, sulfur, potassium, and calcium. The formation of magnesium-based minerals is puzzling because of the relative abundance of calcium in colonic fluids, which would favor the formation of calcium phosphates (apatite) rather than struvite.[89] However, elevated dietary intake of magnesium and protein may play a role. Many horses that develop enteroliths are located in California and are fed a diet consisting mainly of alfalfa hay. Analysis of this hay has revealed a concentration of magnesium approximately 6 times the daily requirements of the horse.[90] Furthermore, the high protein concentration in alfalfa hay may contribute to calculi formation by increasing the ammonia nitrogen load in the large intestine. Enteroliths most commonly form around a nucleus of silicon dioxide (a flintlike stone), but nidi have included ingested nails, rope, and hair.[88] Enteroliths usually are found in the right dorsal and transverse colons.[90] Although enterolithiasis has a wide geographic distribution, horses in California have the highest incidence. In one California study, horses with enterolithiasis represented 28% of the surgical colic population, and Arabians, Morgans, American Saddlebreds, and donkeys were at greatest risk of this disease.[91] In a study of enterolithiasis in Texas, risk factors also included feeding of alfalfa hay and Arabian breed. However, in that study, miniature horses were also at risk.[92] Horses with enteroliths are rarely under 4 years old,[90] although an enterolith in an 11-month-old miniature horse has been reported recently.[93]

Enterolithiasis is characterized by episodic, mild to moderate, intermittent abdominal pain.[90] Progressive anorexia and depression may develop. The amount of pain depends on the degree of obstruction and amount of distention. Partial luminal obstruction allows the passage of scant, pasty feces. Heart rate varies and depends on the degree of pain. In some cases, an enterolith is forced into the small colon, where it causes acute small colon obstruction. One may diagnose enteroliths by abdominal radiography or at surgery. On rare occasions, one may palpate an enterolith per rectum, particularly if it is present in the distal small colon.

In general, these cases require surgery, although enteroliths being retrieved per rectum have been reported. In fact in one study, 14% of horses presented for treatment of enterolithiasis had a history of passing an enterolith in the feces. However, enteroliths typically are located in the right dorsal colon, transverse colon, or small colon. At surgery, one gently pushes the enterolith toward a pelvic flexure enterotomy, but removal frequently requires a separate right dorsal colon enterotomy to prevent rupture of the colon. Following removal of an enterolith, one must conduct further exploration to determine if other enteroliths are present. Solitary enteroliths are usually round, whereas multiple enteroliths have flat sides. The prognosis is good (92% 1-year survival in one study of 900 cases), unless the colon ruptures during removal of an enterolith. In one recent study, rupture occurred in 15% of cases.[91]

13.15.3.4 SAND IMPACTIONS OF THE LARGE COLON

Sand impactions are common in horses with access to sandy soils, particularly horses eating feed placed on the ground. Some horses, especially foals, deliberately eat sand. Fine sand tends to accumulate in the ventral colon, whereas coarse sand may accumulate in the dorsal colon.[94,95] However, individual differences in colonic function may contribute to accumulation of sand, because some horses can clear consumed sand, whereas others cannot. Distention from the impaction itself, or gas proximal to the impaction, causes

abdominal pain. In addition, sand may trigger diarrhea, presumably because of irritation of the colonic mucosa.[96] In horses with sand impactions, clinical signs are similar to those of horses with large colon impactions.[94] One may find sand in the feces, and auscultation of the ventral abdomen may reveal sounds of sand moving within the large colon.[97] However, unlike sand-induced diarrhea, one may not hear sand impactions easily because of the lack of colonic motility. To determine the presence of fecal sand, one places several fecal balls in a rectal palpation sleeve or other container, which subsequently is filled with water. If sand is present, it accumulates at the bottom of the container. In addition, one may detect mineral opacity within the colon on abdominal radiographs, particularly in foals, ponies, and small horses. Abdominal paracentesis typically yields normal fluid and poses some risk because large quantities of sand in the ventral colon make inadvertent perforation of the colon more likely.[95] Peritoneal fluid is often normal but may have an elevated protein concentration.

Initially, medical therapy is warranted. Administration of psyllium hydrophilic mucilloid (0.25 to 0.5 kg/500 kg in 4 to 8 L of water by stomach tube) may facilitate passage of sand. One should administer the solution rapidly because it will form a viscous gel. An alternative method of administration is to mix psyllium with 2 L of mineral oil, which will not form a gel and can be pumped through a nasogastric tube easily. One then pumps 2 to 4 L of water through the tube. The psyllium separates from the oil phase and mixes with the water, forming a gel within the gastrointestinal tract. Psyllium is thought to act by stimulating motility or by agglutinating the sand. However, a recent experimental study failed to show a benefit of this treatment for clearing sand from the colons of otherwise normal horses.[98] If a severe impaction is present, one should not 931
give the psyllium until softening the impaction by administrating intravenous or oral fluids and other laxatives. 932
Perforation is a potential complication in horses with sand impactions because the sand stretches and irritates the intestinal wall and causes inflammation. Therefore if colic becomes intractable, one should perform surgical evacuation of the large colon. The prognosis is generally good.[94,95]

13.15.3.5 LARGE COLON DISPLACEMENT

Displacement of the ascending colon is a common cause of large intestinal obstruction. The ascending colon is freely movable except for the right dorsal and ventral colons. Contact with adjacent viscera and the abdominal wall tends to inhibit movement of the ascending colon from a normal position; however, accumulation of gas and fluid or ingesta may cause the colon to migrate.[99] Feeding practices, including feeding of large concentrate meals, likely plays a role in initiating displacement of the large colon. Large concentrate meals increase the rate of passage of ingesta, allowing a greater percentage of soluble carbohydrates to reach the large intestine,[31] which in turn increases the rate of fermentation and the amount of gas and volatile fatty acids produced. The production of large amounts of volatile fatty acids stimulates the secretion of large volumes of fluid into the colon.[100] The association between feeding concentrate and development of displacements of the large colon is illustrated by studies indicating that ascending colon displacement is more prevalent in horses fed a high-concentrate, low-roughage diet.[101] Abnormal motility patterns of the ascending colon also have been suggested to contribute to the development of colonic displacement. Feeding stimulates colonic motility via the gastrocolic reflex, but large meals may alter normal motility patterns and concurrently allow rapid accumulation of gas and fluid from fermentation.[31,102] Migration of parasite larvae (strongyles) through the intestinal wall also has been shown to alter colonic motility patterns. Other experimental studies also have shown that *Strongylus vulgaris* infection results in reduced blood flow to segments of the large intestine without necessarily causing infarction. Electric activity of the colon and cecocolic junction increases after

infection with *S. vulgaris* and cyathostome larvae, probably reflecting a direct effect of migration through the intestine and an early response to reduced blood flow.[103]

Displacements of the ascending colon generally are divided into three types: left dorsal displacement, right dorsal displacement, and retroflexion. Left dorsal displacement is characterized by entrapment of the ascending colon in the renosplenic space. The colon often is twisted 180 degrees such that the left ventral colon is situated in a dorsal position relative to the left dorsal colon. The entrapped portion may be only the pelvic flexure or may involve a large portion of the ascending colon, with the pelvic flexure situated near the diaphragm. The colon may become entrapped by migrating dorsally between the left abdominal wall and the spleen or may migrate in a caudodorsal direction over the nephrosplenic ligament. Occasionally, one can palpate the ascending colon between the spleen and abdominal wall, lending support to the first mechanism of displacement. Gastric distention is thought to predispose horses to left dorsal displacement of the ascending colon by displacing the spleen medially, allowing the colon room to migrate along the abdominal wall. Right dorsal displacement begins by movement of the colon cranially, medial (medial flexion) or lateral (lateral flexion) to the cecum. According to one author, the proportion of right dorsal displacements with medial versus lateral flexion is approximately 1:15.[104] In either case the pelvic flexure ends up adjacent to the diaphragm. Retroflexion of the ascending colon occurs by movement of the pelvic flexure cranially without movement of the sternal or diaphragmatic flexures.

Displacement of the ascending colon partially obstructs the lumen, resulting in accumulation of gas or ingesta and causing distention. Secretion of fluid in response may exacerbate the distention.[105] Tension and stretch of the visceral wall is an important source of the pain associated with colonic displacement. Tension on mesenteric attachments and the root of the mesentery by the enlarged colon also may cause pain.[99] Ischemia rarely is associated with nonstrangulating displacement of the colon. However, vascular congestion and edema often occur in the displaced segments of colon, resulting from increased hydrostatic pressure from reduced venous outflow. Morphologic damage to the tissues is usually minor.

Clinically, displacement of the ascending colon is characterized by intermittent signs of mild to moderate abdominal pain of acute onset. However, one also may note an insidious onset of colic.[104] One may note dehydration if the duration of the displacement is prolonged. The heart rate may be elevated during periods of abdominal pain but is often normal. Abdominal distention may be present if the colon is enlarged by gas, fluid, or ingesta. Fecal production is reduced because progressive motility of the large intestine is absent. One often diagnoses left dorsal displacements by palpation per rectum. One can feel the left ventral colon in a dorsal position; it often is filled with gas. One can trace the ascending colon to the nephrosplenic space, and the spleen may be displaced medially. Alternatively, one can reach a tentative diagnosis using abdominal ultrasonography. The spleen is visible on the left side of the abdomen, but the gas-distended bowel obscures the left kidney. Evaluation of this technique indicates that false positives occur in few instances, although false 932
negatives occasionally may occur.[106] A definitive diagnosis therefore may require surgery. Right dorsal 933
displacements are characterized by the presence of the distended ventral colon running across the pelvic inlet and may be felt between the cecum and the body wall if a lateral flexion is present. The pelvic flexure is usually not palpable. Retroflexion of the ascending colon may produce a palpable kink in the colon. If the displaced colons are not distended by gas in the instance of right dorsal displacement and retroflexion, the ascending colon may not be palpable and is conspicuous by its absence from a normal position. Peritoneal fluid may increase in amount, but the color, protein concentration, and white blood cell count are usually normal. However, as the displaced segment becomes edematous, fluid leaking through the serosa into the peritoneal fluid increases the protein concentration.

Surgical correction of colon displacement is the most effective means of resolving this disorder. However, nonsurgical intervention has been successful in select cases of nephrosplenic entrapment of the large colon.[106–108] Before attempting such manipulations, the clinician must be certain of a diagnosis. One anesthetizes the horse and places it in right lateral recumbency, rotates the horse up to dorsal recumbency, rocking it back and forth for 5 to 10 minutes, and then rolls the horse down into left lateral recumbency.[109] One should palpate the nephrosplenic space per rectum to determine whether the entrapment has been relieved before recovering the horse from anesthesia. One may administer phenylephrine (3–6 μg/kg/min over 15 minutes) to decrease the size of the spleen.[110] More recently, phenylephrine has been used successfully with 30 to 45 minutes of exercise to reduce nephrosplenic entrapments in four of six horses.[26] The authors suggested that the technique be used on horses with mild to moderate colonic distention, particularly when financial constraints are severe. A number of cases occur in which nonsurgical interventions do not correct the problem and others in which nonsurgical manipulations correct the entrapment but result in large colon volvulus or displacement.[111] One should take horses in such condition to surgery promptly. The prognosis for horses with large colon displacement is good. In one study on horses with nephrosplenic entrapment of the large colon, survival exceeded 90%.[108]

13.15.3.6 FOREIGN BODY AND FECALITH OBSTRUCTION

The horse, particularly young horses, may ingest foreign material that can cause obstruction, such as bedding, rope, plastic, fence material, and feedbags. These foreign bodies may result in impaction with ingesta and distention of the intestine, typically in the transverse or descending colon. Young horses usually are affected. In one study the obstructing mass could be palpated per rectum in three of six horses.[112]

Figure 13.15-4 Intramural hematoma of unknown origin in the small colon of a horse taken to surgery for persistent signs of colic. The lack of a complete physical obstruction suggested a functional obstruction at the site of the hematoma.

Fecaliths are common in ponies, miniature horses, and foals.[113] Older horses with poor dentition also may be predisposed to fecaliths because of the inability to masticate fibrous feed material fully. Fecaliths commonly cause obstruction in the descending colon and may cause tenesmus.[112] Other clinical signs are similar to those of enterolithiasis. Abdominal radiography may be useful in smaller patients to identify the obstruction, especially if gas distention around the foreign body or fecalith provides contrast. The horse usually requires surgical treatment.

13.15.3.7 MURAL MASSES AND STRICTURES

Mural masses such as abscesses, tumors (adenocarcinoma, lymphosarcoma), granulomata, and hematomas (Figure 13.15-4) can cause luminal obstruction and impaction, typically in older horses. Impaction may result from obstruction of the lumen or impaired motility in the segment of intestine with the mass. Abscesses may originate from the lumen of the intestine or may extend from the mesentery or mesenteric lymph nodes. Intramural hematomas form most commonly in the descending colon and cause acute abdominal pain.[114] Once the acute pain from the hematoma subsides, impaction proximal to the hematoma develops because of impaired motility through the affected portion of the colon. Trauma, ulceration of the mucosa, and parasitic damage are speculated causes of intramural hematomas.[114,115] Stricture of the large intestine occurs when fibrous tissue forms in a circular pattern around or within the intestine, reducing the luminal diameter and the 933
ability of the wall to stretch. Strictures may be congenital or may follow peritonitis, previous abdominal 934
surgery, or inflammatory bowel disease. In a report of 11 horses with inflammatory bowel disease, 6 horses had strictures, four of which were in the small intestine and two of which were in the large colon.[116]

Clinical signs vary according to the degree of luminal obstruction. Partial obstruction and impaction tend to produce mild to moderate abdominal pain of insidious onset. Mural hematomas tend to produce signs of acute abdominal pain.[114,115] Per rectal palpation of the abdomen may reveal the presence of a mass or simply the impacted segment but not the mass itself. One may note fever, weight loss, and anorexia if an abscess or tumor is the cause. An elevated white blood cell count; hyperfibrinogenemia; hyperglobulinemia; or normocytic, normochromic anemia may occur with abscesses or tumors. Peritoneal fluid may reflect the cause of the mass. Tumor cells may occur infrequently. One may note evidence of inflammation with bacteria if the cause of colic is an abscess or granuloma, in which case one should culture the fluid. Hematomas may cause hemorrhage into the peritoneal fluid. Treatment usually requires surgical resection of the mass. One may treat abscesses with appropriate antibiotics if the impaction can be resolved medically with oral or intravenous analgesics and laxatives. *Streptococcus* spp, *Actinomyces pyogenes, Corynebacterium pseudotuberculosis, Rhodococcus equi,* anaerobic bacteria, and gram-negative enteric organisms commonly are involved in abscesses.

13.15.3.8 ATRESIA COLI

Atresia of a segment of the colon is a rare congenital abnormality in horses. The heritability and causes of the condition are unknown. One potential mechanism for development of the lesion is intestinal ischemia during fetal life, which results in necrosis of a segment of intestine. Clinical signs include a failure to pass meconium and colic within the first 12 to 24 hours of life. Secondary abdominal distention results from complete intestinal obstruction, and abdominal radiographs may reveal gas-distended colon. One makes the diagnosis at surgery. Any portion of the colon may be absent, but the distal segment of the large colon or the proximal small colon usually is affected most severely. If sufficient tissue is present, one may attempt anastomosis to

the proximal blind end of the colon.[117] The prognosis depends on which segment of the colon is absent but is usually poor because of an absence of distal colon.

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13.16

13.16—Neoplasia of the Alimentary Tract

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Neoplasia in the alimentary tract of the horse is uncommon.[1] Primary and metastatic neoplasia can affect multiple locations within the oral cavity and gastrointestinal tract (Boxes 13.16-1 and 13.16-2). Neoplasia is not limited to older horses. The average age of horses with squamous cell carcinoma is 8.6 to 14.6 years.[2,3] The alimentary form

of lymphoma occurs most commonly in horses less than 5 years of age.[4] Identification of benign versus malignant tumors is imperative to justify treatment and predict survival.

13.16.1 Clinical Signs

Clinical signs associated with alimentary neoplasia are related to the tumor location. Clinical signs of oral neoplasia can include enlargement or ulceration of the mandible or maxilla.[48] Neoplasia of the tongue results in weight loss, quidding, prepharyngeal dysphagia, halitosis, and nasal discharge containing feed material if the oropharynx is involved.[6–8] Tumors of the esophagus cause signs typical of esophageal dysphagia, ptyalism, choke, intermittent colic, fever, weight loss, and halitosis.[10,49,50] Gastric neoplasia can be associated with abnormal chewing and swallowing behavior, anorexia, weight loss, chronic intermittent colic, abdominal distention, and intermittent fever.[16] Abdominal neoplasia has been implicated in 4% of horses with intermittent or chronic colic.[51,52] Altered stool character, weight loss, ventral edema, and recurrent fever have been associated with intestinal neoplasia.[4] Acute signs of abdominal discomfort can occur in intestinal obstructions from malignant and benign neoplastic disease.

Paraneoplastic syndromes may occur in the horse. The most common syndromes are cancer cachexia, ectopic hormone production, anemia, leukocytosis, thrombocytopenia, hypergammaglobulinemia, fever, and neurologic abnormalities.[53] Horses with cancer cachexia have profound weight loss despite adequate consumption of calories.

13.16.2 Diagnostic Evaluation

Diagnosis of alimentary neoplasia can be challenging. Data collected from a complete blood count, biochemistry panel, and urinalysis may support the diagnosis of neoplasia but rarely confirms it. Normocytic normochromic anemia indicates chronic disease and is the most likely cause of anemia associated with neoplasia. Blood loss anemia (via gastrointestinal tract) and immune-mediated hemolytic anemia (lymphoma)[54] are less frequent causes of anemia associated with abdominal neoplasia. Peripheral eosinophilia has been reported in association with multisystemic eosinophilic, epitheliotropic disease with lymphoma.[14] Leukocytosis and hyperfibrinogenemia are common findings.

Serum chemistry can confirm hypoalbuminemia caused by inflammation of the bowel wall. Hyperglobulinemia can be characterized with serum electrophoresis, which is nonspecific and can reveal chronic inflammation. A few cases of lymphoma have been identified with monoclonal hypergammaglobulinemia.[55] Ectopic hormone production may result in hypercalcemia (calcium >14 mg/dl), which is associated with alimentary neoplasia such as lymphoma, multiple myeloma, carcinomata, and ameloblastoma.[2,56] Hypoglycemia (blood glucose <70 mg/dl) can occur with neoplasia of the pancreas or liver.[2]

Rectal examination may detect an abdominal mass, thickening of the intestinal wall, lymph node enlargement, or a gritty texture in horses with carcinomatosis.[2] Rectal biopsy can reveal lymphoma in some cases.[42] Fecal occult blood test is nonspecific for neoplastic disease but can reveal blood loss through the gastrointestinal tract. Occasionally, abdominocentesis can identify neoplasia if the tumor exfoliates cells into the abdomen. One can diagnose squamous cell carcinoma, adenocarcinoma, and mesothelioma from peritoneal fluid.[10,45,46,57] Characterization of peritoneal fluid as an inflammatory exudate or modified transudate without any neoplastic cells present is common. Cytologic analysis of peritoneal fluid samples collected by abdominocentesis accurately

predicted the presence of neoplasia in 11 of 25 cases in one study.[58] Cytologic examination of two or more peritoneal fluid samples increases the sensitivity of this test for detecting abdominal neoplasia. Measurement of peritoneal fluid glucose concentration and pH is valuable to differentiate inflammation in the peritoneum caused by neoplasia from bacterial peritonitis. Abdominal neoplasia typically is associated with peritoneal glucose concentrations similar to blood and pH higher than 7.3. d-xylose absorption tests can reveal malabsorptive diseases that include lymphoma.[42,59]

Immunoglobulin M deficiency is associated with lymphoma in some young adult horses, but the prevalence of immunoglobulin M deficiency in horses with lymphoma and the value of measuring serum immunoglobulin M concentrations for the diagnosis of lymphoma have not been evaluated.[60] DNA cell cycle analysis of suspect 937
neoplastic cells has been used to detect lymphoma in equine patients confirmed with the disease. This method of 938
evaluating fluid or tissues aspirates may increase the accuracy for diagnosing neoplasia in the future.[61]

13.16.2.1 BOX 13.16-1 NEOPLASIA OF THE ORAL CAVITY

13.16.2.1.1 Odontogenic Tumors (Originate From Dental Tissue)[5]

Ameloblastic odontoma

Ameloblastoma

Cementoma

Complex odontoma

Compound odontoma

Fibromyxoma

Odontoblastoma

13.16.2.1.2 Osteogenic Tumors (Originate >From Bone)[5]

Myxoma

Osteoma

Osteosarcoma

13.16.2.1.3 Soft Tissue Tumors[5]

Epulis

Fibrous dysplasia

Juvenile ossifying fibroma

Melanoma

Papilloma

Salivary adenocarcinoma

Sarcoid

Squamous cell carcinoma

13.16.2.1.4 ### Tongue

Lymphosarcoma[6]

Multiple myeloma[7]

Rhabdomyosarcoma[8]

Paraneoplastic bullous stomatitis[9]

A complete evaluation of the oral cavity may include using a full-mouth speculum, radiographs, and endoscopy of the pharynx. Evaluation of the esophagus and stomach with a 3-m endoscope can reveal intralumenal masses.[11] Pleuroscopy has been used to obtain biopsy samples of extralumenal masses surrounding the esophagus.[49] Contrast radiography can assist in the diagnosis of neoplasia within the wall or outside of the esophagus.[49,62] Ultrasonography of the stomach, small intestine, cecum, and large colon is useful in detecting intestinal wall thickness, abdominal masses, and excessive peritoneal fluid.[63] Identification of neoplasia in the liver, kidney or spleen may support metastasis to other parts of the gastrointestinal tract or lymph nodes. Laparoscopy and exploratory laparotomy often are required to obtain a final diagnosis.[64]

13.16.2.2 ## BOX 13.16-2 NEOPLASIA OF THE GASTROINTESTINAL TRACT

13.16.2.2.1 ### Esophagus

Squamous cell carcinoma[10,11]

13.16.2.2.2 ### Stomach

Gastric polyp[12]

Leiomyosarcoma[13]

Lymphoma (lymphosarcoma)[14,15]

Squamous cell carcinoma[10,16,17]

13.16.2.2.3 ### Small Intestine

Adenocarcinoma[18]

Adenomatous polyposis[19]

Ganglioneuroma[20]

Intestinal carcinoid[21]

Leiomyoma[22–24]

Leiomyosarcoma[25]

Lipoma[26]

Lymphoma (lymphosarcoma)[27]

Neurofibroma[28]

13.16.2.2.4 Cecum

Adenocarcinoma[29]

Intestinal myxosarcoma[30]

Stromal tumor[31]

13.16.2.2.5 Large Colon

Adenocarcinoma[32,33]

Lipomatosis[34]

Lymphoma (lymphosarcoma)[35]

Neurofibroma[36]

13.16.2.2.6 Small Colon

Leiomyoma[37,38]

Lipoma[26,39]

Lipomatosis[34]

13.16.2.2.7 Rectum

Leiomyosarcoma[40]

Lipoma[41]

Lymphoma (lymphosarcoma)[42]

Polyps[43]

13.16.2.2.8 Peritoneum

Disseminated leiomyomatosis[44]

Mesothelioma[45,46]

Omental fibrosarcoma[47]

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13.16.3 Specific Neoplasia

13.16.3.1 LYMPHOMA (LYMPHOSARCOMA)

Lymphoma is the most common neoplasia in the horse and has been divided into four categories. This section covers only the intestinal/alimentary form. In the past, lymphoma has been called lymphosarcoma, but the preferred term by oncologists is *lymphoma* because no benign form of this disease exists.[2] Lymphoma originates from lymphoid tissue and predominantly affects the small intestine and intestinal lymph nodes. Chronic weight loss from malabsorption, intermittent colic, and fever are the most common clinical findings.[27,65] Chronic diarrhea has been reported in some cases.[66] Paraneoplastic pruritus and alopecia have been identified in one case of diffuse lymphoma.[67] One generally does not note peripheral lymphadenopathy, but one may palpate enlargement of the intestinal lymph nodes on rectal examination.[4] Large colon resection for treatment of lymphoma in horses has increased short-term survival in two horses.[35] Chemotherapy in two mares that were pregnant extended their lives long enough to foal normally.[68] Long-term prognosis for intestinal lymphoma is poor.

13.16.3.2 SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma (SCC) is a malignant tumor of the gastrointestinal epithelium. SCC is the second most common neoplasia in the horse and is the most common oral neoplasia. However, the incidence of SCC is rare.[10,16,50] In the oral cavity SCC may affect the lips, tongue, hard palate, pharynx, and mucosa.[69,70] Treatment for SCC in the oral cavity may involve surgical resection, iridium-192 brachytherapy, 5-fluorouracil, or intralesional cisplatin.[5,71–73] The prognosis for survival is good if complete removal of the tumor is possible. Metastasis beyond the regional lymph nodes is rare for oral SCC.

Squamous cell carcinoma is the most common tumor of the stomach and esophagus[11,16] and can invade these areas and metastasize to the lymph nodes and lungs. Abnormal masses were palpated on rectal examination in four of five cases of gastric SCC.[16] Treatment by surgical resection is not possible in most cases and the horses die or are euthanized.[2]

13.16.3.3 ADENOCARCINOMA

Adenocarcinoma is a malignant tumor that can occur in the small intestine, cecum, and large colon.[18] The tumor arises from the glandular crypts of the gastrointestinal tract and has been reported in middle-aged and older horses. Metastasis to the lymph nodes, liver, and lungs can occur. Intestinal adenocarcinoma has been reported to metastasize to the bone and was diagnosed using nuclear scintigraphy following injection of technetium-99m hydroxymethylene diphosphate.[29,32] No reports of successful surgical resection have been published.

13.16.3.4 LEIOMYOSARCOMA

Leiomyosarcoma is a malignant tumor of the smooth muscle lining the gastrointestinal tract and has been reported in the stomach, small intestine, and rectum.[13,22,23,40,58] In one case report, gastroscopy could not identify the mural mass in the stomach that was found during exploratory surgery. Another report describes a favorable outcome for surgical resection of a leiomyosarcoma that was protruding from the anal sphincter in a 4-year-old Quarter Horse.[40] Prognosis for survival is favorable if surgical resection is possible.

13.16.3.5 LEIOMYOMA

Leiomyoma is a benign tumor of the smooth muscle of the gastrointestinal tract that can occur in the stomach, small intestine, and small colon.[37,38] Clinical signs are consistent with intestinal obstruction. Surgical resection and anastomosis of the affected portion of the intestine have been performed without complications.

13.16.3.6 LIPOMA

Lipoma is a benign tumor that occurs in older horses (10 to 26 years) and arises from mesenteric adipocytes. The tumor grows on a stalk that wraps around the intestine, causing a strangulating lesion manifested clinically by acute obstructive colic. Intestinal injury caused by pedunculated lipomata may occur in the small intestine, small colon, and rectum. Long-term survival with surgical resection and anastomosis of the affected segment has been reported to be 40% to 50%.[26,74]

13.16.3.7 ORAL NEOPLASIA

Oral cavity neoplasia may involve the dental tissue (odontogenic tumors), bone (osteogenic tumors), or soft tissues (see Box 13.16-1). Ameloblastoma occurs in horses greater than 10 years old and mainly affects the mandible. Ameloblastic odontoma affects younger horses and usually involves the maxilla. Both are benign but locally invasive. Radiographs may distinguish the difference between an ameloblastoma (radiolucent lesion) and ameloblastic odontoma (radiolucent lesion with partially mineralized density). The best treatment option is surgical resection and radiation therapy regardless of the type.[48]

Juvenile mandibular ossifying fibroma occurs in the rostral mandible of young horses between the ages of 2 months and 2 years. The fibroma may cause significant distortion of the bone. With early diagnosis and surgical excision of the mass, the horse has a good prognosis.[75]

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Melanomas, sarcoids, and oral papilloma occur on the mouth and lips. Melanomas rarely metastasize, but they commonly are found in the parotid salivary glands and lymph nodes. Sarcoids are the most common skin tumor that can involve the mouth. Ulcerations of the buccal mucosa are difficult to treat. Intralesional cisplatin, cryosurgery, radiation, and laser excision have been tried with limited success.[5] Equine papilloma virus is responsible for the common skin wart found on the lips and muzzle of young horses. These lesions are usually self-limiting but may be removed successfully by cryosurgery or excision.

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13.17

13.17—Peritonitis

Charles Dickinson

13.17.1

Structure and Function

A number of detailed and informative reviews are available describing the anatomy, physiology, and pathophysiology of the equine peritoneum.[1–5] The peritoneum consists of a single layer of mesothelial cells lining the peritoneal cavity and serosal surfaces of the intraabdominal viscera. The mesothelial lining of the diaphragm, abdominal walls, and pelvic cavity is termed *parietal peritoneum*. The visceral peritoneum includes

the serosal surfaces of the intraabdominal organs. The parietal and visceral portions of the peritoneum are contiguous with each other through the omentum, mesenteries, and ligaments. Caudally, the peritoneum reflects over the surfaces of the pelvic organs (portions of the urogenital tract and rectum), excluding them from the peritoneal space, and thus much of the pelvic cavity and contents are described as retroperitoneal. The peritoneal space communicates with the lumen of the uterus (and thus the external environment) via the fallopian tubes in females. In males the peritoneum forms a true blind sac. The vascular supply and nervous innervation of the visceral peritoneum are supplied by the splanchnic vessels and visceral autonomic nerves, respectively. Branches 941
of the intercostal, lumbar, and iliac arteries supply the parietal peritoneum, and the phrenic and intercostal nerves 942
provide nervous innervation. The clinical relevance is that inflammation of the parietal peritoneum is perceived as somatic pain, resulting in a splinted abdominal wall, pain on external palpation, and reluctance to move. Visceral pain is mediated by small type C sensory fibers, which are believed to be stimulated by bowel distention, smooth muscle spasms, tension on the mesentery, and ischemia.

The peritoneal lining functions as a semipermeable barrier to the diffusion of water and low-molecular weight solutes between the blood and the abdominal cavity.[1] The peritoneum secretes a serous fluid that lubricates the abdominal cavity, inhibits adhesion formation, and has minor antibacterial properties.[1,2] Macrophages, mast cells, mesothelial cells, and lymphocytes provide immune function within the peritoneum.[2,3] Peritoneal macrophages impart antibacterial activity via complement receptors, phagocytic activity, interaction with T lymphocytes, neutrophil chemotaxis, and fibroblast activation. The peritoneal surface maintains a high level of fibrinolytic activity through the production of plasminogen activators by mesothelial cells. This function, together with the lubricant properties of the peritoneal fluid, helps to maintain gliding surfaces within the peritoneum and prevent adhesion formation. In quadrupeds, peritoneal fluid produced by the mesothelium tends to move ventrally and cranially, aided largely by diaphragmatic movement. Peritoneal fluid, waste products, and foreign material (including bacteria) exit the peritoneal cavity to enter the lymphatic system through diffusely distributed subendothelial pores or via the large diaphragmatic stomata, depending on particle size. Large molecules and particles greater than approximately 40,000 MW (such as bacteria) exit through the diaphragmatic stomata and ultimately enter the thoracic duct.

Peritonitis is inflammation of the mesothelial lining of the peritoneal cavity and is characterized by desquamation and transformation of mesothelial cells; chemotaxis of neutrophils; release of several soluble mediators of inflammation; exudation of serum, fibrin, and protein into the peritoneal cavity; and depression of fibrinolytic activity.

13.17.2 Etiopathogenisis

Peritonitis occurs in association with a variety of disorders that result in mechanical, chemical, or infectious insult to the peritoneal lining.[1–4] Any process resulting in disruption or irritation of the peritoneal lining, inflammation or infection of abdominal organs, or compromise of the intestinal wall can result in peritonitis (Box 13.17-1). Common mechanical injuries include blunt or perforating trauma to the abdominal wall, breeding and foaling accidents, and abdominal surgery. A variety of traumatic insults of iatrogenic origin can cause 942
peritonitis, such as abdominocentesis, enterocentesis, splenic puncture, bowel trocharization, liver biopsy, 943
uterine biopsy, castration, and rectal tear. Chemical insults of endogenous origin include blood, urine, pancreatic enzymes, bile, gastric juice, chyme, and chyle. Talc, contrast agents, antibiotics, and lavage solutions are additional examples of chemical insults. Traumatic events often involve bacterial contamination at the time of injury and mechanical and chemical injuries can become infected secondarily. The most common manifestation of peritonitis is acute, diffuse, septic peritonitis following inflammation, vascular insult, perforation, or surgical manipulation (enterotomy, resection, anastomosis) of the gastrointestinal tract. The septic process in such cases

involves mixed bacteria of gastrointestinal origin. Penetrating abdominal wounds also result in mixed infections. Less commonly, singular bacterial forms gain access to the peritoneum though hematogenous spread, extension from a contiguous organ, or through the female genital tract. Primary, monomicrobial infections involving *Streptococcus equi, S. zooepidemicus, Actinobacillus equuli,*[6] *Rhodococcus equi,* and *Corynebacterium pseudotuberculosis* are examples. Septicemia, septic omphalophlebitis, ascending urinary tract infections, and uterine infections are additional examples. Parasites also play a role in peritonitis. Verminous arteritis caused by strongylosis can lead to vascular damage (thromboembolism, infarction) to the intestine. The activities of strongyles, ascarids, and tapeworms can result in perforation of the bowel and damage to other abdominal organs. Peritonitis has been associated with viral infections, including influenza, viral arteritis, and African horse sickness virus. Neoplastic diseases also can result in peritoneal inflammation. Although a number of potential causes of peritonitis exist, sepsis is a common and serious complication, and the identification and control of bacterial sepsis is critical for a successful outcome.

13.17.2.1 BOX 13.17-1 CAUSES OF PERITONITIS IN FOALS AND HORSES

13.17.2.1.1 Foals

Meconium impaction

Ruptured bladder

Urachal infection

Gastric/duodenal ulcer perforation

Septicemia

Enteritis

Intestinal vascular accident

Ascarid impaction

Intussusception

Streptococcus abscess

Rhodococcus equi abscess

Neoplasia

13.17.2.1.2 Adults

13.17.2.1.2.1 Iatrogenic

Rectal tear

Enterotomy

Trocharization

Enterocentesis

Castration

Vaginal perforation

13.17.2.1.2.2 Trauma

Foreign body penetration

Gunshot

Capture dart

Fence post

Uterine/vaginal perforation during foaling

Vaginal perforation during breeding

Splenic tear

13.17.2.1.2.3 Vascular accident

Verminous arteritis

Intestinal strangulation

Nonstrangulating infarction

Thromboembolism

Ruptured uterine artery

13.17.2.1.2.4 Bowel contamination

Rupture of stomach, cecum, or colon

Strangulating intestinal obstruction

Nonstrangulating intestinal obstruction

Foreign body perforation

Anastomosis leakage/dehiscence

Intestinal mural abscess/neoplasia

Perforating colitis

13.17.2.1.2.5

Other

Mesenteric abscess

Pyometra

Cholelithiasis

Pancreatitis

Retroperitoneal abscess

Neoplasia

Bowel leakage (as well as external trauma) results in contamination of the peritoneum with large numbers of many types of bacteria. The intestinal tract contains a mixed population of bacteria, and the quantity of bacteria and prevalence of anaerobic species increase in the distal segments.[1–7] There are approximately 1×10^9 anaerobic and 1×10^5 aerobic bacteria per milliliter of cecal and colonic fluid, thus the potential for bacterial contamination of the peritoneum is great. High mortality is associated with contamination from the lower bowel because of the large numbers of bacteria present.[8] Hirsch and Jang[9] reported isolation of an infective agent from equine peritoneal fluid in approximately 25% of attempts. Obligate anaerobic bacteria were cultured most frequently, followed by members of the Enterobacteriaceae family *(Escherichia coli)*. Penicillin-resistant *Bacteroides fragilis* was isolated from 10% to 20% of cases. In another study in which bacteria were identified in equine abdominal fluid by cytologic examination or culture, *E. coli* was the organism most commonly isolated.[10] In human beings and laboratory animals the well-established fact is that despite the variety of organisms initially introduced subsequent to these events, established infections are characterized by only a few types of bacteria, which are often gram-negative aerobes and anaerobic bacteria.[2] This selectivity occurs through the processes of selective reduction of bacterial populations and bacterial synergism. A well-known example of synergism in human beings and laboratory animals is peritonitis involving *E. coli* and *B. fragilus*. The presence of each organism is beneficial to the survival of the other, and each is important in the overall pathogenesis of the disease. *E. coli* is associated with septicemia and early mortality, whereas *B. fragilis* infection tends to result in chronic abscessation with delayed morbidity and mortality. Some evidence suggests that in horses, in addition to coliforms and anaerobes, streptococci and perhaps *C. psuedotuberculosis* may survive selective reduction and participate in synergistic infection following polymicrobial contamination.

Biologic events resulting from contamination of the abdomen or injury to the mesothelial cells have been described[1–4] and include release of catecholamines, histamine, and serotonin from peritoneal mast cells; vasodilation and hyperemia; increase in peritoneal vascular permeability; secretion of protein-rich fluid into the peritoneum; transformation of mesothelial cells into macrophages; and influx of polymorphonuclear cells, humoral opsonins, natural antibodies, and serum complement into the peritoneal cavity. Additionally, depression of the peritoneal fibrinolytic activity, fibrin deposits on the peritoneal surface, and sympathetic-mediated ileus of the gastrointestinal tract can occur.

These processes benefit the animal by confining contamination and infection, and indeed, with clean, minimally invasive procedures such as enterocentesis or trocharization, this is effective. However, with greater severity of peritoneal contamination or irritation, these processes are magnified and become deleterious, resulting in problems such as hypovolemia, hypoproteinemia, ileus with resultant bowel distention, ischemia of the bowel

wall with subsequent absorption of bacteria and toxins, and ultimately adhesion and abscess formation. Additionally, systemic responses to bacterial toxins, particularly lipopolysaccharide,[11,12] can compromise the metabolic condition of the patient further. Equine peritoneal macrophages release several mediators when exposed to bacterial lipopolysaccharide,[13] undoubtedly an important component of septic peritonitis.

Pathologic description of peritonitis includes origin (primary or secondary), onset (peracute, acute, chronic), distribution (localized versus diffuse), and presence of bacteria (septic versus nonseptic).[3,4] Clinically, viewing 943

944 the pathogenesis of peritonitis as a series of stages, as reviewed and described by Trent, is useful.[2] The contamination stage, lasting 3 to 6 hours, involves introduction of bacteria into the peritoneum and initiation of the acute inflammatory response previously described. If the organisms are not eliminated and infection is established, the process evolves to the stage of acute diffuse peritonitis. Although the overall movement of contaminants is toward the diaphragmatic stomata and into the thoracic duct, the nature of the peritoneal circulation is such that regardless of the location of the initial contamination, bacteria spread throughout the peritoneum within several hours. The stage of acute diffuse peritonitis lasts up to 5 days. The inflammatory response persists and escalates with continued exudation of proteinaceous fluid and influx of inflammatory cells. Offending organisms are delivered to the lymphatic system and may be eliminated by the immune system. Organisms, however, may gain access to the systemic circulation in sufficient numbers to result in clinically relevant septicemia. In human beings and laboratory animals having undergone polymicrobial contamination of the peritoneum, the organisms causing septicemia at this stage are usually coliforms, *E. coli* in particular. This stage of the disease process has the highest mortality because of the effects of severe peritoneal inflammation, endotoxemia, and septicemia. If the animal survives this stage but fails to eliminate the infection in the peritoneal cavity, the disease enters a transitional phase referred to as the acute adhesive (or localizing) stage. This stage occupies a time frame of perhaps 4 to 10 days after the initial insult. Neutrophils are still active, macrophages are increasing in numbers, and fibrin aggregates are being organized or lysed. In human beings and laboratory animals, selective reduction and synergism continue such that anaerobes and gram-negative aerobes predominate. If infection persists beyond this point, organization of fibrin proceeds and organisms become isolated from host defenses. At this point, the disease process enters the stage of chronic abscessation. This stage can begin as early as 8 days after inoculation and persists indefinitely.

13.17.3 Clinical Signs

Clinical signs of peritonitis depend on the primary disease process, the duration of the problem, and the extent of peritoneal inflammation. Localized peritonitis may have few or no systemic manifestations, whereas severe localized or generalized peritonitis often is accompanied by severe toxemia or septicemia or both. Septic peritonitis usually causes more severe clinical signs because of the inflammatory mediators released in response to bacterial toxins and because of the presence of endotoxin when gram-negative organisms are involved. Most clinical signs are nonspecific and include fever, depression, inappetance, decreased borborygmi, and dehydration. Additional signs, reported in 30 horses (ages 2 months to 16 years) with peritonitis, were colic, ileus, weight loss, and diarrhea.[14]

Horses with peracute peritonitis, as occurs with rupture of the bowel or rectal tear, have severe toxemia, weakness, depression or severe colic, tachycardia, tachypnea, and circulatory failure. Fever may not be present depending on the degree of shock. Typical clinical findings include sweating, pawing, muscle fasciculations, weak peripheral pulses, red to purple mucous membranes, prolonged capillary refill time, and decreased skin elasticity. Parietal pain, characterized by reluctance to move, splinting of the abdominal wall, and sensitivity to external abdominal pressure occur in some acute cases. Urination or defecation may be painful for the horse, and urine and fecal retention may be evident on rectal examination. Palpation of the abdomen externally may elicit

flinching, aversion movements, or groaning. With extensive abdominal fecal contamination, rectal examination may reveal a gritty feeling of the serosal and parietal surface of the peritoneum because of fibrin deposition and a dry texture of the peritoneum. In horses with more chronic peritonitis, rectal examination findings can include pain on palpation of fibrinous or fibrous adhesions, intestinal impaction or distention following ileus and dehydration, an abdominal mass (abscess or neoplasia), or an impression of bowel floating in fluid. In many cases, one can detect no abnormalities on rectal examination.

Horses with localized, subacute, or chronic peritonitis may have signs of chronic or intermittent colic, depression, anorexia, weight loss, intermittent fever, ventral edema, exercise intolerance, decreased or absent intestinal sounds, and mild dehydration. Heart rate and respiratory rate may be normal. Fecal output may be normal; however, horses with chronic diarrhea and weight loss have been reported.[14]

Foals with peritonitis usually exhibit signs of colic (acute or chronic) and are febrile, depressed, and inappetant. In some foals with primary peritonitis, pleural effusion occurs. In young foals, peritonitis can cause rapid metabolic deterioration, and determination and correction of the primary problem requires immediate attention. In older foals, peritonitis may occur insidiously, as occurs following *S. equi* or *R. equi* infections.

13.17.4 Clinicopathologic Findings

13.17.4.1 HEMATOLOGY AND SERUM CHEMISTRY

Clinicopathologic abnormalities vary depending on the time of onset and severity of peritonitis. Horses with 944
acute, septic peritonitis can have leukopenia, hemoconcentration, metabolic acidemia, azotemia, and 945
electrolyte imbalances reflective of systemic inflammation from endotoxemia and hypovolemia. Horses with peritonitis of a few days' duration may have leukocytosis and hyperfibrinogenemia. Plasma protein levels vary depending on the hydration status, degree of exudation into the peritoneum, and type of underlying problem. In chronic peritonitis, hyperproteinemia with hyperglobulinemia may be present.

Neonates with uroperitoneum caused by urinary bladder rupture or urachal disease tend to develop azotemia, hyponatremia, hypochloremia, hyperkalemia, and acidosis. Foals with peritonitis following septicemia, severe enterocolitis, severe meconium impaction, intussusception, small intestinal volvulus, gastric or duodenal rupture, or ascarid impactions usually have clinicopathologic findings reflective of systemic inflammation, such as inflammatory leukogram or leukopenia, hemoconcentration, and acidosis. Chronic abscessation, as occurs in foals with *R. equi* and streptococcal infections, results in clinicopathologic findings reflecting chronic inflammation (anemia, hyperfibrinogenemia, hyperglobulinemia).

13.17.4.2 PERITONEAL FLUID

Abnormalities in the composition of peritoneal fluid occur with peritoneal inflammation, and peritoneal fluid analysis is principal to the diagnosis of peritonitis. One collects peritoneal fluid through puncture of the abdomen on the ventral midline. One should clip and prepare an area aseptically. Usually, the lowest point of the abdomen, 5 to 10 cm caudal to the xiphoid cartilage, is prepared for puncture; although in some cases one may perform paracentesis more caudally, particularly when one suspects a specific area of sequestered fluid or abscessation. In addition, one may choose a site to the right of midline in an attempt to avoid the spleen. One can perform peritoneal puncture using a 1½-inch, 18-gauge needle or, following local anesthesia and a stab incision with a No. 15 scalpel blade, using a sterile cannula. One collects fluid by gravity flow and should collect fluid in a tube containing anticoagulant, preferably EDTA for cytologic examination, and in a sterile

tube without anticoagulant for visual inspection and, if desired, for culture. One should fill the EDTA tube to half its volume, because the EDTA will alter the refractive index of the fluid, resulting in a falsely elevated value for total solids when one collects only a small volume and tests it with a refractometer.

One should evaluate peritoneal fluid routinely as to color, turbidity, total protein, white blood cell (WBC) count and differential, and the presence of bacteria as determined by Gram stain. Normal peritoneal fluid is clear and straw-colored and does not coagulate spontaneously. Peritoneal fluid becomes turbid when increased numbers of white blood cells and concentration of protein are present. Pink or red fluid indicates free hemoglobin or hemorrhage. Blood introduced into the peritoneal fluid iatrogenically in some cases may be differentiated from blood from internal hemorrhage based on the presence of platelets and hematocrit. Fluid with iatrogenic blood contamination contains platelets, whereas fluid with blood following internal hemorrhage or diapedesis often does not have platelets. Blood contamination resulting from splenic puncture often results in the packed cell volume of the sample being greater than that of the blood. Large volumes of dark brown or green fluid with a fetid odor obtained from several sites strongly suggest bowel rupture, but one should perform cytologic examination for confirmation.

The distribution of polymorphonuclear and mononuclear cells varies widely, and one should interpret the results of cell counts and differentials as supporting a number of disorders rather than a specific diagnosis. Normal equine peritoneal fluid contains fewer than 5000 nucleated cells per microliter.[2,15]

WBC counts in acute peritonitis (>100,000/μL) are reported to be higher than those in chronic peritonitis (20,000 to 60,000/μL)[14–16]; however, this is not always the case, and the WBC count depends most on the cause of the peritonitis. The WBC level does not always correlate with severity of peritonitis or the prognosis. The peritoneal fluid WBC count can be greater than 100,000/μL following enterocentesis, with no clinical signs or problem.[17] Conversely, peritoneal WBC counts of fewer than 100,000/μL may be found in foals or horses with intraabdominal abscesses.[18] The peritoneal WBC count can increase to greater than 150,000/μL following celiotomy[19] and can be higher if an enterotomy is done. Postoperatively, the WBC count normally continues to decline and returns to near normal by 5 to 7 days. Failure of the WBC count to decrease suggests peritonitis resulting from a postoperative complication. Finally, peritoneal fluid WBC counts greater than 500,000/μL indicate severe focal or generalized peritoneal sepsis.

The distribution of polymorphonuclear and mononuclear cells varies in normal peritoneal fluid,[2,15] but polymorphonuclear cells usually predominate. With acute peritonitis, polymorphonuclear cells typically increase to a greater degree than mononuclear cells, but this depends on the cause. In horses that have bowel disease accompanied by endotoxemia, the number of peritoneal mononuclear cells increases, as does transformation of mesothelial cells to macrophages. In chronic cases, one easily may mistake transforming mesothelial cells for neoplastic cells, which can make diagnosis difficult, particularly when the presenting problem is compatible with a neoplastic process. In such cases, consultation with a clinical pathologist regarding cytologic findings is prudent. 945

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Normal peritoneal fluid protein concentration is less than 1.5 g/dl.[15] Protein levels between 1.5 g/dl and 2.5 g/dl can be difficult to interpret, but one should consider levels greater than 2.5 g/dl to be elevated abnormally. Fibrinogen concentration increases with inflammation, and levels greater than 10 mg/dl in the peritoneal fluid suggest that an acute inflammatory process is present.[20] Fibrinogen content will also increase from blood contamination.

The presence of free and phagocytosed bacteria in peritoneal fluid indicates generalized suppuration, abscessation, or compromised bowel. If one observes numerous microorganisms of mixed types free in the peritoneal fluid or if one observes plant material, massive bacterial contamination of the abdomen following bowel rupture likely has occurred. The presence of toxic or degenerate neutrophils and bacteria within polymorphonuclear cells helps to distinguish peritoneal fluid from intestinal contents in such cases. Enterocentesis yields discolored fluid containing mixed microorganisms and plant material and that is largely devoid of white blood cells. Bacterial contamination of a sample can occur during collection of the sample, and iatrogenic contamination of a sample can result in free and intracellular bacteria in peritoneal fluid, particularly if processing is delayed. In such cases the bacterial numbers are few and the neutrophils appear healthy. In some cases of gastrointestinal perforation the luminal material, inflammatory cells, and protein may be sequestered by the omentum and further contained by fibrinous adhesions. Abdominal fluid obtained via standard ventral paracentesis may have low cellularity and protein content but large numbers of mixed bacteria indicating bowel rupture.[5] Examples include gastric rupture along the greater curvature of the stomach between the omental layers (omental bursa) and perforated gastric or duodenal ulcers in foals. Correlating all cytologic findings with clinical and clinicopathologic findings is important for interpreting the results of peritoneal fluid cytologic examination.

Biochemical analysis of peritoneal fluid may be useful in detecting sepsis when cytologic examination and culture are negative or otherwise unavailable. In a prospective study by Van Hoogmoed, Rodger, Spier, et al., peritoneal fluid pH and glucose concentrations from horses with septic peritonitis were significantly lower than horses with nonseptic peritonitis and healthy horses.[21] Peritoneal fluid pH less than 7.3, glucose less than 30 mg/dl, and fibrinogen concentration greater than 200 mg/dl were considered highly predictive of septic peritonitis. Serum to peritoneal glucose concentration differences of greater than 50 mg/dl was considered the most diagnostically useful test for septic peritonitis in the study. Increased activities of alkaline phosphatase, lactic dehydrogenase, creatine kinase, aspartate aminotransferase, tumor necrosis factor, and interleukin-6 have been measured in the peritoneal fluid of horses with abdominal disorders, but the diagnostic and prognostic implications of the presence or absence of these enzymes and analytes is limited.[20–22]

One should submit peritoneal fluid samples in appropriate media (Port-A-Cul Vial, BBL Microbiology System) for aerobic and anaerobic cultures in an attempt to identify the pathogenic organism(s). Obligate anaerobic bacteria such as *Bacteroides* are difficult to culture, because one must collect, transport, and culture the sample under strict anaerobic conditions. Frequently, bacterial cultures are negative when bacteria are present in peritoneal fluid. To enhance recovery of bacteria, one can inoculate peritoneal fluid into blood culture medium (Septi-Chek Columbia, Hoffmann-LaRoche Inc., Nutley, New Jersey), and if the horse has received antimicrobial treatment, one first should pass fluid through an antimicrobial removal device (A.R.D., Becton Dickinson & Co., Cockeysville, Maryland).

13.17.5 Treatment

Early and aggressive therapy is required if treatment of peritonitis is to be successful. The goals of treatment are to resolve the primary problem, minimize the inflammatory response, and prevent long-term complications. In the acute phase, one gives primary consideration to the arrest of endotoxic, septic, or hypovolemic shock; correction of metabolic and electrolyte abnormalities and dehydration; and management of pain. In the absence of blood gas and electrolyte determinations, adequate volumes of a balanced electrolyte solution are required to correct dehydration and support the cardiovascular system. If the plasma protein concentration of the horse is less than 4.0 g/dl, one should consider administration of plasma or synthetic colloids.

One should administer flunixin meglumine (Banamine) for its local and systemic antiinflammatory effects. Dosages vary depending on the severity of peritonitis, degree of toxemia, severity of pain, and hydration status of the horse and range from 0.25 mg/kg intramuscularly or intravenously every 6 to 10 hours to 1.0 mg/kg intramuscularly or intravenously every 12 hours. The higher dosage provides greater visceral analgesia, whereas the lower dosage is effective in modifying the effects of experimental endotoxemia.[23] In addition to analgesic and general antiinflammatory effect, flunixin meglumine may be effective in retarding adhesion formation when administered early in the acute, diffuse stage of septic peritonitis.[2]

Heparin therapy has been recommended to prevent adhesion formation and to render bacteria more susceptible to 946
cellular and noncellular clearing mechanisms. In experimental models using laboratory animals, heparin therapy 947
was associated with decreased adhesions in septic peritonitis.[24] Heparin has not yet been demonstrated clearly to have similar efficacy in horses, although it may. Suggested dosages range from 20 to 40 IU subcutaneously every 12 hours for 48 hours[4] to 40 to 80 IU/kg subcutaneously every 8 hours.[5] One should note that heparin induces red blood cell aggregation in horses,[25] which may adversely affect capillary blood flow.

One should initiate antimicrobial therapy after making a diagnosis of peritonitis and before the results of peritoneal culture are available, because isolating an organism may take several days and often culture fails to isolate the organism(s). Intravenous administration of antimicrobials is preferred over oral or intramuscular routes in acute, diffuse, septic peritonitis because more reliable levels of drug are achieved in the tissues and peritoneal fluid than otherwise would be obtained in horses with hypovolemia or decreased intestinal motility.[26] The combination of a β-lactam antibiotic with an aminoglycoside is appropriate in most circumstances and certainly in the acute diffuse stage of septic peritonitis. These drugs act synergistically to provide a broad spectrum of activity against a variety of gram-positive and gram-negative aerobic and anaerobic bacteria.[27] Potassium penicillin (22,000 to 44,000 IU/kg intravenously every 6 hours) combined with gentamicin (6.6 mg/kg every 24 hours) is an appropriate regimen. In most cases, peritonitis will have resulted from bowel contamination, and thus one should presume a mixed infection with gram-negative aerobic bacteria and gram-positive and gram-negative anaerobic bacteria.[2] One also should presume the same in many cases of traumatic peritonitis, as occurs with foreign body puncture, breeding trauma, or foaling trauma. Therefore a strong possibility exists of infection involving penicillin resistant *Bacteroides fragilis,* so that adding metronidazole (15 mg/kg orally every 6 to 8 hours) to the regimen is prudent. Combination therapy with β-lactam and aminoglycoside antibiotics (and metronidazole when indicated) is a standard and generally effective protocol. One can modify this antimicrobial regimen when culture and antimicrobial sensitivity results become available.

Aminoglycosides and nonsteroidal antiinflammatory drugs have the potential to induce acute renal tubular damage, particularly when dehydration and decreased renal perfusion are present. Therefore adequately rehydrating the patient and ensuring that renal function is intact before initiating treatment with these drugs are important. Furthermore, maintaining hydration and monitoring renal function during the course of treatment are important. Monitoring serum creatinine concentration; performing serial uninalysis observing for pigment, red blood cells; and casts; determining the ratio of γ-glutamyltransferase to creatinine in the urine; and therapeutic drug monitoring[26] of aminoglycoside levels are useful in this regard.

Sodium ampicillin and ceftiofur sodium are β-lactam antibiotics that may be useful in combination therapy. These drugs have an extended gram-negative spectrum compared with penicillin. However, as a third-generation cephalosporin, ceftiofur is less effective against anaerobes than penicillin. One also may consider ceftiofur, trimethoprim-potentiated sulfonamides, amikacin, and enrofloxacin for treatment of gram-negative infection based on culture and sensitivity results. Enrofloxacin is a quinolone drug with excellent activity against gram-

negative pathogens, including *Pseudomonas,*[27] and also can be effective against resistant staphylococci (personal observation). Such staphylococci may be involved in infections caused by traumatic puncture of the abdominal wall. Enrofloxacin has a variety of potential toxic effects, including cartilage damage in young growing animals.[29] However, a recent study concluded the drug was safe when administered to adult horses intravenously at 5 mg/kg every 24 hours for 3 weeks.[30] One probably should avoid using the drug in young, growing animals until the issue of cartilage damage is resolved. Administration of enrofloxacin to horses constitutes off-label usage.

One should treat horses with acute, diffuse, septic peritonitis with antibiotics until the white blood cell count, plasma fibrinogen, and abdominal fluid analysis are normal. In horses that respond to therapy, this process takes a variable amount of time depending on the offending organisms and stage of disease at the time treatment is initiated. Horses with abdominal abscessation resulting from polymicrobial infection may require many months of antibiotic treatment. Abdominal abscesses caused by streptococci and *Corynebacterium pseudotuberculosis* also may require long-term treatment (weeks to months). Long-term antibiotic treatment generally necessitates the use of oral antibiotics, and the options are limited. Trimethoprim-potentiated sulfonamides are administered orally and are effective against a variety of gram-positive and gram-negative organisms, although some streptococci are resistant. Metronidazole is an orally administered drug effective against anaerobic bacteria, as previously discussed. Other orally administered antimicrobials one may consider for long-term use include doxycycline (broad spectrum), erythromycin (gram-positive spectrum), and enrofloxacin (mostly gram-negative spectrum). Importantly, rifampin, when used with other drugs, can be effective in penetrating and resolving abscesses. Combination therapy with erythromycin and rifampin is the standard treatment for *Rhodococcus equi* infection in foals.

Peritonitis caused by *Actinobacillus equuli* usually is manifested as a diffuse, supporative peritoneal exudate.[6] 947
The same is true for some cases involving streptococci (personal observation). These infections generally 948
respond well to combination therapy with penicillin and gentamicin. If streptococci are involved as the sole pathogen, then penicillin alone should be effective. Streptococci potentially can be involved in mixed, synergistic peritoneal infections in horses.[2]

Drainage or lavage of the peritoneal cavity may be of benefit in removing toxic bacterial by-products and products of inflammatory cells.[31] High numbers of inflammatory cells and release of their mediators can persist even after the primary stimulus of the inflammatory response has resolved. Infusing large volumes of isotonic, warmed fluid into the peritoneal cavity also dilutes the inflammatory mediators, possibly reducing their deleterious effects. When successful, peritoneal lavage decreases the peritoneal fluid WBC count and total protein, potentially reflecting a decrease in diffuse inflammation. The benefits of peritoneal lavage are controversial, and a positive effect may be more likely during the acute, diffuse stage of disease.[2,4] Some studies suggest peritoneal lavage, along with heparin therapy, may reduce the incidence of adhesions.[2]

One should perform peritoneal drainage and lavage using a drain of no less than 24F diameter. Foley-type catheters can be used, but “mushroom” drains provide a larger area for fluid to enter the drain. Two approaches to peritoneal lavage are (1) retrograde irrigation through a ventrally placed ingress-egress drain and (2) placement of ingress catheter(s) in the paralumbar fossa(e) for infusion of fluids, with a drain placed ventrally for removal of infused fluid. One must recognize that thorough peritoneal lavage can be achieved only via ventral midline laparotomy.

Complications associated with the use of abdominal drains or repeated peritoneal penetration to drain fluid include retrograde infection, local irritation, pneumoperitoneum, and subcutaneous seepage around the drain and

resultant cellulitis. If the patient is hypovolemic or hypoproteinemic, one should consider volume replacement and administration of plasma before removing large quantities of fluid from the abdomen.

In horses with suspected parasite involvement, such as verminous arteritis, one should give larvicidal doses of an anthelmintic once the condition of the horse is stabilized. Ivermectin, fenbendazole, and thiabendazole have been recommended as larvacidal therapies.

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14 CHAPTER 14 DISORDERS OF THE LIVER

Michelle Henry Barton

14.1 The Normal Liver

14.1.1 ANATOMY

The liver is the largest organ in the body, constituting approximately 1% of the body weight in the adult horse.[1] The location of the liver between the gastrointestinal tract and the heart is functionally suited for its metabolic, secretory, excretory, and storage properties. In the normal horse the liver lies mostly to the right of the median, is contained completely within the rib cage, and does not contact the ventral abdominal floor. The most cranial portion of the liver is located in the ventral third of the sixth to seventh intercostal spaces and extends caudad to the right kidney (fifteenth rib). In disease processes resulting in hepatomegaly and in the normal equine neonate the liver may extend beyond the caudal border of the last rib. Right liver lobe atrophy has been described as an uncommon normal anatomic variation in adult horses. However, in 1994, Jakowski[2] hypothesized that right hepatic lobe atrophy in horses is a pathologic condition resulting from long-term compression of the right lobe of the liver by abnormal distention of the right dorsal colon and base of the cecum.

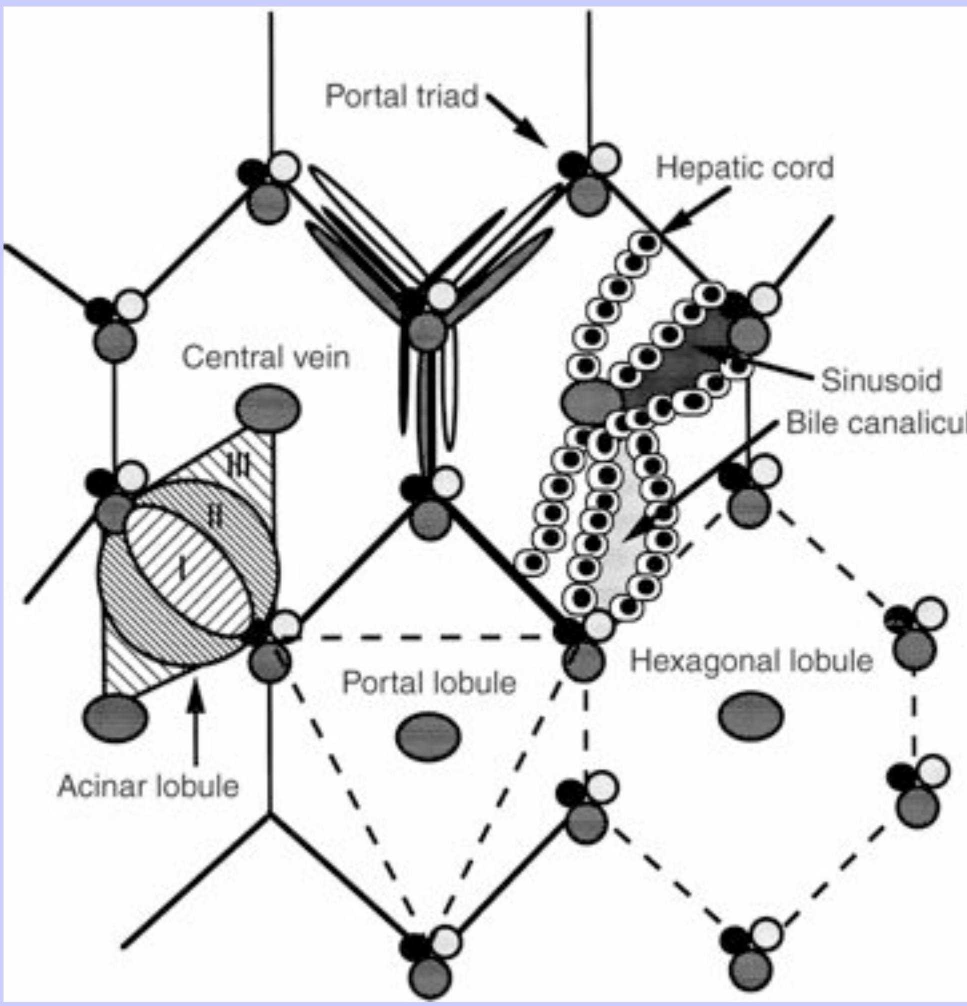
The equine liver consists of two surfaces, diaphragmatic and visceral, and is divided by fissures into four lobes: right, left, quadrate, and caudate. The visceral surface of the liver in situ is malleable and contains impressions of the organs with which it is in contact. The visceral surface also contains the hilum, or *porta* (door), of the liver, through which blood vessels, lymphatics, and nerves enter and the hepatic duct exits. In the horse, six ligaments secure the liver in the abdominal cavity.[1] The *coronary ligament* has two laminae, right and left, that attach the diaphragmatic surface of the liver to the caudal vena cava and the abdominal esophagus. The two laminae of the coronary ligament unite ventrally to form the *falciform ligament*. The falciform ligament, a remnant of the fetal ventral mesentery that extends from the diaphragm to the umbilicus, attaches the quadrate and left lobes to the sternal diaphragm and ventral abdominal floor. The *round ligament*, the remnant of the fetal umbilical vein, is contained within the free border of the falciform ligament. The right and left *triangular ligaments* attach the dorsal right lobe to the right costal diaphragm and the dorsal left lobe to the tendinous center of the diaphragm. The *hepatorenal ligament* connects the caudate process of the quadrate lobe to the right kidney and the base of the cecum.

14.1.2 HISTOLOGIC FINDINGS

At the hilum of the liver a tree of connective tissue consisting of collagen and fibroblasts enters the hepatic parenchyma. The parenchymal cells, or *hepatocytes*, compose approximately 50% to 60% of the mass of the liver and are epithelial cells.[3,4] The hepatocytes are arranged in rows, or *cords*, at least two cells thick that anastomose to form blood passageways called *sinusoids* (Figure 14-1). Hepatic sinusoids are larger than capillaries and are lined with endothelial cells and *Kupffer's cells*. Kupffer's cells are tissue-fixed macrophages and are estimated to make up 20% of the mass of the liver. The endothelial cells make up approximately 20% of the mass of the liver.[4] A cleft, called the *space of Disse*, lies between the hepatocytes and the cells lining the sinusoids. The space of Disse contains fluid similar to the composition of blood but does not contain erythrocytes.

The afferent hepatic blood vessels, bile ducts, lymphatics, and nerves follow the branching connective tissue tree into the hepatic parenchyma. The liver receives approximately one third of the cardiac output. Two separate sources of blood supply the liver and empty into the hepatic sinusoids: the *portal vein* and the *hepatic artery*. The portal vein contains poorly oxygenated blood that carries nutrients absorbed from the gastrointestinal tract to the liver for storage, metabolism, transformation, or packaging for export to other tissues. The hepatic artery contains oxygen-rich blood to support the metabolic and energy-generating activities of the liver. The sinusoids drain into terminal hepatic venules or *central veins*, which connect with the hepatic vein and caudal vena cava. 951 952

Figure 14-1 Histology of the liver. Roman numerals I, II, and III represent zones 1, 2, and 3, respectively, of the acinar lobule.



The space between contiguous hepatocytes in a cord forms a *bile canaliculus* through which bile excreted by the hepatocytes drains into bile ductules and ducts. The bile canaliculi thus are formed solely by the cell membranes of the hepatocytes. The bile ductules and ducts are lined with cuboidal and columnar epithelial cells, respectively, that make up approximately 7% of the mass of the liver.[4] The bile ducts run in the connective tissue tree, adjacent to branches of the portal vein and hepatic artery, toform a distinct *portal tract, radicle, canal*, or

triad (see Figure 14-1). The bile ducts converge at the hilum to form the *hepatic duct*, which drains into the duodenum just distal to the pylorus. Because the horse does not have a gallbladder or a sphincter at the entry site of the hepatic duct into the intestine, the bile is unconcentrated and flows continuously in a direction opposite that of the blood flow in the portal vein and hepatic artery.[1]

The liver can be divided anatomically or functionally into lobules to facilitate histopathologic description of lesions[3] (see Figure 14-1). The *classic hepatic lobule* is delineated by abundant interlobular connective tissue that in cross section roughly appears hexagonal. The corners of the hexagon are defined by three to eight portal tracts with a central vein in the center of the lobule. In contrast, the *portal lobule* is a functional unit describing the exocrine duties of the liver. The three corners of the portal lobule are defined by central veins, with a portal tract situated in the center. The *acinus lobules* describe the vascular supply to the hepatic parenchyma, divided according to the tissue oxygen content. Zone I of the acinus lobule is located immediately adjacent to branching hepatic arteries and portal veins, is the most metabolically active zone, and receives the best oxygen supply. Zone III is located adjacent to central veins, has high mixed-function oxidase activity, is least favorably situated respecting oxygen content, and thus is most susceptible to toxic and hypoxic damage. Zone II is situated between zones I and III.

14.1.3 PHYSIOLOGY

The liver is the main organ involved in regulating nutrient distribution.[5] The majority of nutrients absorbed from the gastrointestinal tract pass directly to the liver via the portal circulation. The incoming nutrients are metabolized for energy, transformed to other nutrient classes, packaged and exported to peripheral tissues, or stored by the liver. The liver is capable of adjusting to the carbohydrate, protein, and lipid load from the gastrointestinal tract and of maintaining consistent blood levels of nutrients between feedings and in response to special needs. In addition to its role in nutrient metabolism and homeostasis the liver is involved in excretion (bile), detoxification, and metabolism of endogenous and exogenous substances and in hematopoiesis.[4]

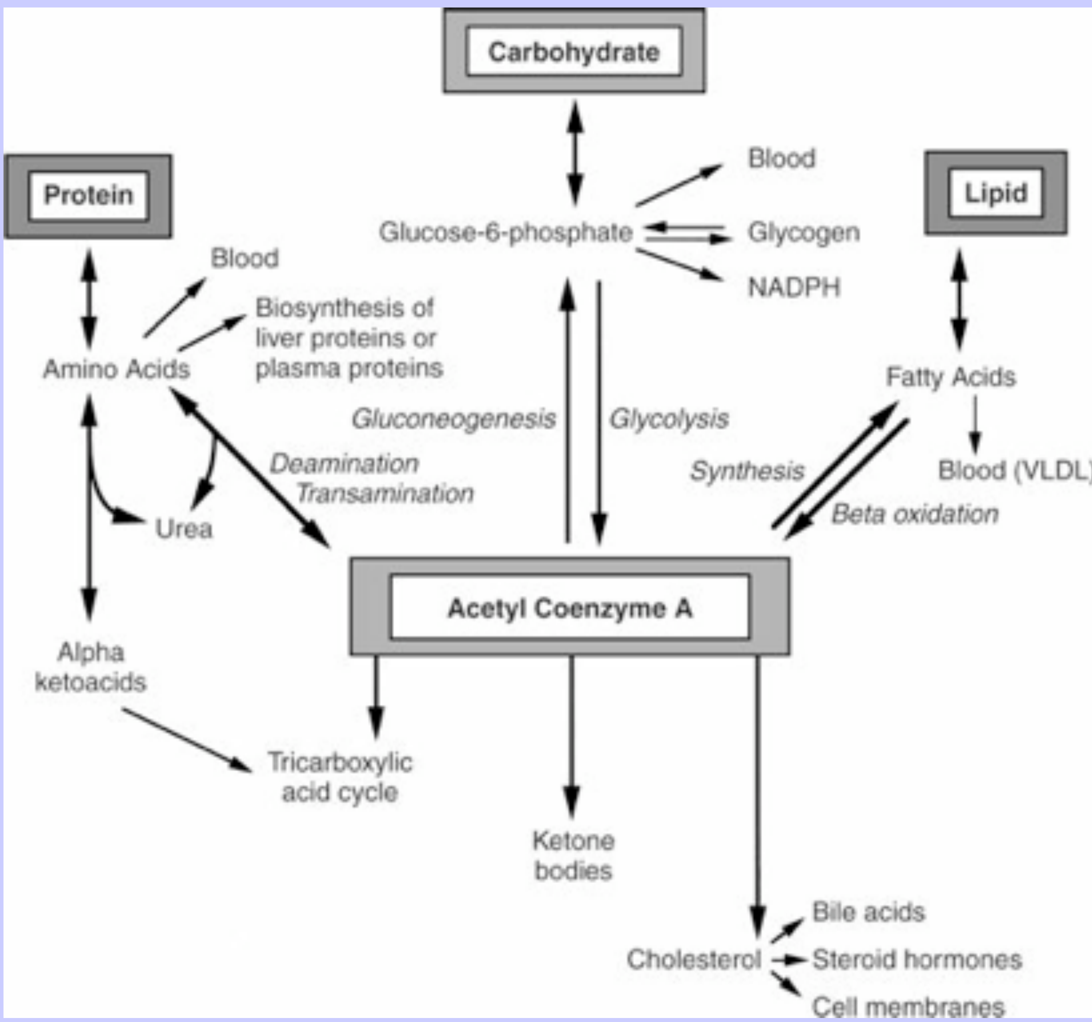
14.1.3.1 Protein Metabolism

Amino acids, which are transported to the liver via the portal or hepatic blood, may be used in the biosynthesis of intrinsic hepatocellular proteins, plasma proteins, porphyrins, polyamines, purines, and pyrimidines.[5] The liver synthesizes 90% of the plasma proteins, including albumin, factors involved in coagulation and fibrinolysis (fibrinogen and factors II, V, VII to XIII, antithrombin III, protein C, plasminogen, plasminogen activator inhibitor, α_2-antiplasmin, α_2-macroglobulin, and α_1-antitrypsin), transport proteins (haptoglobin, transferrin, ceruloplasmin, hormone transport proteins), and acute phase reactant proteins (α- and β-globulins).[4] The liver is the only site of synthesis of albumin and fibrinogen.

The liver is also capable of transamination, or the reversible transfer of an amino group on one amino acid to 952
an α-keto acid, thus forming a new amino acid and a new keto acid. If the liver receives an excess of amino 953
acids or if carbohydrates are unavailable as an energy source, the liver deaminates the amino acids and converts them to pyruvate, acetoacetate, and intermediates of the tricarboxylic acid cycle[5] (Figure 14-2). These intermediates may be oxidized for energy or used as precursors in *gluconeogenesis*, the synthesis of glucose from noncarbohydrate precursors. Endogenous and exogenous glucocorticoids, glucagon, and thyroid hormone act directly on the liver to increase gluconeogenesis(Figure 14-3). Simultaneously, glucocorticords indirectly influence liver gluconeogenesis by promoting peripheral protein catabolism, thus increasing the availability of amino acids. Insulin inhibits gluconeogenesis in the liver.[6]

In addition to protein synthesis and gluconeogenesis the liver plays an important role in eliminating the major toxic by-product of amino acid catabolism, *ammonia*.[7,8] All tissues and intestinal microflora generate ammonia, which subsequently is released into the circulation. One method by which the liver, as well as certain peripheral tissues, eliminates ammonia is by synthesizing nonessential amino acids from α-keto acids and ammonia in a reversal of deamination. A fundamental reaction in the synthesis of nonessential amino acids is the formation of *glutamate* from α-ketoglutarate and ammonia (Figure 14-4). Subsequently, glutamate is used in transamination reactions to form other amino acids. Glutamate also participatesin the conversion of cytotoxic free ammonia into a nontoxic transport form, *glutamine*. Glutamine may be delivered to the kidney, converted back to free ammonia and excreted, or delivered to the liver for urea synthesis.

Figure 14-2 Role of the liver in the metabolism of nutrients. *VLDL*, Very-low-density lipoprotein; *NADPH*, nicotinamide adenine dinucleotide phosphate.



The liver has sole responsibility for converting free ammonia or glutamine into *urea*, the principal form of amino group nitrogen excretion by mammals.[4] Urea is formed by the irreversible condensation of two ammonia molecules with carbon dioxide (see Figure 14-4). The reaction takes place in the hepatocyte mitochondria viathe Krebs-Henseleit cycle.[5] The newly formed urea is released from the hepatocyte, secreted into the sinusoidal blood, and transported to the kidney as *blood urea nitrogen (BUN)* for excretion.

14.1.3.2 Carbohydrate Metabolism

The liver is responsible for the synthesis, storage, and release of glucose.[5] Monosaccharides absorbed from the
gastrointestinal tract are delivered via portal blood tothe liver. In the hepatocyte the majority of glucose is 953
phosphorylated to *glucose-6-phosphate* by the enzyme hexokinase (see Figure 14-2). The remaining glucose is 954
released into the systemic circulation. Other monosaccharides (fructose, galactose) are phosphorylated and converted in the liver to glucose-6-phosphate. The majority of glucose-6-phosphate is converted to glycogen for storage. A small amount of glucose-6-phosphate is oxidized to form adenosine triphosphate, though the major source of adenosine triphosphate in the liver is amino acid and fatty acid oxidation. Approximatelyhalf of the liver glucose enters the phosphogluconate pathway for generation of nicotinamide adenine dinucleotide phosphate, which is required as a reducingagent in the biosynthesis of fatty acids and cholesterol.
Glucocorticoids, catecholamines, glucagon, and thyroid hormone increase gluconeogenesis and 954
glycogenolysisin the liver, whereas insulin inhibits gluconeogenesis[6] (see Figure 14-3). 955

Figure 14-3 Hormonal control of metabolism. The sign (–) represents an inhibitory effect;(+) represents a stimulatory effect. *VLDL*, Very-low-density lipoprotein.

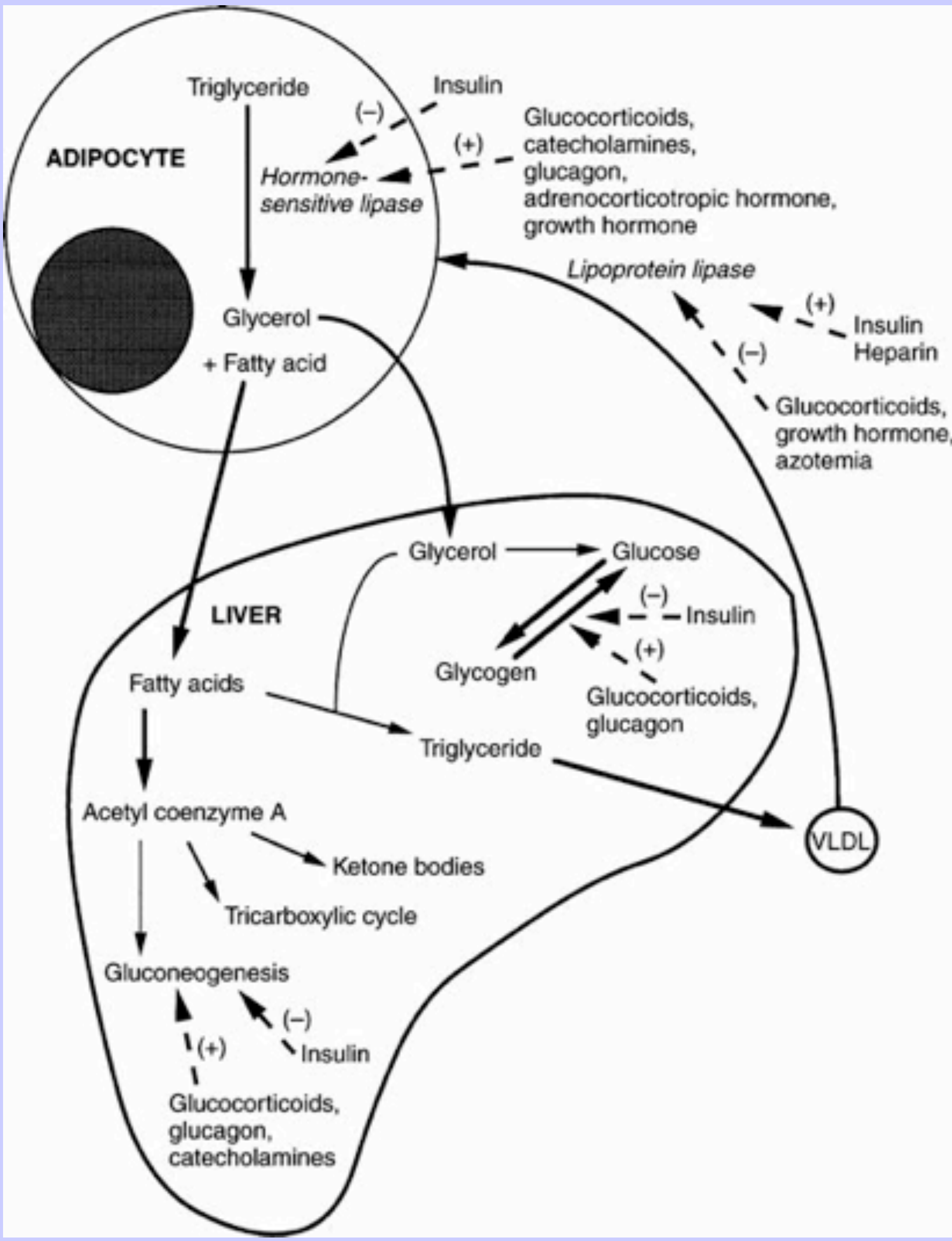
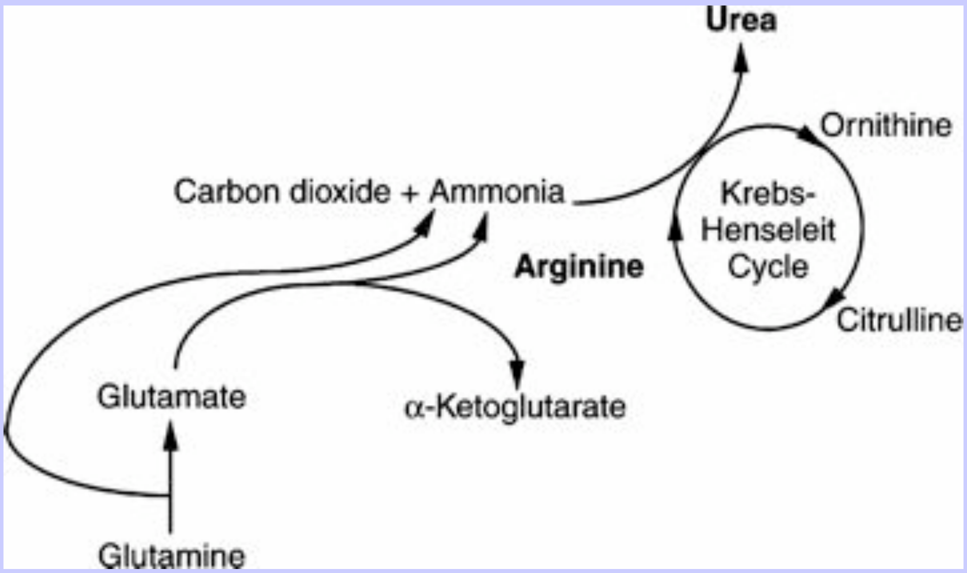


Figure 14-4 The urea cycle.



14.1.3.3

Lipid Metabolism

Short-chain fatty acids (fewer than 10 carbon atoms) can be absorbed directly from the gastrointestinal tract, bound to albumin, and delivered to the liver via the portal circulation.[5] However, most short-chain fatty acids are incorporated into phospholipid or triglyceride by the intestinal epithelium and transported to the liver viathe portal blood. The remaining fatty acids absorbed from the gastrointestinal tract are transported as triglyceride in *chylomicrons*. After formation in the intestinal epithelial cells and absorption into lymphatics, chylomicrons enter the systemic circulation via the thoracic duct and subsequently are delivered to the liver. The liver also may take up albumin-bound fatty acids released from adipose tissue.

The fate of fatty acids in the liver depends on the state of energy demand, the rate of fatty acid delivery, and hormonal influences. The primary role of the liver in lipid metabolism is to esterify free fatty acids into triglycerides for export to other tissues[5] (see Figure 14-3). The triglycerides are packaged with protein, carbohydrates, and cholesterol in the endoplasmic reticulum of the hepatocyte into *very-low-density lipoproteins (VLDLs)*, which primarily contain triglyceride, and *high-density lipoproteins*, which primarily contain protein and phospholipid.[9] The VLDLs and high-density lipoproteins are released into the hepatic sinusoids. Once VLDLs are in the systemic circulation, adipose tissue takes them up or endothelial cell lipases alter their composition by removing triglyceride, forming *intermediate*- and *low-density lipoproteins*.

In addition to exporting plasma lipoprotein, the liver can oxidize free fatty acids for energy to acetyl coenzyme A, a fundamental compound in the tricarboxylic acid cycle (see Figure 14-2). The acetyl coenzyme A thus formed also may be used to synthesize other fatty acids, cholesterol, steroids, and ketone bodies, acetoacetate, and β-hydroxybutyrate.[10] Furthermore, through the synthesis of acetyl coenzyme A from glucose and most amino acids, the liver is capable of converting carbohydrates and proteins into lipids. Ketone bodies can be exported from the liver and used for energy by peripheral tissues, especially the brain, when glucose is deficient. However, overproduction of ketone bodies can be detrimental, resulting in ketoacidosis.[9]

Insulin and glucocorticoids closely regulate lipid metabolism[6] (see Figure 14-3). Glucocorticoids function primarily to increase fatty acid mobilization from the periphery, whereas insulin decreases adipose tissue release of fatty acids by activating lipoprotein lipase and inhibiting hormone-sensitive lipase. Insulin acts on the liver to increase fatty acid synthesis from glucose.

14.1.3.4 Excretion of Bile

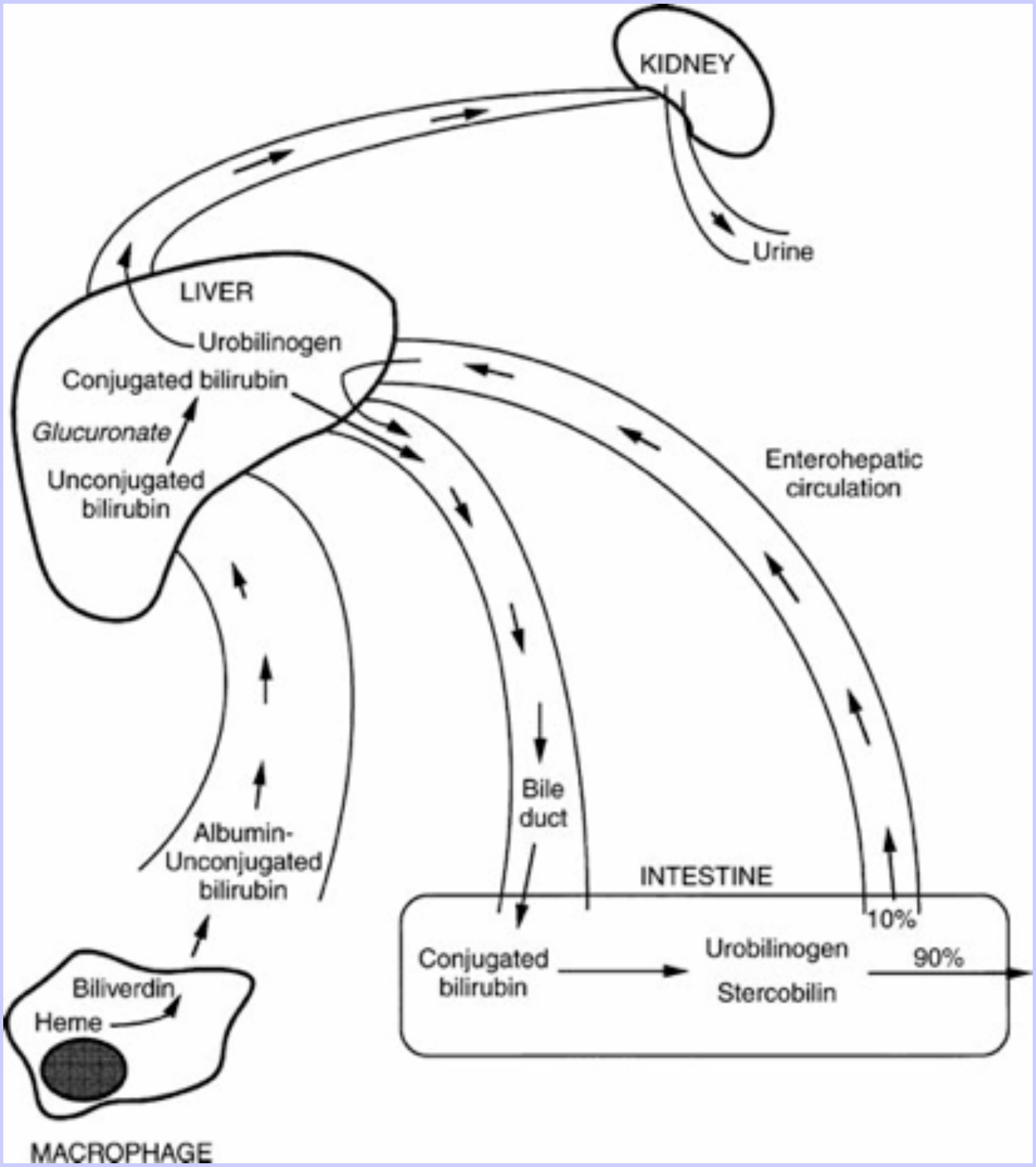
Bile consists of several components, including conjugated bilirubin, bile acids, cholesterol, lecithin, water, and electrolytes.[4] Bile is released by hepatocytes into the bile canaliculi where water diffuses passively. Bile then is transported by large bile ducts and the hepatic duct to the intestine. Water and electrolytes exchange takes place between the bile and the bile duct epithelium; however, isotonicity is maintained. Because the horse does not have a gallbladder or a sphincter at the site of entryof the hepatic duct into the duodenum, the bile is unconcentrated and flow is continuous.[1]

Bile acids make up 90% of the organic portion of bile. Bile acids are amphoteric molecules that act as detergents. These detergents facilitate the excretion of cholesterol and phospholipid from the liver into bile and facilitate the absorption of lipids and lipid-soluble compounds (vitamins A, D, E, and K) from the intestinal tract. The principal *primary bile acids* (i.e., nondegraded) in the horse are *cholate* and *chenodeoxycholate*, which are conjugated with taurine. Once secreted into the lumen of the intestinal tract, cholate and chenodeoxycholate may be reabsorbed or degraded by bacteria, forming the *secondary bile acids deoxycholate* or *lithocholate*, respectively. More than 95% of the conjugated bile acids excreted in bile and released into the intestinal lumen are reabsorbed bythe ileum and returned to the liver via the *enterohepatic circulation*. Deoxycholate acts as a normal bile acid and can undergo enterohepatic circulation, whereas lithocholate is reabsorbed only once. Bile acids are estimated to be recycled at least 38 times a day in healthy ponies.[11]

Bilirubin is the breakdown product of tetrapyrroles that function as electron transport pigments.[4] Most bilirubin is formed from hemoglobin and myoglobin, but nonheme pigments such as the cytochromes also serve as a source of bilirubin. Macrophages in the spleen, bone marrow, and liver (Kupffer's cells) engulf the pigments, convert them to biliverdin (Figure 14-5), convert biliverdin to bilirubin, and then release it from the cellas free, insoluble bilirubin. This form of bilirubin also is referred to as *indirect reacting* or *unconjugated bilirubin*. Unconjugated bilirubin is bound with albumin in the plasma to decrease its hydrophobicity and is delivered to the liver. At the surface of the hepatocyte, the bilirubin is transferred from albumin to *ligandin*, an intrahepatic transport and storage protein.[4,11] Within the hepatocyte the bilirubin is conjugated with glucuronide in the endoplasmic reticulum. *Conjugated bilirubin*, also called *direct reacting bilirubin*, is water soluble and is excreted into the bile canaliculi. Under normal circumstances, little conjugated bilirubin escapes into the general circulation.

Microflora in the intestinal tract reduce conjugated bilirubin to *urobilinogen* and *stercobilin* (see Figure 14-5), 955
which impart a yellow-brown color to feces. In herbivores the presence of chlorophyll pigments in the 956
fecesmasks the color of urobilinogen.[12] Only in the neonatal herbivore receiving a milk diet are the feces yellow. Urobilinogen is absorbed by the intestinal mucosa and transported back to the liver via the enterohepatic circulation. A small amount of conjugated bilirubin in the intestinal lumen is hydrolyzed to unconjugated bilirubin and subsequently is reabsorbed. The liver extracts most of the urobilinogen; however, a small amount spills over into the urine. Urobilinogen is concentrated in the normally alkaline urine of horses and thus is detectable.[13]

Figure 14-5 Metabolism and excretion of bile.



14.1.3.5

Detoxification

The liver is responsible for the *biotransformation* of numerous endogenous and exogenous compounds. Biotransformation involves a series of enzymatic reactions that alter the physical properties or activity of compounds. Biotransformation occurs in two phases.[14] In phase 1, polar groups are added to the compound or existing polar groups are exposed by oxidation, hydroxylation, deamination, or reduction. In phase 2 the product of phase 1 is conjugated, usually with glucuronate or sulfate. Substrates for detoxification usually are

water insoluble, and biotransformation renders them more susceptibleto renal or biliary excretion.[4,15] Examples of endogenous substances biotransformed by the liver include ammonia, bilirubin, and steroid hormones (estrogen, cortisol, aldosterone). The liver biotransforms countless exogenous substances; for example, drugs, plant toxins, insecticides, and mercaptans.

Phase 1 of biotransformation occurs primarily on the enzyme-bound systems of the endoplasmic reticulum, called *microsomes*.[4,15] Most of these enzymes are iron-containing enzymes of the P-450 system, thus named because they absorb light at 450 nm. The P-450 enzymes also are called *mixed function oxidases*. Some substrates, referred to as *inducers*, are capable of saturating the enzymes involved in biotransformation. Enzyme saturation and induction causes hypertrophy of the endoplasmic reticulum and all contained enzymes, 956
thus accelerating substance removal rates. Inducers not only accelerate their own removal rate but also may 957
accelerate the biotransformation of other endogenous and exogenous substances. Examples of enzyme inducers are the barbiturates, phenylbutazone, and chlorinated hydrocarbons. Other agents presented for biotransformation may inhibit microsomal enzymes—for example, chloramphenicol, cimetidine, organophosphates, morphine, and quinidine—thus prolonging the effect of other substrates. Hepatic biotransformation sometimes results in the formation ofa toxic metabolite from a nontoxic parent compound, examples being aspirin and halothane.[15]

14.1.3.6 Mononuclear Phagocyte System

Hepatic macrophages, or Kupffer's cells, make up a major portion of the mononuclear phagocyte system. Cellsof the mononuclear phagocyte system are derived from bone marrow myeloid progenitors and serve two main functions: phagocytosis and to act as antigen-processing cells for lymphocytes. Kupffer's cells respond to opsonins and synthesize a vast array of inflammatory mediators, including interleukins, tumor necrosis factor, and eicosanoids. Unlike other macrophages in the mononuclear phagocyte system, Kupffer's cells function mainly in phagocytosis and are located strategically along the hepatic sinusoids, where portal blood can be cleansed, for example, of bacterial endotoxin, before exposure to the hepatocytes and subsequently the systemic circulation.[16] Kupffer's cells also help cleanse systemic blood entering via the hepatic artery by removing fibrin degradation products, tissue plasminogen activators, hemoglobin, microbes, foreign antigens, and other particulate debris.

14.1.3.7 Miscellaneous Functions

The liver serves as a storage site for several vitamins and trace minerals, including vitamins A, D, and B_{12}, copper, and iron. Vitamin D is converted first in the liver to25-hydroxycholecalciferol and exported to the kidney, where it is transformed into 1,25-dihydroxycholecalciferol, the active form of the vitamin.[6] In the fetus the liver is involved in *hematopoiesis*.[4] In the adult the bone marrow serves as the primary site for hematopoiesis; however, the liver may serve as an extramedullary site of hematopoeisis under intense conditions of erythrocyte regeneration or if a large portion of the bone marrow is destroyed.

14.2 Hepatic Insufficiency

14.2.1 DEFINITION

Hepatic insufficiency or failure refers to the inability of the liver to perform its normal functions properly. Because the liver is involved in such a diverse array of physiologic activities, any pathologic process may hinder one or several functions without impeding others. Furthermore, most hepatic functions are not impaired until greater than 80% of the hepatic mass is lost.[4,12,17] The liver also has the capability to regenerate under certain conditions. If hepatocyte loss is gradual and regeneration parallels destruction, hepatic failure does not necessarily ensue. Thus hepatic disease may be present without accompanying hepatic failure. Consequently, hepatic disease does not always manifest clinically.

14.2.2 PATTERNS AND PATHOLOGIC FINDINGSOF HEPATIC INJURY

The severity of the accompanying clinical signs andthe course of hepatic disease vary depending on the pattern, location, rate, and extent of hepatic damage. Hepatic injury may be reversible (fatty degeneration, cloudy swelling), irreversible (necrosis), focal or zonal, generalized, acute, chronic, inflammatory, anatomic, or functional.

14.2.2.1 Acute Focal or Multifocal Hepatic Injury

Focal hepatic injury occurs with uniform damage to one small area of the liver. Examples of focal hepatic injury include hepatic abscesses, solitary infarctions, and neoplastic growths. Because adequate hepatic reserve exists in the unaffected regions, focal hepatic injury rarely is accompanied by clinical signs of hepatic failure, though evidence of hepatic disease may be demonstrable.[14] Acute multifocal hepatic injury is more likely to result in clinically significant hepatic disease. Acute hepatic injury may be degenerative, necrotizing, or inflammatory. Hepatic *degeneration* refers to a toxic or immunologic insult that causes hepatocytes to swell and take on an edematous appearance. *Ballooning degeneration* is used to describe irregularly clumped cytoplasm with large clear areas. If biliary material has been retained, the hepatocytes appear foamy and swollen *(foamy degeneration)*. *Ischemic coagulative necrosis* refers to poorly stained and mummified hepatocytes with lysed nuclei, whereas *lytic necrosis* describes osmotically swollen and ruptured cells. Necrosis of contiguous hepatocytes that spans adjacent lobules in a portal to portal, portal to central, or central to central fashion is called *bridging necrosis*.[18]

Hepatic injury may be zonal, that is, affecting certain zones of the liver uniformly throughout the entire organ.[14] The liver often appears pale with an enhanced lobular pattern on the cut surface. The two most common types of zonal hepatic injury are centrilobular and periacinal. In *centrilobular zonal injury*, the area adjacent to the central veins (zone III) is affected uniformly, whereas in *periacinal* or *(paracentral) zonal injury*, cellular degeneration involves only a wedge around the central vein (see Figure 14-1). Hepatocytes in these locations 957
are most susceptible to anoxic damage, because the normal oxygen tension is lowest and mixed function 958
oxidase activity is the greatest in these areas. Examples of disease states resulting in centrilobular injury are severe acute anemia, passive congestion caused by congestive heart failure *(nutmeg liver)*, and toxic hepatopathies. Periportal (zone I acinar lobular) injury is rare but may occur with infarction of hepatic vessels, as may occur during verminous arteritis, or exposure to toxins that do not require metabolism by mixed function oxidases (e.g., phosphorus).

14.2.2.2

Acute Generalized Hepatic Injury

Acute generalized hepatic injury often is accompanied by clinical signs of hepatic failure, with the extent of damage dictating the severity of the clinical signs.[14] Typically, the liver appears pale and enlarged and is often friable. Acute generalized hepatic injury may result from infection, necrosis, inflammation, or hepatotoxic agents.[4] Bacterial or viral infections, parasitic infestations, or immune disorders may cause acute generalized necrosis or inflammation. Despite its cause, any process that results inan inflammatory response in the hepatic parenchyma is referred to as *hepatitis*. Acute inflammation most commonly accompanies necrosis and is characterized by the presence of neutrophils and lymphocytes in the areas of cell death or surrounding portal triads. An inflammatory process primarily involving the biliary system is called *cholangitis*, usually resulting from ascending infection from the intestinal tract or following cholestasis.

14.2.2.3

Chronic Generalized Hepatic Injury

Chronic hepatic injury is accompanied by clinical signs of hepatic failure when greater than 80% of the hepatic mass is destroyed or replaced by fibrosis.[4,17] Fibrosis, the presence of collagen and fibroblasts, occurs when the rate of ongoing necrosis exceeds the rate of regeneration. Typically, the liver appears smaller than normal. Fibrosis commonly follows conditions resulting in chronic hypoxia, chronic inflammation, chronic cholangitis or cholestasis, metastatic neoplasia, trauma, or ingestion of antimitotic agents such as plants containing pyrrolizidine alkaloids. *Cirrhosis*, or an *end-stage liver disease*, refers to chronic hepatic disease characterized by the presence of widespread fibrosis, nodular regeneration, and biliary hyperplasia.[14] *Nodular regeneration*, or islands of hepatocytes, occurs when the normal architecture and blood supply of the liver are disrupted or destroyed by the presence of fibrosis. *Bridging fibrosis* implies fibrosis that extends from one portal area to another or from portal areas to central areas.[18] The cause of biliary hyperplasia during chronic liver disease is unknown. One form of chronic hepatic disease, called *chronic active hepatitis*, is characterized by the presence of cirrhosis and an acute inflammatory response.[14]

14.2.2.4

Anatomic or Functional Injury

Anatomic or functional shunts cause liver injury by anoxic damage. Additionally, if blood habitually bypasses the liver, the liver cannot perform its normal metabolic regulatory or detoxifying functions, thus clinicalsigns of hepatic failure become imminent. Anatomic shunts can be congenital or acquired, intrahepatic or extrahepatic.

14.2.3

CLINICAL SIGNS OF HEPATIC INSUFFICIENCY

The clinical signs of hepatic insufficiency vary greatly, are nonspecific, and depend on the extent and duration of hepatic disease (Box 14-1). Usually, greater than80% of the liver mass must be lost before clinical signs become apparent, regardless of the cause of hepatic disease. Thus despite the duration of hepatic disease, the onset of clinical signs is often abrupt. The most common clinical signs of hepatic insufficiency in horses are depression, anorexia, colic, hepatic encephalopathy, weightloss, and icterus.[19–22] Less commonly reported clinicalsigns 958
include hepatogenic photosensitization, diarrhea, abdominal pain, bilateral laryngeal paralysis, and hemorrhagic 959
diathesis. Rarely reported clinical signs of hepatic insufficiency in horses are ascites, dependent abdominal edema, steatorrhea, tenesmus, generalized seborrhea, pruritus, endotoxic shock, polydipsia, and hemolysis. The

appearance of specific clinical signs of hepatic disease often reflects the type of hepatic function(s) that is altered.

14.2.3.1 BOX 14-1 CLINICAL SIGNS OF LIVER DISEASE

14.2.3.1.1 Common Signs

Depression

Anorexia

Colic

Hepatic encephalopathy

Weight loss

Icterus

14.2.3.1.2 Less Common Signs

Photosensitization

Diarrhea

Bilateral laryngeal paralysis

Bleeding

Ascites

Dependent edema

14.2.3.1.3 Rare Signs

Steatorrhea

Tenesmus

Generalized seborrhea

Pruritus

Endotoxic shock

Polydipsia

Pigmenturia (yellow-brown with bilirubinuria; red-brown with hemoglobinuria)

14.2.3.2

Hepatic Encephalopathy

Hepatic encephalopathy (HE) is a complex clinical syndrome characterized by abnormal mental status that accompanies severe hepatic insufficiency.[23–25] Clinical signs vary greatly but represent manifestations of augmented neuronal inhibition. This syndrome occurs in patients with advanced decompensated liver disease of all types and may be a feature of acute, subacute, or chronic hepatocellular disease. HE generally is considered a potentially reversible metabolic encephalopathy.[24] Whether multiple episodes of HE could lead to irreversible neuronal damage is uncertain.

14.2.3.2.1

Clinical Signs and Laboratory Findings

No specific features of HE allow one to distinguish this syndrome from other causes of cerebral dysfunction. The earliest phase of HE probably is missed in most equine patients because it represents minimal behavioral changes with subtle impairment of intellect caused by bilateral forebrain dysfunction[26] (stage I; Table 14-1). In human beings these early signs are more apparent to close friends and family members than to a physician. As encephalopathy progresses, motor function, intellectual abilities, and consciousness become impaired, and generally at this stage (corresponding to stage II) horses become obviously affected. Clinical signs include depression, head pressing, circling, mild ataxia, aimless walking, persistent yawning, and other manifestations of inappropriate behavior. Somnolence develops next: the horse is rousable but responds minimally or excessively to the usual stimuli. At this stage (III) the horse often manifests aggressiveor violent behavior interspersed with periods of stupor. Finally, consciousness fades, the horse becomes recumbent, and coma ensues. Occasionally, seizures occur during the later stages of HE, but in general they are atypical. The severity of encephalopathy corresponds to the degree of hepatic dysfunction; however, neither of these parameters correlates with type or reversibility of the underlying hepatic disease.

TABLE 14-1 Clinical Stages of Hepatic Encephalopathy

STAGE	MENTAL STATUS
I	Mild confusion, decreased attention, slowed ability to perform mental tasks, irritability
II	Drowsiness, lethargy, obvious personality changes, inappropriate behavior, disorientation
III	Somnolent but rousable, marked confusion, amnesia, occasional aggressive uncontrolled behavior
IV	Coma

Adapted from Gammel SH, Jones EA: Hepatic encephalopathy, *Med Clin North Am* 73:793–813, 1989.

14.2.3.2.2

Cause and Pathophysiology

By definition, the cause of HE is insufficient hepatocellular function, irrespectiveof the cause of the liver disease. Whether a normally functioning liver is necessary to maintain normal brain neuron and astrocyte function is unclear. The pathogenesis of HE remains unclear, and considering the numerous proposed

hypotheses, the cause almost certainly is multifactorial. The following mechanisms have been suggested for the development of HE, and any or all factors may be involved to greater or lesser degree:

1. Gastrointestinal-derived neurotoxins
2. False neurotransmitter accumulation following plasma amino acid imbalance
3. Augmented activity of γ-aminobutyric acid (GABA) in the brain
4. Increased permeability of the blood-brain barrier
5. Impaired central nervous system (CNS) energy metabolism

Perhaps the oldest and most predominant hypothesis for HE involves the accumulation of toxic materials in the blood, derived from the metabolism of nitrogenous substrates in the gastrointestinal tract, that bypass the liver through functional or anatomic shunts.[23,27,28] Accordingly, HE may be caused primarily by failure of the liver to remove certain substances adequately from the blood that have the direct or indirect ability to modulate function of the CNS. Ammonia, following the degradation of amino acids, amines, and purines by enteric bacteria, has been supported widely as a major neurotoxin of hepatic disease.[28–30] In patients with liver failure, ammonia is metabolized insufficiently, thus plasma concentrations increase and ammonia enters the CNS, where it may cause encephalopathy.[28,31] Ammonia has a toxic effect on cell membrane neurons by inhibition of Na^+,K^+-ATPase (adenosine triphosphatase) activity in nerve cell membranes, causing depletion of adenosine triphosphate.[30,32] Hyperammonemia also is associated with a disturbance in CNS energy production caused by alterations inthe tricarboxylic acid cycle that result in a decrease inα-ketoglutarate formation and increased synthesis of glutamine.[33] Astrocytes in the brain also detoxify ammonia by converting it to glutamate and glutamine. Glutamine accumulation in astrocytes is a major cause of cell swelling and generation of cerebral edema in acute fulminate hepatic failure. Another effect of prolonged exposure of neuronal tissue to ammonia is downregulation of glutamate receptors. Because 959
glutamate is the major excitatory neurotransmitter on the mammalian brain, decreased glutamate receptor 960
activity likely contributes to the decreased excitatory transmission in HE.[32] Hyperammonemia also induces generation of nitric oxide, which leads to accumulation of peroxides, oxidative stress, and nerve cell damage. These cumulative effects of ammonia on neural tissue certainly play an important role in the pathogenesis of HE. Experimentally, ammonia can induce encephalopathy,[34] and children with hyperammonemia caused by congenital enzyme deficiencies have encephalopathy.[29] Furthermore, therapy aimed at reducing the absorption of ammonia from the intestine tends to ameliorate HE.[26] Points argued against the role of ammonia in the pathogenesis of HE are that plasma ammonia concentrations correlate poorly with the severity of HE and ammonia does not induce the electroencephalogram changes typical of the encephalopathy of liver disease.[24,35] Thus the actions of ammonia on the CNS are complex, and ammonia likely is involved in but is not solely responsible for HE.

The synergistic neurotoxins hypothesis for the pathogenesis of HE implicates not only ammonia but also other gut-derived neurotoxins, specifically mercaptans, short-chain fatty acids, and phenols. Members of each of these classes of substances increase in the blood of patients with hepatic failure in concentrations that alone are insufficient to induce encephalopathy. However, the combination of some or all of them may induce encephalopathy by their synergistic actions and by augmenting endogenous metabolic abnormalities,[26,27] mostly centering around inhibition of brain Na^+,K^+-ATPase with subsequent impaired

neurotransmission.[24] As with ammonia, blood and brain concentrations of mercaptans correlate poorly with the stage of HE.[36]

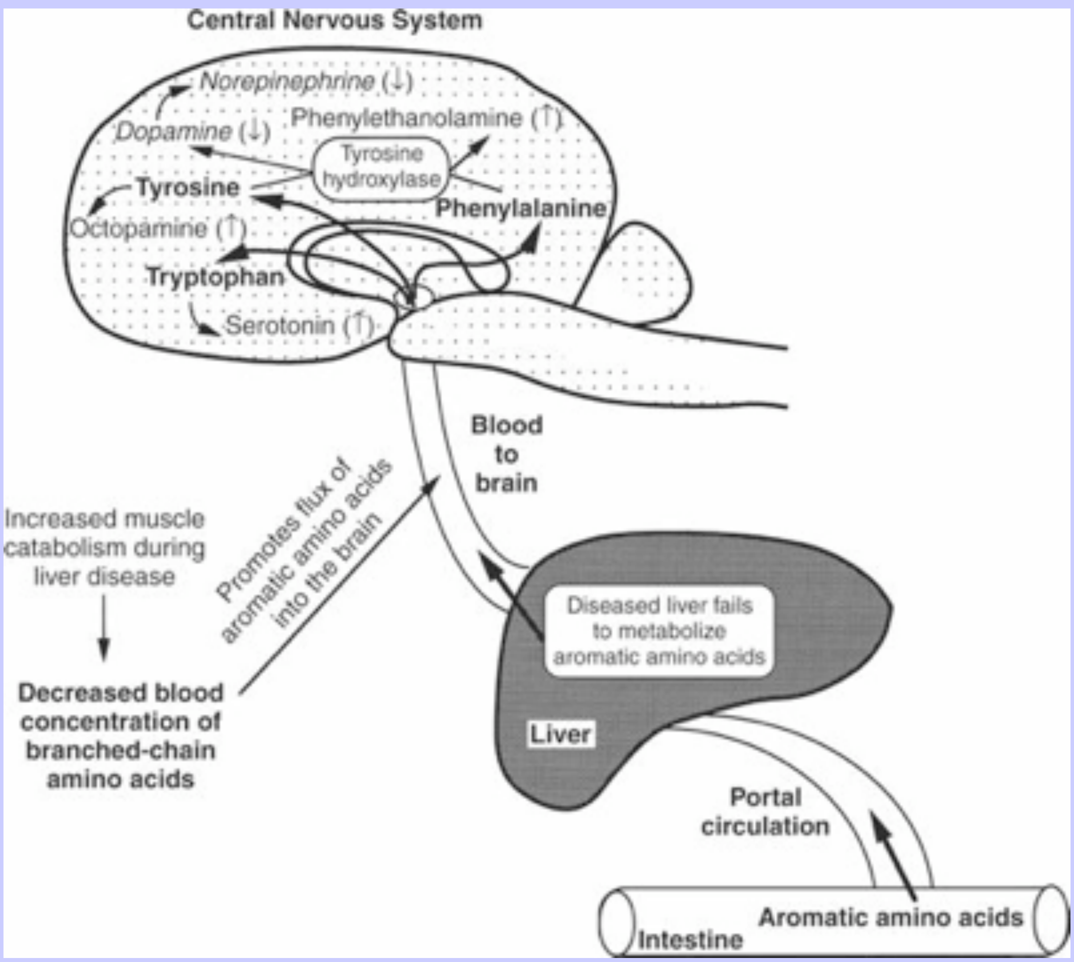
A separate hypothesis holds that during liver failure, true neurotransmitters in the CNS such as norepinephrine and dopamine become depleted and false neurotransmitters, especially octopamine and phenylethanolamine, increase.[25,37] The net neurophysiologic effect of such changes is reduced neuronal excitation and increased neural inhibition. The mechanism of this effect is related to the increased serum concentrations of aromatic amino acids (AAAs: phenylalanine, tyrosine, tryptophan) and decreased concentrations of branched-chain amino acids (BCAAs: valine, leucine, isoleucine) that occur in liver failure.[38,39] Serum glucagon increases in hepatic failure, leading to muscle catabolism and release of amino acids. However, hepatic metabolism of AAAs is reduced, and because BCAAs are metabolized by muscle and adipose tissue, a relative increase in AAAs and decrease in BCAAs occurs. The decreased plasma BCAA:AAA ratio during liver failure and increased brain glutamine concentration (presumably a consequence of ammonia retention) are considered to promote an influx of AAAs into the brain and efflux of glutamine from the brain by exchange transport processes at the blood-brain barrier.[24] Phenylalanine can compete with tyrosine for tyrosine hydroxylase, resulting in decreased production of dopamine[39] (Figure 14-6). The displaced tyrosine may be decarboxylated to tyramine and then converted to the false neurotransmitter octapamine. Accumulated tyrosine also competes for dopamine β-oxidase and reduces the formation of norepinephrine. Phenylalanine and tryptophan in the CNS ultimately are converted to phenylethanolamine and serotonin, a false neurotransmitter and a neuroinhibitor, respectively. Tryptophan also is metabolized to serotonin and oxindole, which has a strong sedative effect.[32]

Consistent with this theory are the observationsof increased serum concentrations of AAAs accompanied by increased cerebrospinal fluid concentrations of octopamine, serotonin, and phenylethanolamine in patients with HE.[37] However, octapamine alone cannot induce encephalopathy, the plasma BCAA:AAA ratio correlates poorly with HE in human beings,[40] and controlled clinical trials of oral or intravenous BCAA therapy do not indicate consistent amelioration of signs of HE.[39]

A recently popular theory regarding the pathogenesis of HE involves augmented activity of inhibitory neurotransmitter systems, GABA-benzodiazepine and serotonin, and depression of the function of the excitatory glutamatergic system.[32] When released from presynaptic neurons, GABA binds to specific receptors on postsynaptic neurons, resulting in increased chloride ion conductance across the postsynaptic neural membrane, membrane hyperpolarization, and generation of an inhibitory postsynaptic potential.[41] The GABA receptor is a chloride ionophore supramolecular complex that has interactive binding sites for three classes of synergistic ligands: GABA and agonists, benzodiazepines, and barbiturates.[24,42] The binding of benzodiazepine or a barbiturate on its binding site of the GABA receptor potentiates GABA-induced sedation. The GABA hypothesis of HE originally was based on a series of observations using a model of HE in rabbits in which increased GABA-like activity was identified in the serum and cerebrospinal fluid along with an increased density of GABA receptors.[41] Increased brain levels of natural benzodiazepines probably constitute one mechanism for the increased GABAergic tone in HE.[32] Ammonia, which acts synergistically with natural benzodiazepines, also may enhance GABAergic neurotransmission. Agonists of benzodiazepine increase the frequency of GABA-induced chloride channel openings, and barbiturates lengthen the average time that channels are open.[43] Results of studies using electrophysiologic and in vitro techniques to study an animal model of HE provide strong evidence for a functional increase in 960
GABAergic tone that is mediated allosterically through the benzodiazepine receptor by an endogenous 961

diazepam-like substance.[44,45] Clinical studies that showed improved consciousness and reduced electroencephalogram changes of HE in patients treated with the benzodiazepine receptor antagonist flumazenil support this suggestion.[46] Recent evidence also points to zinc deficiency, which is important for the urea cycle, in the pathogenesis of HE.[32]

Figure 14-6 Role of aromatic amino acids in the brain in hepatic encephalopathy. Aromatic amino acids (tryptophan, tyrosine, phenylalanine) enter the CNS, where they are metabolized, altering the balance of neurotransmitters. True neurotransmitters (dopamine, norepinephrine) decrease *(down arrow)*, whereas false neurotransmitters (octopamine, phenylethanolamine) increase *(up arrow)*. The net effect is increased neuronal inhibition and reduced neuronal excitation.



14.2.3.2.3

Diagnosis

The diagnosis of HE is based on neurologic signs of cerebral dysfunction in a horse with physical examination and laboratory findings compatible with liver disease (see Diagnosis and Laboratory Findings of Hepatic Insufficiency). *One should exclude other possible causes for the neurologic signs because no specific features of HEallow one to distinguish this syndrome definitively from other encephalopathies*. A partial list of conditions to be ruled out includes trauma, viral encephalomyelitis, rabies, moldy corn toxicity (leukoencephalomalacia), brain abscess, equine protozoal myeloencephalitis, parasite larval migrans, blister beetle toxicosis, organophosphate toxicity, nigropallidal encephalomalacia, botulism, fluphenazine or other sedative overdose, and heavy metal toxicosis. Many of these conditions have other characteristic clinical signs, the absence of which would exclude them from the differential diagnosis. One carefully should determine access to potential toxins or drugs from the history.

One should perform serum electrolyte testing, including calcium, and obtain creatinine values and a complete blood count to aid in ruling out other encephalopathies. Serologic testing for the viral encephalitides and toxicologic screening for organophosphates and heavy metals may be appropriate. A lumbar spinal tap for cerebrospinal fluid analysis may be indicated if other causes of encephalopathy are highly suspect. The cerebrospinal fluid is normal in horses with HE. In human beings, electroencephalogram changes of symmetric generalized slowing of cerebral electrical activity are sensitive indicators of HE but are not specific for this disorder because other metabolic encephalopathies can cause 961
similar abnormalities.[23,25] Visual evoked potentials are superior to conventional electroencephalograms in 962
terms of specificity and ease of quantitation.[47] An average visual evoked potential reflects the pattern and magnitude of postsynaptic neuronal activity evoked by a visual afferent stimulus. Hepatoencephalopathy in human beings is attended by a distinctively abnormal visual evoked potential trace; however, this testing would be technically difficult in horses and has not been explored. As a general rule, the brain shows no definite light or electron microscopic structural changes; however, some patients with hepatic cirrhosis and portosystemic shunts have an increase in the number and size of astrocytes (Alzheimer's type II) in the gray matter of the cerebrum and cerebellum.[24] These changes appear to be reversible and specific for portosystemic shunting of blood. The relevance of these changes, if any, to behavioral dysfunction of HE is unknown.

14.2.3.3

Icterus

Icterus, or *jaundice*, is caused by *hyperbilirubinemia* with subsequent deposition of the pigment in tissues causing yellow discoloration. Icterus is most apparent in nonpigmented skin; mucous membranes, especially the vulvar mucosa; and the sclerae. Approximately 10% to 15% of horses normally have slightly yellow sclerae.[20] Disease states that result in hyperbilirubinemia can be categorized as follows: increased production of bilirubin, impaired hepatic uptake or conjugation of bilirubin, and impaired excretion of bilirubin.[17] Increased production of bilirubin occurs with hemolysis, intravascular and extravascular, and following reabsorption of erythrocytes after massive intracorporeal hemorrhage. This form of hyperbilirubinemia, often called *hemolytic* or *prehepatic icterus*, occurs despite a normally functioning liver, because the rate of bilirubin production by the reticuloendothelial system temporarily exceeds the ability of the liver to conjugate and excrete. Classically, this form of icterus is caused by the increased concentration of unconjugated bilirubin in the blood. However, on occasion the concentration of conjugated bilirubin also mildly increases in the blood because of hepatic spillover when the liver processes the excessive bilirubin or the enterohepatic

circulation reabsorbs it. The presence and intensity of hemolytic icterus are determined by the rate and extent of erythrocyte destruction and the rate of uptake of bilirubin by the liver.

Impaired uptake and conjugation of bilirubin also result in increased blood levels of unconjugated bilirubin with subsequent icterus. This form of icterus is referred to as *retention* or *hepatic icterus* and is the most common form in horses with liver disease, usually the result of acute hepatocellular disease.[12,17,20] In horses the presence of icterus is highly inconsistent with chronic hepatocellular disease.[11,22] In addition to hepatocellular disease, certain drugs, anorexia, or prematurity can impede bilirubin uptake and conjugation by hepatocytes, despite an otherwise normally functioning liver.[4,11,12,17] Steroids can inhibit bilirubin uptake in all species. Heparin administration to horses sometimes results in icterus and is believed to be caused in part by impaired uptake of bilirubin by hepatocytes.[11] Anorexia in horses causes variable degrees of hyperbilirubinemia and may be related to the half-life of ligandin.[11,20] Ligandin is the intrahepatic protein responsible for extracting unconjugated bilirubin from albumin in the sinusoidal blood. The half-life of ligandin is short (days), and starvation in other species reduces the store of ligandin in the hepatocytes, thus impeding bilirubin uptake.[4] Premature and neonatal foals are also more susceptible to retention icterus in the absence of hepatic disease. The cause of icterus in equine neonates presumably is lower hepatocellular ligandin concentrations compared with the adult.[48] In human beings, inherited or congenital deficiencies in enzymes responsible for conjugation (bilirubin-uridine diphosphate glucuronyl transferase) may result in intermittent or persistent icterus (Gilbert's syndrome or Crigler-Najjar syndrome type II) that often is not recognized until the patient is several years of age. The icterus occurs without other clinical or laboratory evidence of liver diease.[20] Persistent hyperbilirubinemia without anorexia, hemolysis, or evidence of acquired liver disease was reported in an otherwise healthy 4-year-old Thoroughbred gelding.[49] Conjugated bilirubin concentrations were normal, but total serum bilirubin concentrations ranged from 9.0to 12.3 mg/dl during a 2½-year monitoring period. The case most closely resembled Gilbert's syndrome.

If the excretion of conjugated bilirubin into the biliary tract is impeded, *regurgitation icterus* occurs.[17] Blockage of bile flow with resultant regurgitationicterus can accompany cholangitis, hepatitis, obstructive cholelithiasis, neoplastic infiltration, fibrosis, or hyperplasia of the biliary tract. Because conjugated bilirubin is water soluble, this form of icterus may be accompanied by bilirubinuria.

In hepatocellular disease, icterus most often results from a combined increase in unconjugated and conjugated bilirubin.[17] Of the two fractions the majority of the increase in the total bilirubin is from unconjugated bilirubin. Increases in the conjugated fraction greater than 25%of the total usually indicate hepatocellular disease, and increases greater than 30% usually indicate cholestasis.[12]

14.2.3.4 Weight Loss

Significant weight loss and failure to thrive are most consistently present during chronic hepatic insufficiency. However, chronic liver disease may be present without apparent weight loss. Weight loss is caused by anorexia and the loss of normal hepatocellular metabolic activities. 962

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14.2.3.5 Hepatogenic Photosensitization

Photosensitization refers to abnormally heightened reactivity of the skin to ultraviolet sunlight because of the increased blood concentration of a photodynamic agent. In the case of hepatogenic photosensitization, the

photodynamic agent is *phylloerythrin*. Phylloerythrin normally is formed in the gastrointestinal tract as a result of bacterial degradation of chlorophyll and is absorbed into the general circulation, conjugated, and excretedby the liver.[50] During hepatic insufficiency, the blood concentrations of this photodynamic agent increase. Subsequent exposure of phylloerythrin to ultraviolet light causes activation of electrons within the molecule to an excited state, with resultant free radical formation. The local production of free radicals causes cell membrane damage and necrosis. Unpigmented areas absorb ultraviolet light most efficiently, thus the lesions of photosensitization are restricted to white skin. The skin first appears erythematous and edematous. Pruritus, pain, vesiculation, ulceration, necrosis, and sloughing may ensue.

14.2.3.6 Colic, Diarrhea, Tenesmus, Ascites, and Steatorrhea

Abdominal pain associated with acute hepatocellular disease may result from acute hepatic swelling or biliary obstruction (cholelithiasis).[4,51] Signs of anterior abdominal pain include anorexia, bruxism, sitting like a dog, recumbency, and rolling up onto the dorsum. Palpation over the last few ribs (especially on the right) or immediately caudal to the rib also may elicit a pain response. Alterations in intestinal motility also may cause concurrent signs ofcolic with liver disease. McGorum, Murphy, Love, et al.[22] recently reported that 10 of 25 horses with liver disease and signs of colic had clinically significant gastric impactions. Of the horses with gastric impactions, seven also had signs of HE and none of these horses survived.

Diarrhea infrequently may accompany chronic hepatic insufficiency in horses.[52] Alterations in the intestinal microflora, portal hypertension, and deficiency of bile acids may be involved in the pathogenesis.[4] Though uncommon in horses, portal hypertension can lead to increased hydrostatic and oncotic pressure in the intestinal mucosa, with resultant water and protein loss into the lumen of the bowel and the peritoneal cavity (ascites). Tenesmus may result from constipation but also has been reported to be a sign of hepatoencephalopathy.[22]

Decreased excretion of bile may result in lipid malabsorption and excessive amounts of fat in the feces, or *steatorrhea*,[4] which subsequently may cause osmotic diarrhea. Because the normal equine diet is low in fat, steatorrhea is rare in horses. Chronic cholestasis may cause clay-colored feces because of lack of fecal urobilinogen and stercobilin. This coloration rarely is observed in adult herbivores, because the normal fecal color is generated primarily by plant chlorophylls and not by bilirubin metabolites.[12]

14.2.3.7 Hemorrhagic Diathesis

Because the liver is responsible for synthesizing numerous factors involved in coagulation and fibrinolysis, abnormal hemostasis may be a sequela to hepatic insufficiency. Clinical signs may vary from petechial or ecchymotic hemorrhages to hemorrhage after trauma or venipuncture to spontaneous hemorrhage (epistaxis, melena, hemoptysis, hematuria, or hematomas).[4,12,17,53,54] Especially sensitive to hepatic disease is the synthesis of fibrinogen and the vitamin K–dependent factors (II, VII, IX, X, and protein C), which have short half-lives. Factor VII has a half-life of only 4 to 5 hours. Other vitamin K–dependent factors and fibrinogen have half-lives in the range of 4 to 5 days. Because vitamin K is fat soluble and requires bile acidsfor proper absorption from the intestinal tract, vitamin K–dependent factors are affected particularly during hepatic insufficiency when bile excretion is decreased.

During hepatic insufficiency, the synthesis of protein C and antithrombin III may be altered. Decreased plasma concentrations of these two anticoagulants would resultin uncontrolled clot formation and

consumption of other coagulation factors. In chronic hepatic disease the plasma concentration of protein C is normal or decreased; however, antithrombin III may be normal, increased, or decreased. Pregnant women with fatty livers have decreased antithrombin III activity, but patients with biliary cirrhosis or biliary obstruction have increased antithrombin III activity.[53] Horses with liver disease have increased antithrombin III activity and theoretically should tend to bleed.[55] Alterations in the factors controlling fibrinolysis vary in chronic liver disease.[53] Conditions that promote fibrinolysis—such as increased plasminogen and plasminogen activator, or decreased plasminogen activator inhibitor, α_2-antiplasmin, and α_2-macroglobulin—result in bleeding tendencies. Conditions that aid thrombus formation, such as decreased plasminogen, further promote consumptive coagulopathy. The fibrinolytic factors have not been evaluated in horses with liver disease.

Finally, the liver plays an important role in balancing normal hemostasis by Kupffer's cell removal of activated coagulation factors and fibrin degradation products from the general circulation.[53] Failure to remove activated coagulation factors further promotes coagulation, and fibrin degradation products interfere with platelet function and fibrin clot formation.

14.2.3.8 Fever

Horses with hepatic abscesses, acute hepatitis, chronic active hepatitis, obstructive cholelithiasis, fatty liver failure, or neoplasia may have constant or intermittent fevers.[51,56–61] 963

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14.2.3.9 Hemolysis

Hemolysis is a rarely seen but grave prognostic indicator of fulminant hepatic failure in horses.[19] The exact cause of hemolysis is not known but is believed to result from increased erythrocyte fragility.

14.2.3.10 Pruritus and Seborrhea

Retention of bile acids and accumulation in the skin may cause pruritus and seborrhea. This finding is reported rarely for horses.[19,62]

14.2.3.11 Edema

Hypoalbuminemia and water retention can occur with chronic liver failure and may result in dependent edema. Because the half-life of albumin is long (19 to 20 days) in the horse, edema is a rare clinical sign.[63] Ponies with hyperlipemia may develop dependent abdominal edema following vascular thrombosis.[60] Dependent abdominal edema also may form if significant portal hypertension and ascites exist.

14.2.3.12 Endotoxemia

The Kupffer's cell plays an important role in removing bacterial endotoxin that normally is absorbed from the lumen of the intestinal tract and carried to the liver via the portal circulation.[16] Failure of Kupffer's cell phagocytosis of endotoxin may result in clinical and laboratory evidence of endotoxemia.

14.2.3.13 Polydipsia, Polyuria, and the Hepatorenal Syndrome

Alterations in renal function, including deranged sodium concentrations, impaired water excretion, and urine concentrating ability, may accompany severe liver disease.[4,64] Sodium retention results from increased blood aldosterone concentrations caused by failure of hepatic biotransformation and a decrease in the effective circulating blood volume resulting from portal hypertension and hypoalbuminemia. Sodium retention raises the osmolality of the extracellular fluid, thereby stimulating the thirst center. Polydipsia has been reported in horses with chronic liver disease.[65,66] Despite the potential increase in exchangeable sodium, the serum sodium concentration is usually normal or decreased, resulting from superimposed water retention. The mechanism for water retention is multifactorial, but increased antidiuretic hormone, reduced effective circulating volume, and altered renal prostaglandin synthesis most likely are involved.[64] The urine concentrating ability is impaired sometimes because of reduced medullary interstitial urea, the net effect being polyuria or isosthenuria or both.

Hepatorenal syndrome is characterized by acute azotemia and anuria and may occur in ponies with hyperlipemia and hepatic lipidosis (see Hyperlipemia and Hepatic Lipidosis). The pathogenesis is obscure, but speculative causes include reduced effective circulating volume, decreased hepatic inactivation of renin, and endotoxemia.[64]

14.2.4 DIAGNOSIS AND LABORATORY FINDINGSOF HEPATIC INSUFFICIENCY

Historical information, as discussed under Specific Hepatic Diseases, may be useful in diagnosing certain types of hepatic insufficiency. The definitive diagnosis of hepatic disease in horses is confounded by nonspecific clinical signs and variable laboratory findings. Paramount to the laboratory diagnosis of hepatic insufficiency in horses is knowledge of the sensitivity and specificity of the tests (Table 14-2). Because massive hepatic disease must be present before alterations are apparent with some laboratory tests and because different liver functions are altered variably by disease, the specificity of the laboratory diagnosis of hepatic disease increases with the magnitudeof abnormal findings. Laboratory findings also may be useful for therapeutic and prognostic considerations.

14.2.4.1 Evaluation of Bilirubin

Serum bilirubin concentration is not a sensitive indicator of liver disease in horses. In one report on serum biochemical changes in horses with liver disease, only one fourth of the horses had increased serum bilirubin concentrations.[22] The *total bilirubin* concentration in the blood, as determined by the van den Bergh test, is a combination of unconjugated and conjugated bilirubin. Because the diagnostic value of the bilirubin concentration, when used for evaluation of hepatic disease, depends on which subfraction is increased, one must determine the concentrations of unconjugated and conjugated bilirubin.

The serum bilirubin concentration is stable for several days, if one protects the sample from sunlight. One first determines the total bilirubin concentration in a chromogenic assay by reaction for 30 minutes with a diazo reagent (sulfanilic acid and sodium nitrite) and methyl alcohol.[12] One similarly determines *conjugated* or *direct reacting bilirubin* over 5 minutes without the additionof methyl alcohol. One then determines the amount of *unconjugated bilirubin* by the difference between the total bilirubin concentration and the direct reacting bilirubin. Because one determines the unconjugated fraction arithmetically, the fraction is called

appropriately *indirect reacting bilirubin*. In normal horses the total bilirubin concentration ranges from 0.2 to 5.0 mg/dl (3.4 to 85.5 μmol/L) with conjugated bilirubin ranging from 0 to 0.4 mg/dl (0 to 6.8 μmol/L).[17] As discussed previously, increases in the unconjugated bilirubin fraction may occur without hepatic disease. 964
Hemolysis, anorexia, intestinal obstruction, cardiac insufficiency, Gilbert's syndrome, and the administration 965
of certain drugs (steroids, heparin, halothane) may cause an increasein the unconjugated bilirubin concentration.[11] If the unconjugated bilirubin concentration is increased, one should evaluate the erythron concurrently to rule out hemolysis as the causative factor. Hemolysis may cause the unconjugated bilirubin concentration to rise as high as 80 mg/dl (1368 μmol/L). Complete anorexia can cause an increase in the unconjugated bilirubin concentration within 12 hours; however, the concentration is unlikely to rise to greater than 6 to 8 mg/dl (102.6to 136.8 μmol/L) in horses suffering purely from anorexia[12]; however, values as high as 10.5 mg/dl have been reported in anorexic horses.[62] Finally, one must consider the age of the horse and concurrent drug therapy. Neonates normally have more unconjugated bilirubin than adults. The higher bilirubin concentration in foals most likely is caused by the turnover of fetal hemoglobin to adult hemoglobin and the deficiency of liver-binding and conjugating enzymes compared with the adult.[48] In the absence of liver disease, concurrent prematurity or illness can increase the unconjugated bilirubin fraction in foals further.

TABLE 14-2 Clinical Pathology of Liver Disease in Horses

TEST	ABERRATION	NORMAL VALUE
SPECIFIC INDICATORS OF LIVER DISEASE		
Serum sorbitol dehydrogenase	Increases	<8 units/L
Serum γ-glutamyl transferase	Increases	<25 units/L
Serum bile acids concentration	Increases	<15 mol/L
Arginase	Increases	
Glutamate dehydrogenase	Increases	
Direct bilirubin	>25% of total	0–0.4 mg/dl (0–6.8 μmol/L)
Ammonia	Increases	Laboratory dependent
Bromosulphthalein half-life	Prolonged	2.8 ± 0.5 minutes
Branched-chain amino acid to aromatic amino acid ratio	Decreases	3.5–4.5
Urine bilirubin	Increases	
NONSPECIFIC INDICATORS OF LIVER DISEASE		
Total bilirubin	Increases	
Indirect bilirubin	Increases	
Lactate dehydrogenase-5	Increases	
Aspartate aminotransferase	Increases	
Alanine aminotransferase	Increases	
Alkaline phosphatase	Increases	
Blood urea nitrogen	Decreases	
Globulins	Increases	
Albumin	Decreases	
Glucose	Decreases	
Prothrombin time	Prolonged	
Activated partial thromboplastin time	Prolonged	
Triglyceride	Increases	
White blood cell count	Increases with infection or inflammation; decreases with endotoxemia	

Keeping the foregoing limitations of interpretationin mind, increases in the unconjugated bilirubin fraction in horses with hepatic disease are most likely to occur with acute hepatocellular disease.[11,12,17] Rarely does the unconjugated bilirubin fraction in acute hepatic disease exceed 25 mg/dl (427.5 μmol/L).[12] An increase in the

bilirubin concentration may indicate hepatic disease but not necessarily hepatic failure. Furthermore, a normal bilirubin value, as commonly occurs in chronic hepatic disease, does not necessarily preclude the diagnosis of hepatic insufficiency.

An increase in the conjugated bilirubin fraction in horses more reliably indicates hepatic disease.[11,12,17] If the conjugated bilirubin concentration is greater than 25% of the total bilirubin value, one should suspect hepatocellular disease. If the conjugated bilirubin concentration is greater than 30% of the total value, one should suspect cholestasis.[12] Conjugated bilirubin is water soluble and detectable in the urine of horses only if blood concentrations sufficiently increase to surpass the renal threshold, thus when urine tests positive forthe presence of bilirubin, one should suspect cholestatic disease. Urine dipstick analysis is less sensitive than 965
analysis with a diazo tablet. One may detect urobilinogen by dipstick analysis in normal horse urine, and its 966
presence indicates a patent bile duct.[13] Because urobilinogen is highly unstable, one must determine the concentration in a fresh urine sample. Dilute or acidic urine may interfere with accurate determination of urobilinogen. Reagent strips are not sensitive enoughto detect the absence of urobilinogen: one must use Ehrlich's reagent. The absence of urobilinogen doesnot necessarily indicate liver disease but may be compatible with failure of excretion of bilirubin into the intestine, biliary obstruction, failure of intestinal bacterial reduction (diarrhea, overuse of oral antimicrobials), or failure to reabsorb it from the ileum. Increased concentrations of urobilinogen in the urine may be caused by the increased production of urobilinogen by intestinal bacteria, failure of the liver to remove it from the enterohepatic circulation, portosystemic shunting, or spillover following severe hemolysis.[4]

14.2.4.2 Serum Bile Acid Concentration

The normal liver removes greater than 90% of bile acids from the enterohepatic circulation. Thus the blood concentration of bile acids may be increased with liver disease, and quantitation provides an excellent screen of liver failure and essentially has replaced foreign dye clearance (e.g., Bromosulphthalein, or BSP) as a functional test of the liver.[11,66,67] Serum bile acids are stable for at least1 month if stored at −20° C and are measured by radioimmunoassay or by an enzymatic colorimetric method. The concentration of total serum bile acids is not affected by short-term fasting (<14 hours) but may be increased by more prolonged fasting.[68] The composition of serum bile acids varies greatly among normal horses, though the major serum bile acids are ursodeoxycholic acid, chenodeoxycholic acid, and deoxycholic acid.[69] Reported normal mean values for horses and ponies as determined by radioimmunoassay are 8.2 ± 1.6 μmol/L (n = 9)[66] and 5.3 ± 6.5 μmol/L (n = 51),[67] respectively, and 5.0 to 28.0 μmol/L[69] by the colorimetric method. Increased serum bile acid concentrations are highly specific for the presence of liver disease (may increase within 24 to48 hours after onset of hepatic disease) but are not specific for the type of liver disease.[11,70] Because bile acids are 90% restricted to the enterohepatic circulation, increases in the blood may result from shunting or decreased blood flow to the liver (first pass effect), failure of the liver to remove bile acids from the enterohepatic circulation, failure of the hepatocytes to conjugate the bile acids for excretion, or failure of excretion with subsequent regurgitation of the bile acids into the blood (biliary obstruction). Increases in the primary (nondegraded) bile acids cholate and chenodeoxycholate account for most of the increase in total serum bile acids concentration in horses with liver disease. In relation to the severity of liver disease, a significant shift in composition of serum bile acid profile from taurocholate to free unconjugated cholate also occurs. This finding suggests that hepatocellular excretion, and not resorption, is the most sensitive step in the enterohepatic circulation of bile acids in horses.[69]

Fasting for longer than 3 days in horses caused an increase in the serum bile acid concentration of 3 times over baseline values. Ligation of the bile duct caused a sixfold increase in serum bile acid concentration compared with fasted horses. Carbon tetrachloride toxicity resulted in a threefold increase compared with fasted horses.[68] Concentrations of serum bile acids greater than 50 μmol/L in horses with pyrrolizidine toxicosis were associated with a grave prognosis.[66] A value less than20 μmol/L appears to be a good predictor in ruling out significant functional liver disease and should be included in the evaluation of horses suspected to have hepatic disease. Serum bile acid concentrations greater than 20 μmol/L appear to indicate chronic liver disease but are less effective in detecting acute hepatic disease.[67] Bile acid concentrations are highest in biliary obstructive diseases and portosystemic shunts.

14.2.4.3 Tests of Protein Synthesis

The blood concentrations of protein or amino acids relate not only to the rate of synthesis by the liver but also to their half-life in the circulation. The half-life of albumin in horses is long (19 to 20 days), thus a decrease in the albumin concentration is rarely detectable until greater than 80% of the liver mass is lost for more than 3 weeks.[12,17,22,63] For the total serum protein to decrease below 5 g/dl (50 g/L) in chronic liver disease is unusual.[16] Hypoalbuminemia is a nonspecific finding in chronic liver disease, for it may occur following endoparasitism, nephrosis, malnutrition, malabsorption, circulatory failure, and many other chronic diseases.[20,63]

The globulin fraction often is increased in chronic hepatic disease because of decreased Kupffer's cell mass. Loss of Kupffer's cell function may result in wider dissemination of enteric-derived foreign antigens. Plasma cells respond to the general increased antigen load, resulting in polyclonal gammopathy.[4,9,12] Although the globulin fraction may be increased, it is a nonspecific finding with chronic hepatic disease, because polyclonal gammopathy can occur following numerous chronic diseases. A decreased serum albumin concentration concurrent with an increased globulin concentration in chronic hepatic disease causes the total plasma protein or serum protein to appear normal. Thus serum protein fractionation is paramount. Protein electrophoresis most accurately determines fractionation of the components making up the total serum protein.[9] 966

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The blood concentration of amino acids may be increased following acute hepatocellular necrosis or during protein catabolic states such as illness or starvation in response to insulin or glucagon.[4] Fractionation of the blood amino acids and determination of the BCAA:AAA ratio rarely is done in clinical practice but is more useful than evaluating either fraction separately (see Hepatic Encephalopathy). Decreases in this ratio indicate hepatic insufficiency. The risk of clinical signs of HE may be projected from the BCAA:AAA (phenylalanine, tyrosine) ratio. A normal ratio falls between 3.5 and 4.5. The risk of HE is low, medium, or high if the ratio is 3.0 to 3.5, 2.5 to 3.0, and less than 2.5, respectively.[38]

Because the liver is primarily responsible for removing ammonia from the circulation and converting it to urea for renal excretion, increases in the blood ammonia concentration or a decrease in the BUN concentration(<9 mg/dl; 6.43 mmol/L) may indicate chronic hepatocellular disease.[16,17,22] Daily blood ammonia levels vary widely in normal horses.[21] The compounding effects of ammonia-generating and urea-using bacteria in the gastrointestinal tract, ammonia generation with blood storage, and the effects of the ration may account partly for these fluctuations. One can refrigerate whole blood in EDTA for up to 6 hours without a significant increase in ammonia content.[71] One can evaluate the effect of the ration and handling by concurrently determining the blood ammonia concentration of a stable mate receiving a similar ration. Oral ammonia

challenge tests have not been evaluated fully in horses; however, the sensitivity of the oral challenge test may be decreased because of the effect of enteric bacteria. Normal values for ammonia vary among laboratories but have been reported in the range of 13 to 108 μg/dl (7.63 to 63.42 μmol/L).[19]

No correlation exists between the blood ammonia concentration and the severity of liver disease in horses, but increased blood ammonia concentration significantly is correlated with the presence of liver disease and with hepatoencephalopathy.[21,22] Although hyperammonemia appears to be a sensitive indicator of liver disease in the horse, it is not specific for liver disease. Encephalopathy associated with hyperammonemia without concurrent evidence of liver disease has been reported in horseswith acute gastrointestinal disease.[72,73] Hyperammonemia also has been reported as a fatal heritable disorder in Morgan foals and is believed to be caused by a defective mitochondrial transporter protein involved in urea synthesis.[74] The syndrome may be accompanied by mild to moderate increases in serum liver enzyme activities.

Because the liver is also responsible for the synthesis of certain coagulation factors, evaluation of hemostatic function may be useful. Changes in hemostatic function values are not specific for liver disease and must be evaluated in light of the other laboratory findings. The vitamin K–dependent factor with the shortest half-life is factor VII, thus abnormalities frequently are observed first in the *prothrombin time (PT)*.[21] However, adequate evaluation of hemostatic function necessitates determination of the *activated partial thromboplastin time (APTT)*, the *fibrinogen* and *fibrin degradation products* concentrations, and a platelet count. In one retrospective clinical review, almost half of the horses with liver disease had an abnormally prolonged PT or APTT.[22] Typically, a 50% to 70% decrease in the blood concentration of the coagulation factors is necessary before a change in these clotting time–based assays is detectable.[17] Daily variation in the normal values for clotting times also hinders accurate detection of the abnormal. The clotting times may be standardized more appropriately if one determines a clotting assay concurrently on a normal horse. If the ratio of clotting time (PT or APTT) of the patient with suspected hepatic disease to the normal value of the horse is greater than 1.3, one may interpret the test as abnormal.[75] The sensitivity of coagulation factor deficiency during hepatic insufficiency may be increased by diluting the plasma[76] or by determiningthe concentration of specific factors by clot-based, chromogenic, or radioimmunologic assays. These latter assays are not widely available.

One cannot detect hypofibrinogenemia accurately by the heat block precipitation method, the most common method for determining the plasma fibrinogen concentration.[9] A clotting assay using thrombin more accurately determines the fibrinogen concentration. Fibrinogen concentrations less than 100 mg/dl (1 g/L) indicate decreased production or increased consumption. The fibrin degradation product concentration, as determined by latex agglutination, may be increased during hepatic insufficiency because of decreased removal by Kupffer's cells. Concentrations of fibrin degradation products greater than 16 μg/dl indicate increased production or reduced removal. The plasma concentration of several factors acting as anticoagulants or involved in fibrinolysis also may be altered in chronic liver failure (see Hemorrhagic Diathesis). Tests for these factors are limited primarily to academic and research institutions.

Thrombocytopenia with associated petechiae or nasal mucosal bleeding has been reported in horses with liver disease, and thus in suspected cases of liver disease, one also should evaluate platelet counts before using invasive diagnostic or therapeutic techniques.[21]

14.2.4.4 Tests of Carbohydrate Metabolism

Changes in the blood glucose concentration rarely occur in horses with liver insufficiency.[16,22] Hyperglycemia may occur with stress-associated catecholamine and glucocorticoid release. Hypoglycemia (glucose <60 mg/ 967
dl;3.33 mmol/L) may occur in acute massive hepatic failure but is more likely in chronic liver disease as 968
anorexia progresses, glycogen stores are depleted, and gluconeogenesis and glycolysis are impaired by increased glucagon concentrations. If one administers glucose, often blood glucose levels remain abnormally increased, indicating tissue insulin resistance.[77] Insulin receptor number and binding affinity for insulin have been found to be diminished in human beings with chronic liver disease. Changes in the blood glucose concentration are not specific for liver disease and must be evaluated in the light of the other laboratory findings.

14.2.4.5 Tests of Lipid Metabolism

The concentration of blood triglycerides may become increased during hepatic insufficiency because of increased mobilization from adipose tissue to support energy-requiring processes and a decreased clearance by the liver.[4,12] In contrast, the VLDL and esterified cholesterol blood concentrations may be decreased because of failure of liver synthesis. Compared with other species, Equidae are thought to possess a greater clearance capacity for triglycerides and a greater hepatic exporting capacity for VLDLs. Thus changes in the blood VLDL, esterified cholesterol, or triglyceride concentrations occur rarely in horses.[20] An exception to this is the significant increase in blood triglyceride levels that occurs with hyperlipidemia syndrome in ponies and miniature horses (see Hyperlipemia and Hepatic Lipidosis). Because blood lipid concentrations may be altered by nonhepatic diseases, these tests are neither sensitive nor specific for liver disease in horses.[12] The blood cholesterol and triglyceride concentrations are normally higher in neonates compared with adults.[48]

Increased mobilization of triglycerides and fatty acid oxidation by the liver may result in increased production of *ketone bodies*, acetoacetate and β-hydroxybutyric acid.[10] Although the peripheral tissues may use ketone bodies, these compounds are weak acids and increased levels in the blood may result in ketoacidosis. The pathway for ketone formation is developed poorly in horses, thus ketoacidosis is not common.[20] One should suspect ketoacidosis in horses that are acidemic and have an abnormally high anion gap. One may quantitate ketones in the blood or urine. Because the renal thresholdof ketone bodies is low, ketonuria usually precedes ketonemia. Routine urine dipsticks only detect acetoacetate.[12]

14.2.4.6 Liver Enzymes

Acute hepatocellular necrosis or changes in hepatocyte membrane permeability result in the release of soluble cytosolic enzymes into the sinusoidal blood. Thus increased blood activity of these cytosolic enzymes may indicate active hepatic disease. One must exercise caution when evaluating increases in these enzymes because not all are liver-specific. Furthermore, some of these hepatocellular enzymes may be increased because of induction by drugs. Most of these enzymes are quantitated colorimetrically, thus hemolysis or lipemia may interfere with accurate evaluation. Furthermore, wide variation in values can exist because of age differences, stage of hepatic disease, and laboratory methodology. In horses the following cytosolic enzymes are liver-specific and are not inducible: sorbitol dehydrogenase (iditol dehydrogenase), arginase, ornithine carbamoyltransferase, and glutamate dehydrogenase.[12,17] Although increases in these enzymes in the blood

are highly specific for heptocellular disease, they are not specific for the type of disease. Significant increases occur following acute hepatic necrosis. Mild increases in these enzymes may occur following hepatic hypoxemia or toxemia resultingfrom endotoxemia, septicemia, transient intestinal disease, hyperthermia, or certain drugs (benzimidazole anthelmintics).[12,16,17]

Sorbitol dehydrogenase (SDH) or iditol dehydrogenase has been used widely to evaluate acute liver disease in horses.[16,48,78] The short half-life of this liver cytosolic enzyme makes it ideal for evaluating acute ongoing disease, for values usually return to baseline within 3 to5 days after a transient hepatic insult.[62] Its short half-life necessitates analysis within hours of collection. Storage of serum in the freezer (−15° C) or refrigerator results in lost of approximately 1% and 3.5%, respectively, of SDH activity per day.[79] Although mild variations exist between laboratories, the normal blood activity of SDH in horses is usually less than 8 units/L.[19] Foals 2 to 4 weeks of age may have SDH activity slightly greater than those of adult horses.[48,78] Increases in SDH have been reported after prolonged halothane anesthesia in horses.[80] *Arginase* is used in the Krebs-Henseleit cycle for urea synthesis and is found in highest activity in hepatocytes, though minute amounts also exist in renal tissue, brain, skin, testicles, and erythrocytes.[20] Increases in arginase are most indicative of acute hepatic necrosis. Like SDH, arginase has a short half-life. *Glutamate dehydrogenase* is found in hepatocytes, renal tissue, brain, muscle, and intestinal cells. Like SDH and arginase, glutamate dehydrogenase has the highest tissue activity in the liver, and increases of this enzyme in the blood can be considered specific for acute liver disease. The half-life of glutamate dehydrogenase is 14 hours.[20]

Other cytosolic enzymes include aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, alanine aminotransferase, and isocitrate dehydrogenase. These enzymes also are found with high activity in other tissues or are inducible. Thus increases in these enzymes are *not specific* for liver disease in horses. 968
Because some of these enzymes frequently are reported in equine biochemical profiles, they may serve as a 969
crude indicator of liver disease; however, one must recognize the limitations of their usefulness.

Aspartate aminotransferase (AST), formerly glutamic-oxaloacetic transaminase, is a cytosolic- and mitochondrial-bound enzyme that catalyzes the reaction responsiblefor aspartate biosynthesis from carbohydrates. Basically, all cells contain AST, but liver and skeletal muscle cells contain the highest activity. Cardiac muscle, erythrocytes, intestinal cells, and the kidney also are sources of AST. Hemolysis and lipemia falsely increase the value of AST.[17] Increases most frequently are associated with muscle damage but may occur following acute hepatic necrosis. Often the AST activity is normal in chronic hepatic disease.[16] The half-life of AST is long, and thus the blood activity may take greater than 2 weeks to decrease following acute hepatic disease. The AST value is most useful when analyzed with other tissue-specific enzymes. For instance, if a muscle-specific enzyme, such as creatine kinase, also is increased, an increase in AST most likely has its origin in muscle. Serial AST and SDH valuescan be useful for determining ongoing hepatic disease. If the SDH and AST values were increased initially but subsequent evaluation reveals a normal or decreasing SDH and elevated AST, then a favorable prognosis is indicated, because hepatic necrosis is most likely subsiding. The normal serum activity of AST is 98 to 278 units/L.[19] *Isocitrate dehydrogenase* has a distribution similar to AST.

Alanine aminotransferase, formerly glutamic-pyruvic transaminase, is responsible for alanine synthesis from carbohydrates. Increases may be evident with acute hepatic disease, but myositis also increases blood levels. Hemolysis falsely increases the value of this enzyme, and microsomal enzyme inducers (i.e., glucocorticoids) increase its production and release in the absence of liver disease. This enzyme is not useful for predicting liver disease in horses.[17]

Alkaline phosphatase (ALP) catalyzes the hydrolysisof monophosphate esters. This enzyme is bound to the mitochondrial membrane and thus does not leak into the blood with changes in cell membrane permeability or necrosis. Increases in the blood activity of this enzyme follow induction.[17] Cholestasis and certain drugs, including glucocorticoids, primidone, and phenobarbital, induce production and release of ALP. The ALP value most likely is increased in chronic or cholestatic liver diseases rather than acute or hepatocellular disease. Other tissues besides the liver contain ALP, including bone, intestine, kidney, placenta, and leukocytes; thus increases do not necessarily indicate cholestasis. Because of increased osteoblastic activity, foals have ALP values2 to 3 times those of adults.[20] Pregnancy, hemolysis, and gastrointestinal disease also cause increases in ALP.

Lactate dehydrogenase (LDH) is the name for five major isoenzymes located in liver, muscle, erythrocytes, intestinal cells, and renal tissue. Increases in LDH are not liver-specific unless the isoenzyme activity is determined. Isoenzyme 5 (LDH-5) is a useful indicator of acute hepatocellular disease in horses because values typically return to baseline within 4 days after a transient hepatic insult.[62,81] LDH-5 is also present in muscle, so increased serum LDH-5 activity is specific for hepatic disease if other indicators of muscle damage (i.e., creatine kinase) are normal.[82] LDH -5 is stable at room temperature for 36 hours.

γ-*Glutamyl transpeptidase* or γ-*glutamyltransferase (GGT)* is involved in glutathione metabolism and transfer of glutamyl groups.[17] γ-Glutamyl transpeptidase is associated primarily with microsomal membranes in the biliary epithelium. Cholestatis induces production and release of GGT. Renal tubule cells contain GGT, but this source is released into urine. The only other potentially significant source of GGT in the blood is pancreatic. Because pancreatic disease is rare in horses, the blood activity of GGT is considered specific for hepatic disease in horses. Some clinicians consider GGT to be the test of highest sensitivity when evaluating horses for evidence of liver disease. The half-life of GGT is approximately 3 days, and GGT is stable in serum for 2 days at room temperature or 30 days if frozen.[20] Mild increases may be evident following acute hepatocellular necrosis and may continue to rise for 1 to 2 weeks despite improvement in clinical signs.[62] Increases are more persistent in chronic disease, especially with cholestasis.[16] Foals 2 weeks to 1 month old may have greater GGT values than adults.[48,78] Normal values for GGT inadult horses typically are less than 30 units/L[19] butmay be 2 to 3 times greater in healthy donkeys, burros, and asses.[83] In chronic, nonactive hepatic fibrosis and focal hepatic disease, GGT may not be increased significantly. Increased serum GGT activity has been reported in horses with cholestasis following colonic displacement.

In summary, evaluation for liver disease in horses should include quantitation of at least SDH (or arginase) and GGT. However, one should not intrepret normal values for these liver enzymes necessarily as absence of liver disease.

14.2.4.7 Clearance of Foreign Dyes

In addition to clearance of endogenous substances from the blood, one can evaluate liver function following injection of an exogenous substance. One such exogenous substance that is removed by the liver, conjugated, and excreted into bile is BSP. Following intravenous injection of 2.2 mg BSP per kilogram body mass, one 969
determines a clearance half-life by periodically obtaining heparinized blood samples over 12 to 15 minutes. 970
One should exercise caution when injecting BSP, for it can be thrombogenic and irritating if administered peri-vascularly. One should collect blood samples for BSP quantitation from a site other than the injection site. Suggested collection times are 3, 6, 9, 12, and 15 minutes after injection. Collection of plasma at the suggested

times is not crucial; however, recording the exact time at which the samples were obtained is important to assure accurate determination of the half-life. One determines the half-life of BSP, thus the rate of extraction by the liver, by plotting the plasma concentrations against the collection times on semilog paper. The normal half-life of BSP in horses is 2.8 ± 0.5 minutes.[12]

The BSP half-life is prolonged when greater than50% of the hepatic function is lost.[12,17] The function test is useful in horses, especially to distinguish hepatoencephalopathy from other causes of abnormal behavior or cerebral signs and to test liver function in chronic liver disease when bilirubin, SDH, and GGT blood levels may be normal. Proper interpretation of the BSP half-life must take into account the state of hepatic blood and bile flow and the bilirubin and albumin concentrations. If the hepatic blood flow is decreased significantly, as may occur with hepatic congestion or portosystemic shunts, the rate of delivery of BSP to the liver will be decreased, thus the half-life of BSP in the plasma will be prolonged. Because BSP is bound to albumin for delivery to the liver, if the blood concentration of albumin is decreased greatly, a higher proportion of unbound BSP will be delivered to the hepatocytes, and the half-life of BSP will be shortened.[17] If the blood concentration of bilirubin is increased greatly, the bilirubin competes with BSP for binding sites and conjugating enzymes in the liver, thus apparently prolonging the half-life of BSP. If significant cholestasis is present, BSP excreted into the biliary tract is reabsorbed into the circulation, causing an apparent prolongation of its clearance. Thus although a BSP clearance test is useful as a test of hepatic function, one must interpret the results in light of these limitations. Furthermore, because pharmaceutical-grade BSP is no longer commercially available, this test is limited basically to academic and research institutions. Quantitation of serum bile acids concentration essentially has replaced BSP clearance as an indicator of hepatic function.

The determination of BSP clearance time has been suggested to be more useful for detecting liver disease than BSP half-life.[84] The proportionality transfer constants of clearance also have been suggested to be more useful in predicting hepatic disease than the BSP half-life alone.[85] The clearance time is the amount of dye irreversibly removed from the plasma per unit time. One gives a dose of 5 mg BSP per kilogram of body mass intravenously and collects heparinized blood samples2, 5, 10, 15, 25, and 30 minutes after injection. The BSP clearance time in fed normal horses is 10 ml/min/kg and in horses fasted 3 days is 6 ml/min/kg.[84]

The *indocyanine green* (Beckmann & Dickinson, Baltimore) clearance test has replaced the BSP clearance test in human beings.[4] Basically, the procedure and the limitations are the same as for the BSP clearance test. The indocyanine green clearance time in fed horsesis 3.5 ± 0.67 ml/min/kg and in fasted horses is 1.6 ± 0.57 ml/min/kg.[84] Although the clearance of indocyanine green is an excellent predictor of hepatic bloodflow and extraction rates, its current expense precludes routine use in horses. Another disadvantage is that quantitation requires a spectrophotometer that reads infrared wavelengths. These limitations have hindered evaluation of indocyanine green clearance times as a diagnostic test of liver insufficiency in horses.

14.2.4.8

Other Nonspecific Laboratory Findings

Increased formation and release of acidic metabolic products, including ketone bodies, lactate, pyruvate, and amino acids, contribute to acidemia. Other factors that may contribute to acidemia include diarrhea and loss of renal acidification because of impaired urea synthesis. Anorexia predisposes horses to hypokalemia.

Although infrequently reported in horses, aldosterone and water retention may cause deranged concentrations of sodium and isosthenuria (see Polydipsia, Polyuria, and the Hepatorenal Syndrome). Azotemia may occur with hyperlipemia.[65]

Cholangiohepatitis, chronic active hepatitis, or a focal hepatic abscess may cause an inflammatory leukogram, anemia of chronic disease, and hyperfibrinogenemia.[61] Primary polycythemia has been reported in horses with hepatocellular carcinoma and hepatoblastoma.[86–88] Because the liver synthesizes fibrinogen, widespread chronic hepatitis may mask an increase in this acute phase reactant protein.

14.2.4.9 Diagnostic Imaging of the Liver

Ultrasonography is a safe, noninvasive imaging technique that uses the reflection of high-frequency sound waves from tissue interfaces to produce a visual image. Ultrasonography of the liver in horses is limited by the ribs, the depth and size of the liver, and its anatomic location deep to the diaphragm and lungs. Mechanical sectoror linear array scanners with 3.0-MHz crystals are most effective.[89] One should use ultrasonography to assess the right and left sides of the liver and the liver shape, size, position, and texture. Hepatic veins are more anechoic than portal veins, and the biliary system is not normally visible. Ultrasonography of the liver is 970 most useful for determining the general size of the liver; changes in the 971 hepatic parenchyma, including abscesses, cysts, and neoplastic masses; and detecting dilated bile ducts or obstructions with choleliths. The common bile duct is not visible in a horse. Abnormal intra- or extrahepatic blood flow or vasculature may be detectable. Ultrasonography is also useful for guiding biopsy instruments into the liver (see Liver Biopsy).

Radionucleotide imaging also may be useful for detecting alterations in the hepatic parenchyma or blood flow.[90] Radionucleotide scanning noninvasively evaluates function and yields structural information. Two radionucleotide scanning techniques are available to evaluate the liver: liver scan and biliary scan. In the liver scan, one injects a technetium 99m–labeled sulfur colloid intravenously. ^{99m}Tc is a γ-emitting radioactive compound detectable in the body by a gamma camera. After injection, Kupffer's cells phagocytize the ^{99m}Tc sulfur colloid. Subsequent scanning with the gamma camera detects the radioactive emissions from the ^{99m}Tc and resolves them into a two-dimensional image. Thus alterations in blood flow (portosystemic shunt) or hepatic masses such as abscesses, cysts, and neoplasia may be detectable. In the biliary scan, one injects ^{99m}Tc-labeled iminodiacetic acid intravenously. The iminodiacetic acid is extracted by the hepatocytes, conjugated, and excreted in the bile.[91] Subsequent scanning images the biliary system and may be useful for detecting biliary obstruction including atresia, cholangitis, and cholelithiasis. Because radionucleotide imaging is expensive and requires a large gamma camera, this procedure is limited to research and academic institutions.

Operative mesenteric portography may be an option when one suspects a portosystemic shunt. In this procedure, one performs a celiotomy, injects radiopaque material into a mesenteric vein, and obtains rapid, sequential survey radiographs. Simultaneous opacification of the portal vein, azygous vein, and caudal vena cava or lack of filling of the intrahepatic portal system indicates portosystemic shunting.[90]

14.2.4.10 Liver Biopsy

A liver biopsy can yield important diagnostic, prognostic, and therapeutic information. One performs the procedure at the right twelfth to fourteenth intercostal spaces at the intersection of a line drawn from the tuber coxae to a point midway between the elbow and the point of the shoulder. One must perform the procedure in a sterile manner. The area is clipped, aseptically prepared, and injected with a local-acting anesthetic subcutaneously; then a stab incision is made with a No. 15 blade. One then inserts a Tru-Cut (Baxter-Travenol, St. Louis) or Franklin modified Vim-Silverman (Mueller & Co., Chicago) biopsy instrument and

directs it craniad and ventrad through the diaphragm into the liver. Semiautomatic biopsy instruments (EZ Core, Products Group International, Lyons, Colorado) and automatic biopsy guns (ProMag 2.2 Biopsy System, Manan Medical Products, Northbrook, Illinois) equipped with 14-gauge, 16-cm needles are helpful for a quick and accurate liver biopsy. One should place samples immediately in formalin for histopathologic evaluation and, if necessary, in transport media for culture of microorganisms. Precautions to consider before performing the procedure include the risk of hemorrhage, pneumothorax, peritonitis from bile leakage and colon or abscess puncture, and spread from infectious hepatitis. One may reduce these complications by performing a hemostasis profile to assess the risk of hemorrhage and by using ultrasonography to guide needle placement. Although subclinical coagulopathy has been reported frequently in horses with liver disease, clinically significant or fatal hemorrhage after performing a liver biopsy rarely is reported.[21,22]

14.2.4.11 Summary of Diagnostic Procedures

The most useful diagnostic tests for evaluating hepatic disease in horses are quantitation of SDH, GGT, and serum bile acids. In the face of clinically significant liver disease, at least one of the three former serum tests typically is abnormal. Although less useful in horses, additional tests of liver disease may include quantitation of bilirubin, ALP, AST, albumin, fibrinogen, globulins, glucose, esterified triglycerides, ammonia, BUN, BSP clearance, and amino acids. Serial testing of these indexes may increase their diagnostic and prognostic value. One should investigate abnormal laboratory findings further with ultrasonography and biopsy, following evaluation of a hemostasis profile.

14.2.5 TREATMENT OF HEPATIC INSUFFICIENCY

Management techniques for hepatic insufficiency are largely supportive. Therapies for specific liver diseases are discussed in more detail under Specific Hepatic Diseases. The basic goal of treatment is to maintain the animal until the liver regenerates enough to provide adequate function. Patients with severe hepatic fibrosis respond poorly because adequate regeneration often is not possible. Horses with signs of HE that are agitated, restless, or uncontrollable must be sedated to enable therapy; however, any medication should be used judiciously because most tranquilizers are metabolized by the liver or potentiate the abnormal neural function of HE. *Xylazine* or *detomidine* in small doses are safest and most effective in these instances. The use of diazepam is contraindicated because it enhances the effect of GABA on central inhibitory neurons and may exacerbate signs of HE.[32] One should correct fluid deficits and acid-base or electrolyte imbalances by administering fluids intravenously. 971
Because most horses with HE are anorectic and blood glucose may be decreased, continuous intravenous 972
infusion of *5% dextrose* at a rate of 2 ml/kg/hr can be beneficial.[92] If infusion continues more than 24 to 48 hours, substituting 2.5% to 5.0% dextrose in half-strength saline or Ringer's solution is recommended. If bicarbonate therapy is necessary to treat acidotic patients with hyperammonemia, the rate of administration should be monitored carefully because too rapid of a rate may increase blood levels of ammonia.[32] Hypokalemia or alkalosis result in increased renal production of ammonia and increased diffusion of ammonia into the CNS, thus treatment with potassium or acidifying fluids may be beneficial.[93]

The veterinarian also should direct therapy at reducing the production of toxic protein metabolites by enteric bacteria or by interfering with their absorption. The administration of *mineral oil* or magnesium sulfateper nasogastric tube is safe and aids in reducing toxin absorption. HE in human beings with acute fulminate hepatic failure almost always is associated with cerebral edema. Thus recommendations for treating severe acute HE in human beings include elective ventilation, mannitol (0.5 mg/kg body mass over 10 minutes), and continuous infusion of acetylcysteine.[94] Methods to reduce the production of ammonia and other enteric toxins include the

oral administration of antibiotics (neomycin, metronidazole, rifaximin, vancomycin) or lactulose or lactitol (not available in the United States).[93] *Neomycin* has been recommended at a rate of 10 to 100 mg/kg orally every6 hours.[57,95] This treatment significantly alters the gastrointestinal flora, may cause diarrhea in some horses, and predisposes horses to salmonellosis because most *Salmonella* species are not sensitive to neomycin. However, in one retrospective study in horses with liver disease, orally administered neomycin therapy subjectively was more helpful that lactulose in alleviating signs of HE.[22] *Lactulose*, a syrup containing lactose and other disaccharides, passes through the upper small intestine and then is metabolized by ileal and colonic bacteria to organic acids, thereby reducing luminal pH. Proposed beneficial mechanisms of action of lactulose include increased bacterial assimilation of ammonia, decreased ammonia production, ammonia trapping in the bowel lumen, enteric microflora changes, and osmotic catharsis.[24,96] A dose of 0.3 ml/kg of lactulose syrup every6 hours per nasogastric tube has been recommendedfor horses[95]; however, this therapy is expensive and may cause diarrhea. In human beings, lactulose also may be given as an enema when the oral route is unavailable.[94] When given orally 3 times a day at 333 mg/kg body mass, lactulose (Duphalac, Solvay Pharmaceuticals, Marietta, Georgia) significantly decreases blood ammonia values in healthy adult horses.[96] None of the horses developed diarrhea, though one horse foundered on the sixth day of treatment. Fecal pH did not decrease. As an alternative or adjunct to antimicrobials or lactulose, one can attempt modification of intestinal flora with probiotics such as *Lactobacillus acidophilus* or *Enterococcus faecium*.[93] Many clinicians prefer not to give antimicrobials or lactulose orally but to rely on a low-protein diet. Oral and intravenous formulations of *BCAAs* are commercially available for use in horses (BCAA Equine Sports Inc, Lake Forest, Illinois; Baxter Healthcare Corp., Deerfield, Illinois), but controlled clinical trials in human beings have not shown this therapy to ameliorate the signs of HE consistently.[39] Zinc is an important cofactor of urea cycle enzymes and may be deficient in human beings with HE. Oral supplemental with zinc is recommended in patients with zinc deficiency.[93,94]

Once the appetite returns, a horse with HE or chronic liver disease is best managed by dietary manipulation. The ration should be high in carbohydrates and low in protein, with the protein source optimally rich in BCAAs. A mixture of two parts beet pulp and one part cracked corn in molasses can be fed at a rate of2.5 kg/100 kg body mass per day divided into six or more feedings.[95] Sorghum, bran, or milo may be substituted for the beet pulp. Multiple small feedings are optimal in horses with liver disease because of impaired gluconeogenesis. Oat hay is the best roughage, followed by other types of grass hay.[92] Horses should be encouraged to graze in grass pastures, provided they are protected from the sun. Alfalfa and legumes should be avoided because of their high protein content; however, any caloric intake is important in liver failure and should be allowed even if it includes legumes. Oats, soybean meal, and fat are also not recommended.[97] One should administer vitamins B_1 and K_1 and folic acid parenterally weekly. Vitamin K therapy is most useful when cholestasis is present.[4]

A number of experimental drugs have been studiedin human beings and may have a future role in management of HE in horses. Regimens to stimulate hepatic regeneration include insulin and glucagon[98] and cytosolic extracts from regenerating liver.[99] None have been shown to be definitively effective in human beings. As discussed previously, benzodiazepine receptor antagonists have been suggested to induce clinical and electrophysiologic remissions of HE in human beings with acute and chronic liver failure.[24,100] This neuropharmacologic approach to treatment of HE holds great promise, although it may not be economically feasible in horses. In human beings with HE, treatment with *flumazenil*, a benzodiazepine antagonist, and *bromocriptine*, a dopamine agonist, have provided inconsistent results.[94]

Antiinflammatory drugs may be beneficial and include *flunixin meglumine*, dimethyl sulfoxide, and pentoxifylline. *Dimethyl sulfoxide* (0.5 to 1 gm/kg body mass given intravenously diluted to 10% for 3 to 5 days) 972

may help dissolve intrabiliary sludge or small calcium bilirubinate stones. *Pentoxifylline* has been shown to reduce hepatic fibrosis in human beings[101] and may be given to horses at 8 to 16 mg/kg every 8 to 12 hours. *Colchicine*, an antigout drug, and *cyclosporine*, a potentT helper cell immunosuppressant have been used toslow hepatic fibrosis in dogs and human beings. These drugs have not been evaluated fully in horses; however, colchicine has been ineffective in cases of pyrrolizidine alkaloid toxicity.[102]

If any drugs are to be administered to a horsewith hepatic insufficiency, altering the dosage may be necessary. Alterations in hepatic blood flow, albumin, and biotransformation during hepatic insufficiency may prolong the half-life, as well as the dosage interval. One should avoid using drugs that rely heavily on the liver for metabolism and excretion, such as chloramphenicol, erythromycin, and corticosteroids.[15]

14.2.6 PROGNOSIS

The prognosis for hepatic insufficiency in horses depends on the severity and type of the underlying disease. Horses with focal or submassive acute hepatic disease have the best chance for hepatic regeneration, thus they have a guarded to fair prognosis if adequate and appropriate supportive care is given. Patients with severe hepatic fibrosis and chronic liver disease have a poorer prognosis because of their inability to compensate for lost hepatic function. Despite the cause of liver disease, the presence of severe encephalopathy, intravascular hemolysis, profound acidosis, clinical signs of coagulopathy, diarrhea, bilateral laryngeal paralysis, greatly increased serum bile acid concentration, BSP half-life greater than 10 minutes, decreased serum albumin, and histologic evidence of bridging fibrosis have been associated independently with a poor to grave prognosis. If a horse survives more than 5 days after an acute transient hepatic insult, the prognosis is fair. Decreasing blood levels of serum bile acids and GGT are more likely to occur in horses that survive.[21] Therapy is generally unrewarding in horses with severe hepatic fibrosis.

14.3 Specific Hepatic Diseases

The variety of clinical signs with hepatic disease, coupled with the fact that the majority of the hepatic parenchyma must be affected before function is lost, makes the clinical distinction between acute and chronic liver disease a challenge. The onset of signs may be sudden, even with chronic disease. A history of progressive weight loss or failure to thrive may indicate chronicity. Although no single blood test is specific for distinguishing acute from chronic hepatic disease, the presence of hyperglobulinemia or hypoalbuminemia or both may suggest chronicity. Histopathologically, the hallmark evidence of chronicity is the presence of fibrosis. For the purpose of discussing specific hepatic disorders in the following sections, diseases have been classified as acute or chronic based on the known cause of the disease (Box 14-2).

14.3.1 ACUTE HEPATIC DISEASES

14.3.1.1 Theiler's Disease

Sir Arnold Theiler first described this disease in South Africa in 1918 following vaccination of horses against African horse sickness with live virus and equine-origin antiserum.[103] In 1934 and 1937, a similar disease was described in the United States following vaccination of horses against western equine encephalomyelitis using live virus and equine-origin antiserum.[58,104,105] Despite numerous accounts of this syndrome, the exact cause of Theiler's disease remains unclear. Today this disease is one of the most commonly described causes of

acute hepatic failure in horses.[19,58,106–108] Theiler's disease also is referred to as *acute hepatic necrosis, serum-associated hepatitis*, and *serum sickness*.

14.3.1.1.1

Clinical Signs

Theiler's disease is limited to adult horses, though one report describes subclinical disease in a2-month-old foal.[107] The onset of clinical signs of hepatic failure in Theiler's disease is acute to subacute and often rapidly progresses over 2 to 7 days. Most horses are anorectic and icteric; HE is reported in most cases. Sudden death, photodermatitis, hemorrhagic diathesis, fever, dependent edema, colic, and bilirubinuria may be present.[58,106,107] Although uncommon, some horses with Theiler's disease have an insidious history of chronic weight loss.

14.3.1.1.2

Epidemiology and Pathogenesis

The disease typically occurs sporadically, but outbreaks involving multiple horses on a single farm over several months have been reported. A pattern of seasonality may occur, with a larger percentage of cases presenting in summer and fall.[58,106,107] Frequently, horses with Theiler's disease have received an equine-origin biologic 4 to 10 weeks before the onsetof hepatic failure, hence the name *serum-associated hepatitis*. Equine-origin biologics that have been associated with Theiler's disease include vaccines or antisera for African horse sickness, eastern and western encephalomyelitis, *Bacillus anthracis*, tetanus antitoxin, *Clostridium perfringens, C. botulinum, Streptococcus equi,* influenza, equine herpesvirus type 1, and pregnant mare's serum.[16,58,106,107] Some reports suggest that lactating broodmares given tetanus antitoxin after parturition are particularly prone to Theiler's disease.[106–108]

Reports of outbreaks associated with parenteral injection of homologous live virus vaccine or antiserum suggest an infectious blood-borne viral cause.[58] The history, onset, clinical signs, and histopathologic 973
findings of Theiler's disease appear most similar to hepatitis B virus in human beings. Hepatitis B virus is 974
present in all body fluids and excreta and is transmitted primarily by parenteral injection.[109] Viral surface antigen or antibody to viral core protein is detectable in 75% to 90% of human patients with hepatitis B.[77] Newer techniques that detect viral DNA or DNA polymerase are even more sensitive and specific tests of infection. To date, these assays have been negative in horses with Theiler's disease. Furthermore, viral isolation on horses with Theiler's disease has been unsuccessful, the disease has not been transmitted experimentally by blood or tissue inoculation, and a large percentage of horses with Theiler's disease do not have a recent history of receiving an equine-origin biologic.[110,111] The sum of these negative findings does not necessarily exclude the possibility of a viral cause. However, ifsuch exists, other modes of transmission, infection, and establishment of disease must exist.

14.3.1.1.2.1

BOX 14-2 LIVER DISEASES OF HORSES

14.3.1.1.2.1.1

Acute Disease

Theiler's disease (serum-associated hepatitis)

Hyperlipemia

Tyzzer's disease

Infectious necrotic hepatitis (black disease)

Cholangiohepatitis

Acute biliary obstruction

- Cholelithiasis
- Colon displacement
- Hepatic torsion

Parasitic hepatitis

- *Parascaris equorum*
- Large strongyles
- *Echinococcus granulosus*
- *Schistosoma*

Toxic hepatopathy

- Plants
- Mycotoxins
- Chemicals
- Drugs
- Iron

Viral hepatitis

- Equine infectious anemia
- Equine herpes virus 1
- Equine viral arteritis
- Giant cell hepatopathy

14.3.1.1.2.1.2

Chronic Disease

Pyrrolizidine alkaloid toxicity

Clover poisoning

Chronic active hepatitis

Cholelithiasis

Gastroduodenal obstruction

Abscess

Neoplasia

- Metastatic infiltration of the liver
- Cholangiocarcinoma
- Hepatocellular carcinoma
- Hepatoblastoma
- Mixed hamartoma

Amyloidosis

Chronic hypoxia

14.3.1.1.2.1.3 Congenital or Heritable

Portosystemic shunt

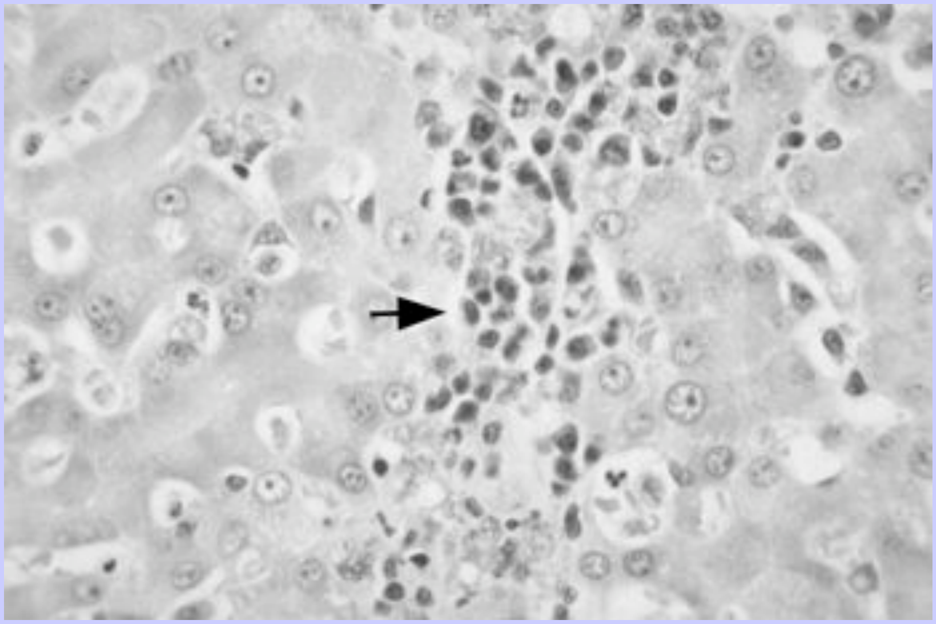
Biliary atresia

Hyperammonemia of Morgans

Other suggested causes of Theiler's disease include exposure to hepatotoxic substances, such as mycotoxins (aflatoxin and rubratoxin), plant toxins (pyrrolizidine alkaloids, alsike clover), drugs, or chemicals.[16,108] However, these causes have not been uniformly demonstrable and usually are considered separate disease entities.

Aside from widespread tissue icterus, the pathologic findings are limited to the liver. The liver appears smaller than normal but may be large in peracute cases. Although not pathognomonic, the histopathologic findings in Theiler's disease consistently include widespread centrilobular to midzonal hepatocellular necrosis with hemorrhage (Figure 14-7),[110] which helps distinguish it from acute pyrrolizidine alkaloid toxicity, which has predominantly periportal changes. Moderate to severe centrilobular hepatocyte vacuolar (fatty) change and granular swelling, hemosiderosis, and bile casts may be evident.[106] A mild inflammatory infiltrate, primarily monocytes and lymphocytes, is present in the portal triads. The occasional finding of 974
mild to moderate biliary hyperplasia and fibroblastic infiltration is evidence of a more chronic disease 975
course than the clinical signs alone suggest.[110]

Figure 14-7 Histopathology of the liver: Theiler's disease. The central area shows hepatic necrosis *(arrow)*.



14.3.1.1.3
Diagnosis

Recent inoculation with an equine-origin biologic, coupled with an abrupt onset of clinical signs and laboratory evidence of hepatic insufficiency, strongly suggests Theiler's disease. No single laboratory test is diagnostic. Bilirubinemia and bilirubinuria are usually present. Liver enzymes, including SDH, arginase, and AST, are increased. The BSP half-life, PT, and APTT often are prolonged, and blood ammonia concentrations are increased. Acidosis, leukocytosis, polycythemia, and increased serum creatine kinase activity also have been reported.[106–108] Hemolysis may occur terminally. The histopathologic findings on a liver biopsy (see preceding Epidemiology and Pathogenesis section) offer the strongest diagnostic evidence of Theiler's disease.

14.3.1.1.4
Treatment

No specific treatment for Theiler's disease exists aside from general supportive care for hepatic insufficiency (see Treatment of Hepatic Insufficiency).

14.3.1.1.5
Prognosis and Client Education

The prognosis is poor to grave in horses with severe HE, hemorrhage, or hemolysis. Horses that survive longer than a week usually recover completely, though death or progressive weight loss have been reported during the ensuing months.[107] A favorable prognosis is warranted if serial SDH activities are decreasing or clinical signs are waning. Increased serum activity of GGT may be sustained for several weeks, despite

clinical improvement and long-term survival.[106] No preventative exists, aside from the judicious use of equine-origin antiserum.

14.3.1.2

Bacterial Hepatitis

14.3.1.2.1

Tyzzer's Disease

Clostridium piliformis (formerly, *Bacillus piliformis*) is a motile, spore-forming, obligate intracytoplasmic bacterium that causes acute necrotizing hepatitis. The disease was first described by E.E. Tyzzer in 1917 in a colony of waltzing mice.[112] The first documented outbreak in foals occurred in Kentucky on a single farm between 1964 and 1973, fatally infecting23 foals.[113]

14.3.1.2.2

Clinical Signs

In horses, Tyzzer's disease is limited to foals between the ages of 7 and 42 days.[114–116] The clinical signs are usually nonspecific and include loss ofa suckle reflex, depression progressing to recumbency, fever, tachypnea, tachycardia, icterus, petechiation, diarrhea, dehydration, shock, seizures, and coma.[113–118] Often the foals are found dead without premonitory signs.

14.3.1.2.3

Epidemiology and Pathogenesis

Outbreaks of Tyzzer's disease are sporadic, thus the disease is not believed to be contagious.[116] Seasonality has not been demonstrated. The disease has been reported in the United States, Canada, South Africa, England, and Australia.[116,117] Mice with Tyzzer's disease are infected by an oral route perinatally, with clinical signs and death occurring between 6 and 44 days of age.[117] *C. piliformis* is excreted in the feces of clinically healthy horses and can survive in the soil for at least 1 year.[115,117] Foals are infected by eating contaminated feces of their dams or by ingesting contaminated soil. The bacteria replicate in the intestinal epithelium and reach the liver and heart by way ofthe lymphatics and blood supply.[115] In rodents and rabbits, administration of corticosteroids or sulfonamides may induce active disease.[117] The presence of antibody to the flagella of *C. piliformis* in the serum of healthy horses suggests that *exposure* to *C. piliformis* is not uncommon.[119]

C. piliformis causes acute multifocal hepatitis and enteritis. Grossly, the liver is swollen with 1- to 5-mm white foci scattered throughout the parenchyma. Tissues are icteric, and petechial hemorrhages are presentin many tissues. Microscopically, the foci are areas of coagulative necrosis with an associated inflammatory infiltrate consisting of neutrophils, macrophages, and lymphocytes. The organisms, best demonstrated atthe periphery of the lesions by Warthin-Starry or Dieterle's silver stains, appear as long bacilli organizedin bundles. In addition to hepatitis, enterocolitis, myositis, pleural effusion, pulmonary congestion and edema, and lymphoid necrosis or depletion may be present.[116,117]

14.3.1.2.4

Diagnosis

The peracute onset and nonspecific signs confound antemortem diagnosis of Tyzzer's disease. Hematologic study may reveal hemoconcentration, leukopenia or leukocytosis, a left shift, and toxic neutrophils.[114–117]

Other nonspecific laboratory findings include hyperfibrinogenemia, acidosis, hyperkalemiaor hypokalemia, and profound hypoglycemia. Liver enzymes are increased, including SDH, GGT, AST, ALP, and LDH-5.[114–118]

One makes the definitive diagnosis at necropsy by demonstrating the organism intracytoplasmically using silver stains. *C. piliformis* is fastidious and can be grown only in living tissue, such as embryonated chick eggs.[118] Positive serologic tests may support evidence of exposure.[119] Differential diagnosis for hepatic diseasein foals includes iron hepatotoxicity, perinatal equine herpesvirus type 1, bacteremia, atresia of the bile duct, and portosystemic shunt.

14.3.1.2.5 Treatment

Tyzzer's disease is highly fatal in foals. In rodents and rabbits, *C. piliformis* is sensitive to penicillin, tetracycline, erythromycin, and streptomycin. A report describes successful treatment of a foal with presumed Tyzzer's disease using penicillin, trimethoprim-sulfonamide (TMS), and partial parential nutrition.[114]

14.3.1.2.6 Prognosis and Client Education

Tyzzer's disease in foals is considered highly fatal. No preventative exists. 975

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14.3.1.3 Infectious Necrotic Hepatitis

Clostridium novyi type B is the cause of *infectious necrotic hepatitis* or *black disease*. The disease is most common in sheep and cattle, but several cases have been documented in horses.[56,120–123]

14.3.1.3.1 Clinical Signs

The onset of disease may be peracute with sudden death. Acute signs progress over 24 to 72 hours and include depression, reluctance to move, fever, icterus, ataxia, colic, petechiae, periods of recumbency, tachycardia, and tachypnea.[56,120–123] Some affected horses remain standing until a short time before death.

14.3.1.3.2 Epidemiology and Pathogenesis

Reported cases of infectious necrotic hepatitis in horses have occurredin or adjacent to areas with a large population of sheep.[56,120–123] In sheep and cattle, infectious necrotic hepatitis results from multiplication of *C. novyi* in areas of liver compromised by the migration of *Fasciola hepatica*. This fluke normally does not infest horses, but migration of any parasite through the liver may predispose the horse to infectious necrotic hepatitis. Some horses with infectious necrotic hepatitis had parasitic infestations, though migration of parasites in the liver at the timeof necropsy was not evident. The onset of disease inone case occurred 48 hours after administration of mebendazole.[56]

The carcass blackens rapidly after death because of engorgement of subcutaneous blood vessels; hence the name *black disease*. Often, necropsy findings include serosanguineous effusion in the pericardial sac, the thoracic and abdominal cavities, widespread hemorrhages and icterus, and multifocal areas (1 to 2 mm) of

coagulative hepatic necrosis. Smears or histologic sectionsof the hepatic lesions reveal large numbers of gram-positive rods.

14.3.1.3.3 Diagnosis

The acute and nonspecific nature of this disease makes antemortem diagnosis improbable. Liver-specific enzymes and bilrubin were mildly to moderately increased.[56,120,123] An abdominocentesis was performed on one horse that revealed serosanguineous peritonitis.[56] Definitive diagnosis is based on positive staining with fluorescein conjugated antiserum specific for *C. novyi*, or isolation of the organism at necropsy.[120,123] The organism is difficult to isolate and requires rapid tissue sampling and anaerobic conditions.

14.3.1.3.4 Treatment

Treatment with high doses of penicillin or ampicillin are indicated, in addition to general supportive care for hepatic insufficiency.

14.3.1.3.5 Prognosis and Client Education

Most reported cases of infectious necrotic hepatitis in horses were fatal.

14.3.1.4 Miscellaneous Causes of Bacterial Hepatitis

Primary bacterial hepatitis is rare in adult horses. Bacterial septicemia in neonates may result in multifocal hepatitis, though the disease rarely is accompanied by clinical signs of hepatic failure. Bacterial endotoxin, released during acute fulminant gram-negative infection or absorbedfrom the gastrointestinal tract following mural damage, may cause hepatic ischemia indirectly through hemodynamic alternations. Because Kupffer's cells normally phagocytose endotoxin, bombardment of the liver with endotoxin temporarily may impede the function ofKupffer's cells.

Primary bacterial cholangiohepatitis has been reported in horses, though more commonly it is secondary.[51,124–127] *Secondary cholangiohepatitis* in horses is a sequela to biliary stasis, cholelithiasis, chronic active hepatitis, hepatic neoplasia, pancreatitis, enterocolitis, intestinal parasitism, and intestinal obstruction. Clinical signs include anorexia, colic, fever, and icterus. HE may be present. Laboratory abnormalities may include leukocytosis; toxemia; hyperfibrinogenemia; increased activity of SDH, arginase, GGT, and direct reacting bilirubin; and septic or nonseptic peritonitis.[125,127] Definitive diagnosis is based on histopathologic findings on liver biopsy and bacterial isolation. Isolates are most commonly enteric organisms such as *Salmonella* species, *Escherichia coli*, and *Citrobacter, Klebsiella, Aeromonas*, and *Acinetobacter* species.[125–127] Treatment includes general supportive care for hepatic failure (see Treatment of Hepatic Insufficiency) and 4 to 6 weeks of antimicrobial therapy. One should base the antimicrobial therapy on culture and sensitivity results. Recommended antimicrobials for bacterial cholangiohepatitis in horses include trimethoprim-sulfonamide, ceftiofur, enrofloxacin, penicillin and gentamicin, ampicillin, and chloramphenicol.[51,124–127]

14.3.1.5 Viral Hepatitis

14.3.1.5.1 Equine Herpesvirus Infection in Foals

Pregnant mares infected with equine herpesvirus type 1 may abort in the third trimester or deliver stillborn or weak foals. Some foals may appear normal at birth but develop clinical signs of respiratory distress, icterus, fever, and severe depression.[128,129] Secondary bacterial septicemia is common. Despite profound hepatic necrosis in herpesvirus-positive foals, serum enzyme activities were not significantly different from premature foals or foals with septicemia.[129] Pathologic lesions of aborted fetuses or neonatal foals are similar and include severe pulmonary congestion, pneumonitis, bronchiolitis, hyaline membrane disease, and intralobular coagulative hepatocellular necrosis.[128,129] Intranuclear, acidophilic inclusions are present in hepatocytes and biliary epithelium. No specific treatment exists for equine herpesvirus infection. General supportive care and prophylactic administration of broad-spectrum antimicrobials are indicated. The prognosis is poor to grave. Most affected foals die within a few days of birth. Vaccination of pregnant mares is only partially efficacious for prevention. 976

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14.3.1.5.2 Equine Infectious Anemia

The causative organism of equine infectious anemia is a retrovirus that has a tropism for mononuclear phagocytes.[130] Although cyclic fever, anemia, icterus, edema, and weight loss are the systemic manifestations of the disease, the Kupffer's cells in the liver are a major site of infection. Icterus is caused by increased erythrocyte destruction and by acute hepatic necrosis.

14.3.1.5.3 Equine Viral Arteritis

Equine viral arteritis is caused bya togavirus and typically is manifested clinically by depression, fever, acute upper respiratory tract infection, abortion, petechiation, and edema. Following ingestion or inhalation, viral septicemia leads to vascular damage. Overt clinical signs of hepatic disease are rare, but vascular damage in the liver may cause icterus.[131]

14.3.1.5.4 Giant Cell Hepatopathy

A disease similar to human neonatal hepatitis has been reported in aborted midterm fetuses. Histologically, disorganization of hepatic cords, multifocal necrosis, a mild mononuclear cell infiltrate, and a large hepatocyte syncytium with 8 to 10 nuclei are evident. The cause in horses and human beings is unknown but is presumably viral. Viral and bacterial isolation, viral immunofluorescence, and serologic testing have been unsuccessful in horses. Giant cell hepatopathy was reported in one foal with prolonged neonatal isoerythrolysis.[132]

14.3.1.6 Parasitic Hepatitis

Parasitic infestation may cause focal hepatic disease but rarely overt hepatic insufficiency. Following their ingestion as embryonated eggs, larvae of *Parascaris equorum* hatch in the small intestine and then migrate through the liver and lungs. Focal or diffuse hepatic fibrosis may result. Migration of *Strongylus edentatus* or

S. equinus through the hepatic portal veins may cause focal hepatitis, subcapsular hemorrhage, and edema followed by focal parenchymal fibrosis and capsular fibrin deposits.[133] Focal hepatic infarcts may occur following thrombotic emboli caused by migrating *S. vulgaris* in mesenteric arteries. Protozoal schizonts consistent with a *Sarcocystis* species other than *Sarcocystis neurona* were reported at necropsy in the liver of a horse with concurrent bacterial osteomyelitis, a plasma cell tumor of the maxilla, and hepatic salmonellosis.[134] The canine cestode *Echinococcus granulosus* may form hydatid (larval) cysts in the liver, which are usually an incidental finding.[135,136]

Fibrosing granulomata were found in the liver, intestinal and diaphragmatic serosae, and lung of several horses submitted for necropsy.[137] The granulomata consisted of dense fibrous tissue surrounding a necrotic, laminated, mineralized center. The periphery of the granuloma contained a small rim of inflammatory cells. The typical architecture of the granulomata, combined with the finding of a residual egg shell typical of *Schistosoma* (a trematode) in one horse, led to the conclusion that the granulomata were caused by chronic schistosomiasis. Although the granulomata were considered to be incidental findings of undetermined origin in most ofthe horses, they caused liver failure in one horse.

14.3.1.7

Toxic Hepatopathy

Numerous chemicals, drugs, mycotoxins, and plant toxins are hepatotoxic, but these rarely cause acute hepatic failure in horses. Clinical signs and routine laboratory diagnostics do not distinguish between these toxins, thus diagnosis largely relies on exclusion of other causes, history of exposure, and in some cases documentationof the toxin in the blood or liver. Some substances cause fatty change, cloudy swelling, necrosis, mild inflammatory infiltration, and fibrosis, primarily in the centrilobular location where the oxygen tension is lowest. Other substances cause the same lesions periportally in thearea of initial exposure. Some substances are directly hepatotoxic; others require biotransformation by the liver to toxic metabolites.

14.3.1.7.1

Hepatotoxic Plants

Plants best known for causing hepatic disease in horses are those containing pyrrolizidine alkaloids. Although pyrrolizidine alkaloid toxicity can cause acute hepatic necrosis, it more often results in hepatic fibrosis and is discussed as a separate entity under Chronic Hepatic Diseases. Other plants that have been reported to cause hepatic necrosis in horses in North America include kleingrass *(Panicum coloratum)*, *Lantana*, lecheguilla *(Agave lecheguilla)*, whitebrush (*Lippia* spp.), sneezeweed (*Helenium* spp.), blue-green algae (*Microcystis* and *Nodularia* spp.), lupine (*Lupinus* spp.), ryegrass, and some poisonous mushrooms (*Amanita* and *Galerina* spp.).[102]

14.3.1.7.2

Chemical Hepatotoxins

Horses rarely are exposed to hepatotoxic chemicals in sufficient amounts to induce hepatic failure. Potential hepatotoxic chemicals include arsenic (pesticide), carbon tetrachloride (fumigant), chlorinated hydrocarbons (insecticide), carbon disulfide (botacide), monensin (ionophore), pentachlorophenols (wood preservative, herbicide, fungicide), phenol (disinfectant, wood preservative), phosphorus (fertilizer), polybrominated biphenyl (fire retardant), and paraquat (herbicide).[138] All of these cause centrilobular necrosis, except phosphorus, which causes primarily periportal changes.

14.3.1.7.3

Drugs

Pharmaceutical agents can have wide range of effects on the liver depending on the drug type, dose, frequency, duration, route of administration, age of the animal, diet, and concurrent treatment.[139] The hepatic damage may be acute or chronic, cholestatic, zonal, vascular, or hypersensitivity-mediated. Certain drugs alter hepatocellular permeability without visible injury or loss of function. 977

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Drugs that are intrinsically hepatotoxic reproducibly cause hepatocellular necrosis.[138,140] Examples of intrinsic hepatotoxic drugs that cause zonal centrilobular necrosis include carbon disulfide and carbon tetrachloride.[138] Hepatocellular damage caused by these drugs is dose-related, and toxicity often results in hepatic failure. Anabolic steroids cause cholestasis with little or no evidence of hepatic damage or inflammation, resulting in mild icterus, which is completely and rapidly reversible once the drug is discontinued.[139,140] Phenothiazines and macrolide antibiotics (erythromycin) cause cholestatic injury, which is accompanied by significant hepatocellular necrosis and periportal inflammation.[140] Recovery usually is expected after discontinuation of the drug. Some drugs, such as tetracycline, cause fatty infiltration of the liver but rarely cause liver dysfunction.

Idiosyncratic hepatotoxicity has been reported following administration of erythromycin, rifampin, tetracycline, isoniazid, halothane, fluothane, phenothiazines, dantrolene, diazepam, sulfonamides, phenobarbital, phenytoin, and aspirin.[102,138,140] Injury varies from mild focal hepatitis to massive necrosis. Idiosyncratic hepatopathy has a low incidence, is not dose related, and often resolves on discontinuation of the drug. Rarely, progressive liver failure ensues. Some idiosyncratic drug hepatopathies may have a pathogenesis similar to drug allergy. Others may be related to the biotransformation properties of the liver. For example, if an individual has enhanced biotransformation of a certain drug and the metabolite of the drug is more cytotoxic than the original unaltered drug, hepatic damage may ensue.

Finally, some drugs induce increased hepatic enzyme activities or alter hepatocellular permeability but do not cause significant hepatic damage or clinical signs. Examples include benzimidazole anthelmintics, phenobarbital, phenylbutazone, and corticosteroids. Compared with human beings and dogs, corticosteroids are apparently less likely to cause hepatopathy in horses; however, a report describes steroid hepatopathy in a horse that received an overdose of triamcinolone for treatment of pruritus.[141] Three weeks after the overdose, the horse developed muscle wasting, depression, polydipsia, and laminitis. Mature neutrophilia, lymphopenia, hyperglycemia, increased serum activities of AST and GGT, and multizonal hepatocellular vacuolation were reported.

14.3.1.7.4

Iron Toxicity

Acute, fatal toxic hepatopathy was reported in numerous newborn foals given an oral microbial inoculum.[52] The syndrome is reproducible if one gives *ferrous fumarate* orally. The incidence of hepatic failure was highest when the foals were given the inoculum before nursing. Colostrum contains abundant vitamin E, an essential cofactor for glutathione. Glutathione protects against free radical damage, a proposed mechanism for iron toxicity. Thus, presumably, foals that are given iron supplements before ingesting colostrum are most susceptible to iron toxicity.[142]

Clinical signs include HE, icterus, and peracute death. Laboratory abnormalities include increased blood levels of bilirubin, SDH, GGT, and ammonia, and reduced BCAA:AAA ratio, decreased glucose, and prolonged PT. At necropsy the liver was abnormally small, and histologic study revealed massive hepatic necrosis with blood-filled reticula. Some livers had mild biliary hyperplasia and periportal fibrosis. Supportive therapy may prolong life, but most foals with iron hepatotoxicosis die. Those foals that survived recovered fully.

Adult horses are less susceptible to iron toxicitiy; however, rare cases of hemochromatosis have been reported.[143,144] Hemochromatosis is characterized by tissue damage and dysfunction caused by deposition of hemosiderin in parenchymal cells. Hemochromatosis was diagnosed in three adult horses by histopathologic examination and use of Prussian blue stain, which detects iron deposition (hemosiderin pigment).[143] Excessive dietary iron was not apparent. All three of the horses had signs and laboratory evidence of liver disease. Serum iron concentration was normal, with no evidence of saturation of the iron transport system in the serum. Hemochromatosis was accompanied by bile duct hyperplasia and hepatic fibrosis. All three horses died or were euthanized because of deterioration. Hemochromatosis was diagnosed in a pony receiving approximately 4 times the daily recommended amount of iron.[144] The diagnosis was confirmed by histopathologic examination (severe periportal fibrosis, hemosiderosis, biliary hyperplasia) and increased serum and liver concentrations of iron.

14.3.1.7.5 Mycotoxins

Mycotoxicosis is more common in ruminants than in horses. The two most common mycotoxins affecting horses are *aflatoxin*, produced by the molds *Aspergillus flavus* and *A. parasiticus*, and *rubratoxin*, produced by the mold *Penicillium rubrum. Aspergillus* species grow on a variety of feedstuffs if the temperature, moisture, and carbohydrate content are sufficient.[138] Aflatoxins impair protein synthesis and carbohydrate metabolism.

The degree of hepatic damage and extent of clinical signs depend on the duration and extent of exposure to the mycotoxin. Most horses refuse to eat moldy feed, thus hepatic failure is uncommon. Pathologic findings include hepatocellular necrosis, fatty change, biliary hyperplasia, periportal fibrosis, and megalocytosis. No specific treatment exists for aflatoxicosis. Administration of activated charcoal or a cathartic shortly after ingestion may impede absorption. Mycotoxicosis is best prevented by avoiding moldy feed.

Fumonisin B1, the mycotoxin of *Fusarium moniliforme*, most frequently is associated with contaminated 978
corn and causes leukoencephalomalacia in horses. The mycotoxin also causes hepatocellular necrosis and 979
periportal fibrosis. Although most horses with leukoencephalomalacia have hepatic disease, signs of leukoencephalopathy predominate and hepatic failure is rare. Recovery from hepatic disease is more likely than from the neurologic disease.

14.3.1.8 Acute Biliary Obstruction

Acute obstruction of the common bile duct causes icterus and signs of abdominal pain. Acute biliary occlusion may occur following cholelithiasis (see Cholelithiasis) or colon displacement. Intense icterus, colic, and increased levels of direct bilirubin (>85.5 μmol/L), GGT (>400 units/L), and bile acids (>150 units/L) were reported in two horses with acute colon displacements.[82] These abnormalities quickly abated after surgical correction of the displaced colon. Torsion of the left lobe of the liver was the cause of acute colic, hepatic

congestion, necrosis, and focal hepatitis in a 14-year-old Arabian gelding.[145] Resection of the affected lobe resulted in complete recovery.

14.3.1.9 Hyperlipemia and Hepatic Lipidosis

Hyperlipemia occurs primarily in ponies and Miniature horses and donkeys and may lead to fatty infiltration of the liver, often accompanied by clinical signs of liver disease and a poor prognosis. *Hyperlipidemia* refers the conditions causing an increase in serum trigylceride concentration (generally less than 500 mg/dl) without grossly lactescent blood or fatty infiltration of the liver. Obesity, stress, inability to satisfy metabolic energy demands, and hormonal imbalance are the major precipitating factors for hyperlipemia.[146–151]

14.3.1.9.1 Epidemiology and Clinical Signs

Ponies, especially Shetland ponies and mare ponies, and Miniature horses and donkeys are most susceptible to hyperlipemia. Rarely, other breeds such as the Quarter Horse, Paso Fino, and Tennessee Walking Horse may be affected. Affected ponies are usually obese, have a recent history of stress or weight loss, and are in late gestation or early lactation during the winter months.[60] In Miniature horses and donkeys, hyperlipemia can occur at any age and frequently develops as a sequela to an underlying primary illness of several days' duration. The most commonly reported primary diseases predisposing Miniature horses to hyperlipemia are enterocolitis, endotoxemia, parasitism, pituitary adenoma, azotemia, and neonatal septicemia.[150,151] The onset of clinical signs of hyperlipemia is often acute and includes icterus, anorexia, weakness, severe depression, ataxia, muscular weakness, recumbency, diarrhea, mild colic, fever, and dependent edema.[60,146,148] In severe cases, clinical signs of hepatic failure may prevail (see Clinical Signs of Hepatic Insufficiency). Sudden death caused by hepatic rupture may occur.[147] Signs of predisposing primary disease in Miniature horses may overshadow signs of hyperlipemia.

14.3.1.9.2 Pathogenesis

The liver serves a unique role in energy homeostasis (see Physiology), especially in large animals in which volatile fatty acids, and not glucose, are a major energy source. Glucose is manufactured primarily in the liver from fatty acids and amino acids and is stored as glycogen for future use. If intake is decreased or energy demands are increased, glycogen stores become depleted, and the major source of energy is provided by fatty acid oxidation. Obesity sets the stage for hyperlipemia by providing excessive adipose tissue stores of fatty acids, available for immediate and rapid mobilization. Fatty acid mobilization is usually first triggered by a stress or inability to maintain energy homeostasis. Concurrentor underlying primary diseases—such as enterocolitis, azotemia, infection, parasitism, or neoplasia or any stressful event such as transport or weaning—may precipitate fatty acid mobilization. Stress increases the release of catecholamines and glucocorticoids, which stimulate fatty acid release from the adipose tissue. A negative energy balance, as may occur during late gestation, early lactation, starvation, or following anorexia induced by some other primary disease, exacerbates hyperlipemia by further promoting fatty acid mobilization.

After lipolysis of adipose tissue triglyceride occurs, free fatty acids, nonesterified fatty acids, and glycerolare released into the blood. Glyceride, fatty acids boundto albumin, and nonesterified fatty acids are carried to the liver, where the glyceride is converted to glucose (see Figure 14-3). In the liver, free fatty acids may be oxidized to acetyl coenzyme A and used in the tricarboxylic acid cycle; used in gluconeogenesis; resynthesized to triglycerides and stored in the liver; or used to make triglycerides that are

released into the sinusoidal blood as VLDLs. Ponies with hyperlipemia have larger diameter VLDLs containing greater concentrations of triglycerides than normal ponies. Thus hyperlipemia in ponies and Miniature horses results from efficient hepatic synthesis of triglycerides from mobilized free fatty acids, with subsequent secretion of triglyceride laden VLDLs into the blood. Endothelial lipoprotein lipase is the rate-limiting enzyme responsible for the removal of VLDLs from the blood back into adipose tissue. In hyperlipemic ponies, the activity of lipoprotein lipase is not impaired and in fact is increased several times. Thus the overproduction of VLDLs by the liver and not impairment of their removal is primarily responsible for hyperlipidemia in ponies.[152,153]

If the oxaloacetate supply is limited when free fatty acids are mobilized to the liver, acetyl coenzyme A is
shuttled away from the tricarboxylic acid cycle and used to make ketone bodies. Because the equine liver is 979
efficient in synthesizing triglycerides and exporting VLDLs, hepatic lipidosis and ketosis are less common 980
than in other species.[149] However, if fatty acid mobilization and synthesis of triglycerides exceed oxidation and VLDL secretion, hepatic lipidosis ensues. Fat infiltration disrupts hepatic function, and excessive amounts of fat in the liver can result in hepatic failure and even hepatic rupture.[147]

Hormonal factors may contribute to the development of hyperlipemia and hepatic lipidosis. Insulin normally impedes the development of hyperlipemia by inhibiting tissue hormone–sensitive lipase, the enzyme responsible for lipolysis of adipose tissue. Insulin also curtails the development of hyperlipemia by stimulating gluconeogenesis in the liver and by activating lipoprotein lipase, the enzyme responsible for uptake of VLDL by adipose tissue. Despite often normal insulin levels, ponies appear to have a tissue insulin insensitivity compared with horses.[147] Fasting in donkeys has been shown to cause a reduction in tissue sensitivity to insulin.[154] Glucocorticoids, catecholamines, adrenocorticotropic hormone, thyroid-stimulating hormone, growth hormone, antidiuretic hormone, and progesterone may contribute to the development of hyperlipemia by opposing the biologic actions of insulin (see Figure 14-3). This may accountfor the high incidence of hyperlipemia during periods of increased cortisol levels (stress, pituitary adenoma, late pregnancy) and increased progesterone (pregnancy). In ponies, norepinephrine linearly stimulates the release of free fatty acids from adipose tissue, an effect which does not occur in horses.[155] Considering the unique effects of insulin and catecholamines on fat metabolism in ponies, not surprisingly ponies are more susceptible than horses to develop hyperlipemia.

Vascular thrombosis can occur following hyperlipemia and fat embolism and may be evident in the lung, kidney, brain, and subcutaneous vessels. Subcutaneous thrombosis causes dependent edema. Renal nephrosis and necrotizing pancreatitis are sometimes present.[60,147] Renal disease most likely is a sequela of occlusive thrombosis. The exact cause of pancreatitis is not known; however, hyperlipemia precedes pancreatitis. Excessive lipid is speculated to be deposited in and around the pancreas, which is hydrolyzed subsequently by pancreatic lipase, and released as free fatty acids. Free (unbound to albumin) fatty acids are cytotoxic, and when the albumin binding capacity is exceeded, pancreatic vascular injury occurs.[156]

Azotemia prevents lipid removal from the blood by inhibiting lipoprotein lipase. The degree of hyperlipidemia correlates directly with the degree of azotemia.[157] Almost half of hyperlipemic Miniature horses are azotemic.[150,151] Compared with ponies and Miniature horses, hyperlipemia rarely develops in horses, though *hyperlipidemia* (trigylcerides <500 mg/dl) may develop during azotemia.[148,157] Mild hepatic lipidosis may ensue, but hyperlipidemia rarely results in clinically recognizable disease.

14.3.1.9.3

Diagnosis

One should consider hyperlipemia in the differential diagnosis for any obese pony or Miniature horse with clinical signs of severe depression, anorexia, ataxia, and icterus. The normal triglyceride level in horses and ponies should be less than 85 mg/dl but may be up to 290 mg/dl in healthy donkeys and up to 250 mg/dl in healthy pregnant pony mares.[153] In hyperlipemia, the blood grossly appears opalescent (Figure 14-8) and the concentrations of all lipids are increased, especially triglycerides (>500 mg/dl), nonesterified fatty acids, and VLDLs. Laboratory evidence of hepatic disease may include increased serum activity of SDH, GGT, increased concentrations of serum bile acids, bilirubin, and ammonia, and decreased glucose, BUN, and albumin.[146] In hyperlipemic Miniature horses, only about half of affected patients have significant hepatic impairment.[150] The BSP half-life is prolonged. Oral and intravenous glucose challenge tests may reveal glucose intolerance because of insulin insensitivity. Metabolic acidosis is frequent, and one should suspect ketoacidosis if the anion gap is increased. Lipemia may increase serum creatinine values falsely and interfere with accurate determination of other serum chemistries. One confirms a diagnosisof hepatic lipidosis by the concurrent demonstration of increased blood concentrations of lipid, laboratory evidence of 980 hepatic dysfunction, and ultrasonographic or histopathologic findings of fatty infiltration in the liver (Figure 981 14-9). One should determine the serum concentrations of creatinine and electrolytes as an adjunctto therapy. Because of the incidence of hyperlipemiain ill Miniature horses and donkeys, monitoring blood triglyceride levels facilitates early recognition of hyperlipidemia in these patients.

Figure 14-8 Hyperlipemia. The spin hematocrit tube on the right is from a Miniature horse with hyperlipemia. The hematocrit tube on the left is from an unaffected horse.

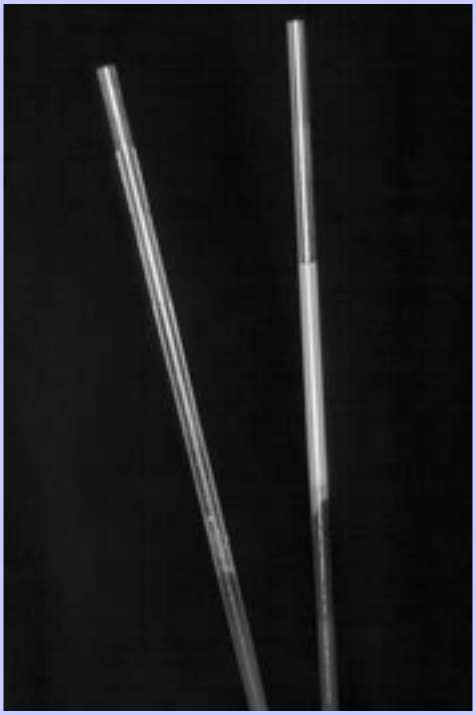
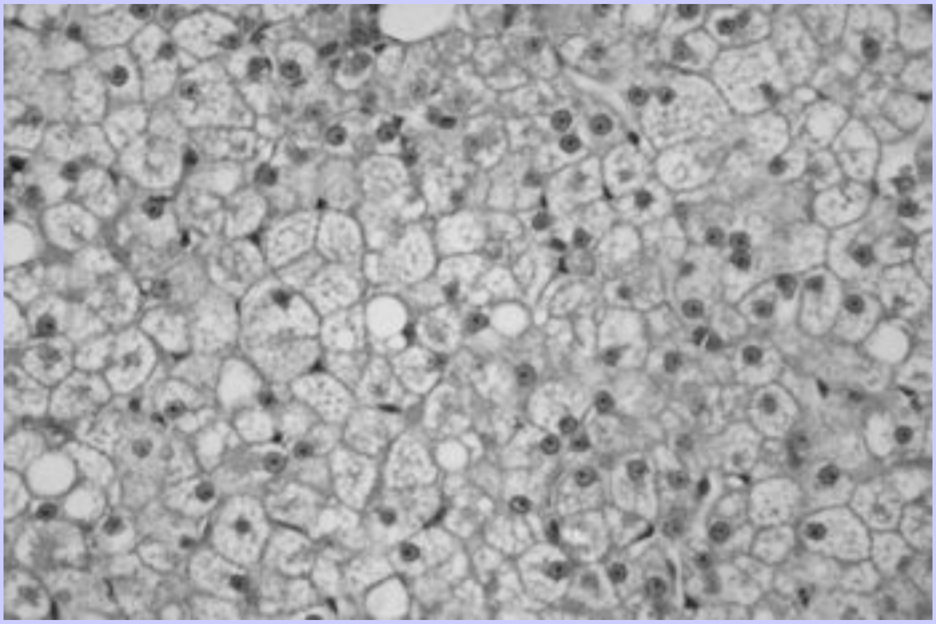


Figure 14-9 Histopathology of the liver: fatty liver.



Necropsy findings in affected ponies include widespread fatty change in the liver, skeletal muscle, kidney, adrenal cortex, and myocardium.[60,147] The liver and kidney are enlarged, yellow, friable, and greasy. One of every five ponies with hepatic lipidosis has a ruptured liver.[147] Microscopically, hepatocytes are engorged with lipids. The nucleus often is displaced, and hepatocellular necrosis may ensue.

14.3.1.9.4

Treatment

The major therapeutic objectives for hyperlipemia and hepatic lipidosis include the following:

1. Treatment of hepatic disease
2. Improvement of energy intake and balance
3. Elimination of stress or treatment of concurrent disease
4. Inhibition of fat mobilization from adipose tissue
5. Increased triglyceride uptake by peripheral tissues

Treatment of hepatic failure is addressed elsewhere(see Treatment of Hepatic Insufficiency). Concentrated carbohydrate feed, such as molasses-coated grain and high-quality pasture or hay, should be encouraged. Anorectic ponies should receive 5% dextrose as a continuous infusion at a rate of 2 ml/kg/hr, and enteral nutrition should be attempted. NutriFoal, NutriPrime (KenVet, Ashland, Ohio) and Osmolyte HN (Ross Laboratory, Columbus, Ohio) have been used successfully as sources of enteral nutrition in hyperlipemic Miniature horses. If enteral nutrition is not possible, providing partial parenteral nutrition with equal volumes of 50% dextrose and 8.5% amino acids is recommended.[150] Monitoring blood glucose

concentrations closely is mandatory because some ponies have glucose intolerance, and excessive glucose worsens acidosis and induces hypokalemia. Weaning suckling foals may help reduce stress and the energy demand imposed on lactating mares. One should treat concurrent diseases amenable to therapy appropriately, such as parasitism, chronic infection, and musculoskeletal disease with anthelmintics, antimicrobials, or analgesics, respectively.

In addition to supplying immediate energy, glucose therapy stimulates the release of insulin. Concurrent carbohydrate and insulin administration have been used successfully to manage hyperlipemia in ponies.[146,158] The following regimen has been suggested for a 200-kg pony[158]:

> Day 1: Administer 30 IU of protamine zinc insulin intramuscularly and 100 g of glucose orally, both twice daily.
>
> Day 2: Administer 15 IU of insulin intramuscularly twice daily and 100 g of galactose once.

Galactose is converted slowly to glucose, thus minimizing lactic acid production. This regimen may be continued for 3 days. One should monitor blood glucose and insulin concentrations closely. Insulin treatment may not lower triglyceride levels, but it may help control hyperglycemia if partial parenteral nutrition is provided.[150] Nicotinic acid, thought to act by inhibiting hormone-sensitive lipase, has been used in cattle with fatty liver; however, nicotinic acid has not been evaluated in ponies with hepatic lipidosis.

Heparin potentiates the activity of lipoprotein lipase and may increase triglyceride removal from the blood. However, because lipoprotein lipase activity is already at maximum rate in affected ponies, whether heparin provides additional benefit is questionable.[153] Recommended dosages range from 40 to 250 IU/kg twice daily.[146,158] Because of the potential for hemorrhage, heparin therapy should be used with discretion and monitored daily by hemostatic testing. If azotemia, acidosis, or electrolyte abnormalities are present, appropriate fluid therapy is suggested.

Lipotropic agents such as choline and methionine are unlikely to be useful in horses with hyperlipemia, because triglyceride synthesis in the liver is usually sufficient.

14.3.1.9.5 Prognosis and Client Education

The prognosis for Equidae with hyperlipemia is poor. Mortality has been estimated to occur in 60% to 100% of affected ponies.[146,147] Survival rate is reported to be better in Miniature horses, and death often results from the underlying disease and not fatty liver failure.[150,151] Most Miniature horses with blood triglyceride levels less than 1200 mg/dl survive.[151] Prevention is achieved best by providing appropriate nutrition without inducing obesity, avoiding stress, and practicing good routine health care.

14.3.2 CHRONIC HEPATIC DISEASES

The distinction between acute and chronic hepatic disease is difficult. Horses with chronic liver disease often 981
have abrupt onset of signs. With the possible exception of hyperglobulinemia, no serum biochemical parameters 982
reliably distinguish acute from chronic hepatocellular disease. Regardless of the cause or site of injury, histopathologic evidence of fibrosis develops only after chronic injury.[159] The following diseases are classified

as chronic based on the presence of fibrosis in the liver or because the cause is known to result in gradual hepatic dysfunction.

14.3.2.1 Chronic Megalocytic Hepatopathy

Megalocytic hepatopathy occurs worldwide and is the most common cause of chronic liver failure in horses in certain parts of the United States.[19] The disease is caused by the ingestion of *pyrrolizidine alkaloid*–containing plants. The intoxication typically results in the delayed onset of chronic, progressive liver failure.

14.3.2.1.1 Clinical Signs

The development of clinical signs of liver disease usually is delayed 4 weeks to 12 months following the consumption of pyrrolizidine alkaloid–containing plants. However, individual differences in susceptibility occur, for not all horses consuming the plants develop clinical signs.[160] The onset of obvious hepatic failure, characterized by HE and photosensitization, usually is abrupt and occurs late in the disease despite the time interval since ingestion. Premonitory signs of insidious onset may include anorexia, weight loss, exercise intolerance, and mild to moderate icterus. Diarrhea, edema, polydipsia, pruritus, laryngeal hemiparalysis, and hemolysis also have been reported to occur late in the disease.[66,160,161] Sometimes oral ulcers and halitosis develop.[162] Although uncommon, if sufficient consumption occurs during a single exposure, abortion or clinical signs of liver disease may develop acutely.

14.3.2.1.2 Epidemiology and Pathogenesis

Numerous species of pyrrolizidine alkaloid–containing plants exist (Table 14-3). In the United States, *Senecio jacobaea* and *S. vulgaris* are found primarily along the Pacific Coast and in the western states, *Amsinckia intermedia* in the West, and *Crotalaria sagittalis* (Figure 14-10), *C. spectabilis*, and *Heliotropium europaeum* primarily in the Southeast.

TABLE 14-3 Pyrrolizidine Alkaloid–Containing Plants

SPECIES NAME	COMMON NAME	ALKALOIDS
Senecio jacobaea	Tansy or common ragwort, stinking willie	Jacobine, jacodine, senecionine
Senecio riddellii	Riddell's groundsel	Ridelline
Senecio longilobus	Threadleaf groundsel	Longilobine
Senecio vulgaris	Common groundsel	Senecionine, seneciphylline, retrorsine
Senecio spartioides	Broom groundsel	Seneciphylline
Senecio integerrimus	Lamb's tongue groundsel	Intergerrimine
Amsinckia intermedia	Fiddleneck, fireweed, or tarweed	
Crotalaria species	Rattlebox	Monocrotaline, fulvine, crispatine
Echium plantagineum	Viper's bugloss	
Heliotropium europaeum	Common heliotrope or potato weed	Heliotrine, lasiocarpine

Pyrrolizidine alkaloid–containing plants are not palatable, and horses typically do not consume them unless pastures are contaminated heavily or no alternative feed source exists. Some herbicides may increase palatability.[161] Pyrrolizidine alkaloids are stable, and many intoxications occur following ingestion of contaminated hay, pellets, or grain. *S. vulgaris* is a common contaminant in alfalfa hay, especially at the first cutting. Not all parts of the plant contain pyrrolizidine alkaloids, and the concentration may vary with the season.[163] *Amsinckia* and *Crotalaria* species concentrate pyrrolizidine alkaloids in their seeds; thus intoxication may occur following ingestion of contaminated oat hay or grain screenings.[161]

Horses and cattle are sensitive to pyrrolizidine alkaloid intoxication compared with goats and sheep. Consumption of the plants at a dose of 2% to 5% of the body weight of the horse, fed at one time or over a few days, can result in acute toxicity.[161] The effects of pyrrolizidine alkaloids are cumulative, thus toxicity more commonly occurs following chronic low-level exposure. Hundreds of different pyrrolizidine alkaloids exist, but only a few are proved to be toxic (see Table 14-3). The toxic pyrrolizidine alkaloids vary among plants, but they all cause the same basic lesion. Ingested pyrrolizidine alkaloids are carried to the liver via the portal circulation and are metabolized by the microsomal enzymes to toxic pyrrole derivatives.[162] Drugs that induce microsomal enzymes, such as mixed function oxidases, increase the toxicity of pyrrolizidine alkaloids. The pyrroles are chemically highly reactive and capable of alkylating nucleic acids and protein; consequently, the pyrroles inhibit cellular replication and protein synthesis. Because the cells cannot divide, hepatocytes enlarge, forming *megalocytes*. When the megalocytes die, fibrosis ensues. When fibrosis becomes extensive, the liver shrinks and develops a firm texture and failure is inevitable. 982

Figure 14-10 A *Crotalaria* spp. plant containing toxic pyrrolizidine alkaloids. (Photograph courtesy Susan White.)

Hepatocytes surrounding the portal triads usually are affected first. When acute massive consumption occurs, extensive centrilobular hepatocellular necrosis occurs. Fibrosis around the portal vessels can cause portal hypertension, ascites, and diarrhea. Endothelial cell swelling in the portal vein is especially common with *Crotalaria* species, further promoting venoocclusion. Despite periportal fibrosis and venoocclusion, portal hypertension rarely develops in horses, though it is a common manifestation of pyrrolizidine alkaloid toxicity in cattle.[161] Additional pathologic findings include myocardial necrosis, colitis, widespread hemorrhages, and adrenal cortical hypertrophy.[66] The toxic pyrrolizidine alkaloidin *Crotalaria* species, monocrotaline, also is pneumonotoxic and may cause hydrothorax, pulmonary edema, epithelialization, and pulmonary arteritis.[162]

Pyrrolizidine alkaloids are excreted rapidly in body fluids such as milk and urine, and they can pass the placenta.[162] A report describes pyrrolizidine alkaloid toxicosis in a 2-month-old foal the dam of which consumed pyrrolizidine alkaloid–containing plants during pregnancy.[164]

14.3.2.1.3

Diagnosis

One can make a presumptive diagnosis of megalocytic hepatopathy from history of exposure to pyrrolizidine alkaloids, clinical signs, and laboratory evidence of hepatic disease. Early in the disease, SDH and AST may be increased, but by the time clinical signs develop, these enzymes are often normal or only mildly increased. Because fibrosis occurs periportally, GGT and ALP are increased persistently. Bilirubin may be increased and the BCAA:AAA ratio may be decreased.[165] The BSP half-life is prolonged and serum bile acids usually are increased late in the disease.[66]

A definitive diagnosis of pyrrolizidine alkaloid intoxication requires a liver biopsy. The histopathologic findings of megalocytosis, biliary hyperplasia, and fibrosis are essentially pathognomonic. One may distinguish pyrrolizidine alkalosis from aflatoxicosis and alsike clover *(Trifolium hybridum)* toxicity. The latter two toxicities are rare compared with pyrrolizidine alkaloids and cause periportal fibrosis and biliary epithelial hyperplasia without megalocytosis. Pyrrolizidine alkaloids in feed can be detected by high-performance liquid chromatography.[66] Some pyrrolizidine alkaloids are identifiable in liver tissue. Unfortunately, because of the prolonged delay in development of clinical signs, often the original source of contamination remains unidentified.

14.3.2.1.4 Treatment and Prognosis

No specific antidote for pyrrolizidine alkaloid intoxication exists. Despite general supportive care, death usually occurs within 10 days of the onset of obvious clinical signs of hepatic failure. A serum bile acid concentration greater than 50 μmol/L suggests a grave prognosis. Because regeneration is not possible, if extensive megalocytosis and fibrosis are present, treatment is not warranted. Treatment with BCAAs may decrease the severity of neurologic signs but does not prevent death.[66] If clinical signs or histologic changes are mild, a low-protein (grass or oat hay), high-energy (molasses concentrate grain) ration may be beneficial.

One should direct attention toward asymptomatic horses that also may have consumed the plants. The source of contamination should be identified by locating the plant in hayfields or pasture or by analysis of feed and then should be discontinued. Progress can be monitored by serial liver enzyme quantitation and biopsies. Serum GGT activity may be helpful in detecting subclinical cases of pyrrolizidine alkaloid toxicity.[166]

14.3.2.2 Clover Poisoning

Alsike clover *(Trifolium hybridum)* and red clover *(T. pratense)* poisoning rarely occurs in horses pastured or fed hay containing these types of clover.[167–169] Photodermatitis and liver disease have been reported. Signsof liver disease may become apparent after 2 weeks of consumption and when the diet consists of at least 20% clover.[167] The likelihood of poisoning is reported to be greater when the pasture is covered heavily with the clover in full bloom during wet seasons. Although the toxic principle is not known, the disease is characterized histopathologically by the presence of biliary hyperplasia and periportal fibrosis. Minimal parenchymal lesions distinguish alsike clover poisoning from pyrrolizidine alkaloid toxicity.

14.3.2.3 Chronic Active Hepatitis

Chronic active hepatitis (CAH) in horses is an idiopathic, chronic, progressive hepatopathy characterized histopathologically by biliary hyperplasia, with concomitant periportal or biliary inflammation or both and associated hepatocellular damage. 983 984

14.3.2.3.1
Clinical Signs

The onset of clinical signs of CAH is insidious. Signs are compatible with progressive liver failure and include depression, exercise intolerance, weight loss, anorexia, colic, icterus, and fever. The signs may be intermittent. Although uncommon, some horses with CAH have moist exfoliative coronary dermatitis.[61]

14.3.2.3.2
Cause and Pathogenesis

The exact cause of CAH in horses is not known. A similar syndrome occurs in human beings and has been linked to autoimmune disease, chronic hepatitis B virus infection, non-A, non-B (viral) hepatitis, Wilson's disease, α_1-antitrypsin deficiency, and drug allergy. Human beings with autoimmune-associated CAH often have significant polyclonal gammopathyand hepatic actin, smooth muscle, and antinuclear antibodies.[170] Histologic study of the liver reveals mononuclear and plasma cell infiltrates, large areas of necrosis, and fibrosis. Extrahepatic signs of autoimmune disease, including dermatitis, arthritis, and glomerulonephritis, may be present. Some horses with CAH have polyclonal gammopathy, but this is a nonspecific finding in many types of chronic hepatic disease and does not necessarily indicate autoimmunity. Occasionally, horses with CAH have predominantly plasma and mononuclear cell infiltrates in the liver, suggesting heightened humoral activity. The presence of coronary dermatitis in horses with CAH may be a manifestation of autoimmune disease, though this has not been confirmed by immunohistologic staining. No reports exist of antinuclear antibodies in horseswith CAH, and viral hepatitis, Wilson's disease, andα_1-antitrypsin deficiency have not been documented. Idiosyncratic drug hypersensitivity has been reported in horses, though not as a consistent feature of CAH.

In addition to the possibility of an autoimmune or hypersensitivity reaction, CAH in horses may be a manifestation of chronic cholangitis. Many horses with CAH have a suppurative inflammatory response involving the biliary system, in addition to periportal inflammation and hepatocellular necrosis.[61] Often the cholangiohepatitis is accompanied by biliary hyperplasia, fibrosis, and cholestasis. In some cases, coliform organisms have been isolated from the liver, suggesting ascending infection from the gastrointestinal tract.

A large number of horses have histopathologic evidence of fibrosis, acute inflammation, and hepatocellular necrosis, but no specific cause can be determined.

14.3.2.3.3
Diagnosis

The diagnostic criteria for CAH in human beings include abnormally increased serum transaminase activity for greater than 6 months, abnormal immunologic findings, and characteristic histopathologic findings.[170] In horses, serum SDH and AST activity may be increased mildly, but GGT and ALP often are increased greatly in CAH. The serum bile acid, total protein, and bilirubin (especially the direct reacting fraction) concentrations may be increased. Bilirubinuria may be present, and the BSP half-life usually is prolonged. Hematologic examination may reveal an inflammatory leukogram, with or without a left shift.[61] Immunodiagnostics, including determination of the antinuclear antibody titer and antiimmunoglobulin immunofluorescent staining of skin lesions, may help confirm an autoimmune phenomenon.

In the face of clinical signs and the demonstration of significant laboratory findings indicative of liver disease, one should obtain a liver biopsy (see Liver Biopsy). One should submit a sample for

histopathologic examination and for bacterial isolation and antimicrobial sensitivity testing. The definitive diagnosis of CAH depends on the presence of characteristic histopathologic findings. Progressive periportal hepatocellular necrosis obscures and distorts the limiting plate, the cord of hepatocytes that surrounds the portal triad. As this destructive process continues, bands of necrotic hepatocytes and inflammatory cells connect one liver lobule to another or extend from the portal tract to the central vein. This feature is called *bridging necrosis*. As bridging necrosis progresses, fibrosis and cirrhosis prevail. Mononuclear cells may be the predominant inflammatory infiltrate; however, neutrophils predominate if cholangiohepatitis is a feature. Biliary hyperplasia may be concurrent evidence of cholangiohepatitis.

14.3.2.3.4

Treatment

General supportive care for hepatic failure (see Treatment of Hepatic Insufficiency) is indicated whenever clinical signs are apparent. Specific therapy forCAH depends on the histopathologic findings. If the liver biopsy reveals abundant plasma cells or other diagnostic tests suggest autoimmune or hypersensitivity disease, corticosteroid therapy may be beneficial. In human beings with autoimmune-associated CAH, corticosteroids improve appetite and attitude, reduce inflammation and serum transaminase activity, and hinder fibrosis. Despite short-term improvement, corticosteroids do not alter survival time, and the long-term prognosis remains poor. In horses, initial treatment with dexamethasone at the dose rate of 0.05 to 0.1 mg/kg/day for 4 to 7 days, followed by a gradual reduction in dose rate over 2 to3 weeks, has been suggested.[171] Additional treatment with prednisolone (1 mg/kg/day) may be necessary for several weeks. In human beings, azathioprine (2 mg/kg every 24 hours) also has been used to control cases refractory to steroids.[170]

If the liver biopsy reveals cholangitis, long-term (4to 6 weeks) antimicrobial therapy is indicated. The choice of antimicrobial is determined best by culture and sensitivity results. Antimicrobials that are excreted in bilesuch as chloramphenicol, ceftiofur, ampicillin, or broad-spectrum antimicrobials such as penicillin 984
and gentamicin are recommended. One should consider carefully the decision to provide corticosteroid 985
therapy in these cases of CAH.

14.3.2.3.5

Prognosis

The prognosis for CAH varies depending on the cause and duration of disease. The presence of cirrhosis warrants a grave prognosis.

14.3.2.4

Cholelithiasis

Cholelithiasis, or the formation of biliary calculi, occasionally results in hepatocellular disease in horses. A *cholelith* is a calculus that develops anywhere along the biliary pathway. A *hepatolith* and *choledocholith* refer to calculi within intrahepatic ducts and the common bile duct, respectively. Hepatic failure and clinical signs occur when multiple stones are present or when they occlude the common bile duct.

14.3.2.4.1

Clinical Signs

Cholelithiasis occurs most commonly in adult, middle-aged horses (6 to 15 years old), though horses as young as 3 years old have been affected.[51,124,172] Broodmares may be predisposed to cholelithiasis.[124] The most frequently reported clinical signs include icterus, abdominal pain, fever, depression, and weight loss.

Signs of hepatic failure, such as photosensitization, petechial hemorrhages, diarrhea, and HE, have been reported less frequently.[51,124,172–174] Clinical signs are often intermittent unless the common bile duct is occluded, whereupon persistent abdominal pain prevails.

14.3.2.4.2 Pathogenesis

The initial step in the formation of a cholelith is precipitation or aggregation of normally soluble constituents of bile: bilirubin, cholesterol, or bile acids. In human beings, 75% of bile stones are composed principally of cholesterol and 25% are composed principallyof calcium bilirubinate. Most calcium bilirubinate choleliths in human beings are associated with bacterial cholangitis.[175,176] Most choleliths in horses consist principally of calcium bilirubinate and are associated with cholangitis.[51,124,172]

The exact sequence of events leading to precipitation of bilirubin in bile is not entirely known; however, soluble, conjugated bilirubin becomes unconjugated by the enzyme β-glucuronidase and combines with calcium, a normal constituent of bile, to form calcium bilirubinate. Excessive formation of unconjugated bilirubin causes the formation of columnar complexes of calcium bilirubinate that subsequently precipitate. β-Glucuronidase is synthesized by the bile duct epithelium, hepatocytes, and certain bacteria. The concentration of β-glucuronidase is normally low in bile, plus an inhibitor, glucaro-1,4-lactone, is usually present.

Whether bacterial, biliary, or hepatic in origin, increased bile concentration of β-glucuronidase has been demonstrated in human beings with obstructive cholangitis.[176] Because most choleliths in horses are composed of calcium bilirubinate and many affected horses with cholelithiasis have documented bacterial cholangitis, cholelith formation in horses most likely occurs following bacterial infection. Furthermore, because enteric organisms are isolated most commonly from horses with cholelithiasis, infection presumably ascends from the intestinal tract. Horses may be more prone to ascending infection because they lack an exit port sphincter to prevent backflow of intestinal contents into the biliary tree. If bile flow decreases for any reason, retrograde flow and infection are likely. However, the correlation of infection with cholelithiasis in horses does not necessarily prove causation. Another plausible explanation is that the sequence of events is the initial presence of a stone that precipitates cholestasis and subsequent infection. Despite its origin, cholangitis is the likely cause of fever. Chronic cholestasis caused by the presence of choleliths results in increased biliary pressure, a likely cause of abdominal discomfort. If biliary pressure is not resolved, pressure-induced periportal hepatocellular necrosis with subsequent fibrosis ensues.

At necropsy, tissues are icteric and the liver is mottled. The liver appears enlarged following acute biliary obstruction and shrunken and firm with chronic cholestasis. Choleliths range in size, are single or multiple in number, are usually green-brown (bilirubinate), and have smooth texture. Histopathologic study reveals periportal hepatocellular necrosis, fibrosis, biliary stasis, and biliary hyperplasia. Suppurative cholangitis is often present. Organisms isolated from the liver or bile consist predominantly of gram-negative coliforms, though *Enterococcus* also has been reported.[51,124,172] Nonobstructive choleliths are subclinical and are recognized only at necropsy.

14.3.2.4.3 Diagnosis

One should consider cholelithiasis in the differential diagnosis in any horse with a history of fever, icterus, and abdominal pain, especially if accompanied by signs of hepatic failure. Hematologic examination often reveals leukocytosis caused by mature neutrophilia, especially if cholangitis is present. Other nonspecific

but frequent findings include hyperproteinemia and hyperfibrinogenemia.[51,124,172] The most common abnormal laboratory findings that suggest cholestatic liver disease include greatly increased GGT (greater than 15 times normal); increased serum bile acids concentration; hyperbilirubinemia, with the direct reacting fraction greater than 25% of the total value; and bilirubinuria.[*] Although not liver-specific, ALP often is increased greatly. Less commonly, SDH, arginase, AST, and LDH-5 are increased mildly to moderately, and clotting times and the BSP half-life are prolonged.[51,124,172–174,177] Affected horses frequently have nonseptic peritonitis or peritoneal transudate.[172]

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Figure 14-11 Ultrasound image of the liver showing a hepatolith *(arrow)* casting an acoustic shadow in a dilated bile duct in a horse with obstructive cholelithiasis.

Although no single blood test was specific for cholithiasis, hepatic ultrasonography or biopsy accurately predicted a diagnosis of cholethiasis in all cases reviewed.[172] In retrospective studies of cholelithiasis, 75% of the cases had visibly dilated bile ducts, which appear as thin-walled, anechoic structures.[51,124,172,178] This finding contrasts with normal horses in which the bile ducts are not detectable. A parallel channel sign of biliary dilation in association with dilation of intrahepatic biliary radicals lying adjacent to portal venous structures may be evident.[178] The liver may appear enlarged with increased echogenicity. Hepatoliths, which occur in 60% of cases, are found most frequently at the level of the right sixth to seventh intercostal space,[51,124,172,178] appear as hyperechoic foci within the bile ducts and maycast acoustic shadow (Figure 14-11). Determination of obstruction of the common bile duct in horses is difficult, because the common bile duct is not visible by transabdominal ultrasonography. Hepatobiliary scintigraphy may be useful to document obstruction of biliary excretion.[179] Otherwise, one can detect choledocholiths only via palpation during exploratory celiotomy.

Obtaining a liver biopsy is useful for diagnostic, therapeutic, and prognostic purposes. The histopathologic findings of periportal fibrosis, biliary stasis and hyperplasia, and cholangitis are not pathognomonic for cholelithiasis; however, a liver biopsy may help rule out other causes of biliary stasis such as CAH or megalocytic hepatopathy. Concentric fibrosis around intrahepatic bile ducts is evidence of occlusion of the common bile duct (Figure 14-12).

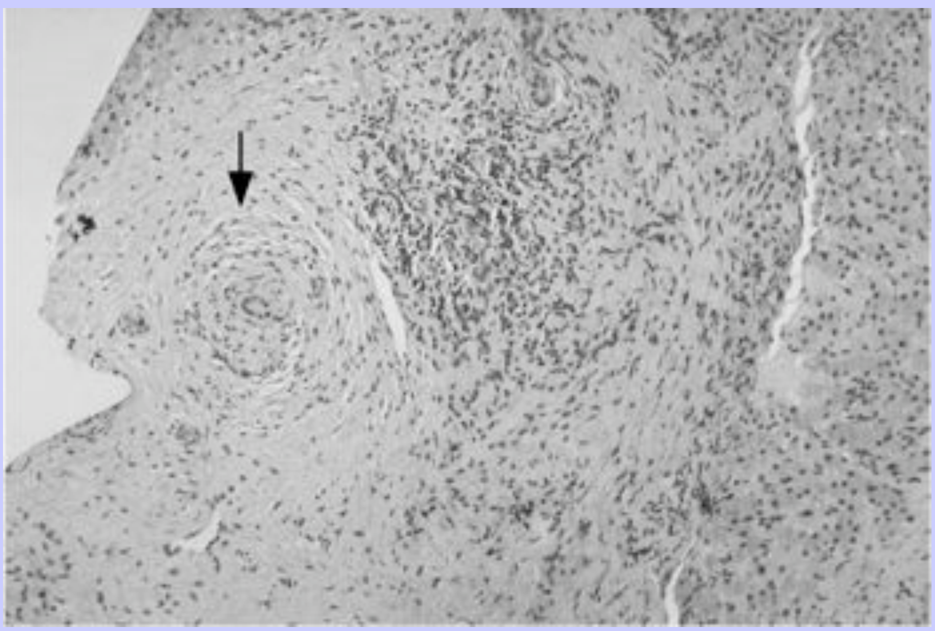
* References 51, 124, 172, 173, 175, 177.

14.3.2.4.4

Treatment

If clinical signs of liver failure are present, one should provide general supportive care (see Treatmentof Hepatic Insufficiency). Long-term (4 to 6 weeks) treatment with antimicrobials is indicated. Antimicrobial selection should be guided by clinical response and by bacterial isolation and sensitivity results obtained via a liverbiopsy or culture of the bile or cholelith obtained at surgery. Penicillin-gentamicin, ceftiofur, chloramphenicol, ampicillin, enrofloxacin, metronidazole, and trimethoprim-sulfonamide have been used successfully[51,124,172]; however, recent reports describe resistance of isolates from affected horses to trimethoprim-sulfonamide.[172] Antimicrobial therapy should continue until complete resolution of clinical signs and laboratory evidence of inflammation. Some have suggested continuing antimicrobial therapy until the serum GGT activity is normal.[124]

Figure 14-12 Histopathology of the liver. Concentric fibrosis *(arrow)* around a bile duct is characteristic of obstruction of the common bile duct.



Dissolution of cholesterol choleliths in human beings has been successful with long-term administration of bile acids.[180] Because choleliths in horses are composed primarily of biliary pigments, treatment with bile acids most likely would be unavailing and potentially harmful. Evidence indicates that dimethyl sulfoxide dissolves calcium bilirubinate stones in human beings, and thusits use may be beneficial to horses with cholelithiasis.[124] Use of other antiinflammatory drugs, such as flunixin meglumine, may help speed resolution of hepatic and biliary inflammation and thus restore biliary excretion.

Surgical intervention may be necessary for occlusion of the common bile duct. The decision for surgery is difficult, because no direct way exists to determine the extent of occlusion of the common bile duct in

horses. In one report, horses with discrete occlusive choledocholiths had persistent fever; more persistent, though mild and intermittent, abdominal pain; and significantly greater serum bile acid concentrations than did horses that were managed successfully with medical therapy.[172] Although cholelithotripsy may be accomplished in human beings percutaneously, via endoscopy or laparotomy, exploratory laparotomy currently is the only available means for reducing a common bile duct obstruction in horses. Choledocholithotripsy (the crushing of choleliths in the bile ducts) and choledochotomy (surgical formation 986

987 of an opening into the bile duct) have been performed in the horse successfully.[175,181,182] In one recent report on horses, external massage of the common bile duct, without choledochotomy, successfully dislodged choledocholiths into the duodenum in all of the cases inwhich it was attempted.[172] If multiple hepatoliths are distributed throughout the biliary tree, these cannot be removed readily by surgery.

14.3.2.4.5 Prognosis

The prognosis of cholelithiasis depends on the extent of hepatic fibrosis, severity of clinical signs, and number and location of choleliths. Extensive or bridging fibrosis, multiple choleliths accompanied by clinical signs of hepatic failure, hepatic atrophy, and severe HE warrant a poor to grave prognosis.[51,124,172] Reports exist of resolution of extensive fibrosis following reestablishment of bile flow, thus one should not consider the presence of extensive fibrosis alone to be lethal.[172,183] In contrast to reports of 30% survival for equine cholelithiasis,[51] recent reviews indicate 77% to 85% survival with medical or medical and surgical treatment.[124,172] If obstruction of the bile duct requires a choledochotomy, the prognosis is guarded because of limited access to the bile duct in a horse and the risk of choleperitoneum.

14.3.2.5 Chronic Cholangiohepatitis and Biliary Fibrosis

Chronic cholangiohepatitis and biliary fibrosis most commonly are associated with CAH, chronic cholangiohepatitis, cholelithiasis, and pancreatitis (see the previous discussion). Liver failure rarely results from primary idiopathic cholangitis or biliary fibrosis.[58] Therapy is supportive. Treatment of bacterial cholangiohepatitis is discussed elsewhere (see Bacterial Hepatitis; Chronic Active Hepatitis; and Cholelithiasis). The prognosis depends on the degree of fibrosis. Extensive fibrosis warrants a poor prognosis, though reports of reversalof hepatic fibrosis exist, especially on relief of the obstruction in cholestatic diseases.[183]

14.3.2.6 Neonatal Acquired Biliary Obstruction

Healing duodenal ulcers located adjacent to the hepatopancreatic ampulla in neonatal foals may result in extrahepatic biliary stenosis.[19,184] Clinical signs are compatible with gastroduodenal obstruction and include reduced suckling reflex, depression, prolonged recumbency, colic, bruxism, and firm feces. Nasogastric intubation often produces voluminous reflux. One may confirm duodenal stenosis radiographically by delayed gastric emptying of barium sulfate. One should suspect biliary stenosis if the serum levels of conjugated bilirubin and GGT are increased or if nuclear scintigraphy indicates delayed biliary excretion. Cholangiohepatitis may develop because of chronic cholestasis and may be confirmed by histopathologic examination of a liver biopsy. One must differentiate acquired biliary stenosis from congenital biliary atresia. Successful surgical correction of acquired biliary stenosis following duodenal stricture has been described.[184]

One should consider chronic cholangiohepatitis without biliary stenosis, another potential sequela to duodenitis or duodenal ulceration, in icteric foals with increased serum conjugated bilirubin and GGT.[19] One confirms the diagnosis by histopathologic examination. Treatment should consist of long-term antimicrobial therapy directed by bacterial isolation and sensitivity results (see Bacterial Hepatitis).

14.3.2.7 Hepatic Abscessation

Liver abscessation is uncommon in horses. Clinical signs of hepatic abscessation depend on the extent and location of the abscess(es); however, abscesses are unlikelyto result in hepatic failure. Small solitary abscesses or multifocal microabscesses usually remain subclinical. Larger abscesses may be accompanied by weight loss, intermittent fever, and anorexia.

Hepatic abscessation in foals may be hematogenousas a sequela to bacteremia or ascending through the umbilical vein as a sequela to omphalophlebitis.[185] In the adult, bacteremia is uncommon, and hepatic infectionor abscessation more likely originates in the intestinal lumen and ascends the bile duct or originates in the intestinal wall or mesenteric lymph nodes and is carried to the liver via the portal blood.[186] The latter is a likely sequela to primary abdominal or mesenteric abscessation caused by *Streptococcus equi*. In one retrospective study of abdominal abscesses in horses, only 2 of 25 horses had solely hepatic abscesses, though some horses had abscesses involving several abdominal structures.[187]

Laboratory diagnosis of hepatic abscessation is difficult. Smaller abscesses may not result in sufficient hepatocellular damage to impede liver function. Laboratory abnormalities characteristic of chronic inflammation and infection, including anemia of chronic disease, hyperfibrinogenemia, hypergammaglobulinemia, and mature neutrophilia, may occur with larger abscesses. Increased serum activity of liver-specific cytosolic enzymes or bile acids rarely occurs unless liver abscessation is extensive. Ultrasonography of the liver may reveal focal hyperechoic or hypoechoic areas.[186] A liver biopsy should be guided by ultrasound to avoid penetration of the abscess. One should direct long-term antimicrobial therapy by culture and sensitivity results. Common isolates from abdominal abscesses in horses include *S. equi, S. zooepidemicus*, and *Corynebacterium pseudotuberculosis*.[187]

14.3.2.8 Hepatic Neoplasia

Primary hepatic neoplasia is rare in horses. A retrospective survey in 1952 indicated that hepatic tumors 987
accounted for only 1% of all equine neoplasms.[188] Of the documented primary hepatic neoplasms in horses 988
(cholangiocarcinoma, hepatocellular carcinoma, hepatoblastoma, and mixed hamartoma), cholangiocarcinoma is the most common.[59,87,88,188–192] Hepatic neoplasia in horses is more likely to occur following metastasis of some other primary tumor, especially lymphosarcoma.[89] Because clinical signs of hepatic failure usually are not apparent and laboratory findings are nonspecifically indicative of chronic inflammatory disease, antemortem diagnosis of hepatic neoplasia is difficult.

In one report, *cholangiocarcinoma* accounted for 9of 10 primary liver neoplasms in horses.[188] Cholangiocarcinoma originates from the bile duct epithelium and is distinguished from hepatocellular carcinoma by its tendency to form multiple foci, its firm texture, and a whitish color produced by abundant fibrous stroma. The primary mass is typically solitary, with multiple intrahepatic secondaries. Extrahepatic metastasis is common with transperitoneal lymphatic spread to the peritoneum and diaphragm and

hematogenous spread to the lungs. In human beings, dogs, and cats, an etiologic relationship exists with liver fluke infestation or a history of biliary tract disease.[190] A report describes cholangiocarcinoma in a horse after treatment for prolonged septic cholangiohepatitis.[179] Aberrant expression of the protein products of the tumor suppressor gene, p53, was reported at necropsy in one horse with cholangiocarcinoma.[193] Loss of function of p53 has been described in other types of carcinomata and may have contributed to malignancy. Microscopically, cholangiocarcinoma is adenocarcinomatous, producing cuboidal or columnar-lined ductules and acini. The neoplastic bile ducts do not contain bile but may contain mucus.

The clinical presentation and progression of cholangiocarcinoma in horses is not well documented but appears to be more common in older horses.[188] One case report describes a 10-year-old mixed-breed horse in which the presenting signs included anorexia, weight loss, pyrexia, mild icterus, tachypnea, severe dependent edema, and abdominal distention.[192] Abnormal clinicopathologic findings included mature neutrophilia; hyperfibrinogenemia; anemia; moderately increased serum bilirubin, GGT, and SDH; and nonseptic peritonitis and pleuritis. Neoplastic cells were not detected in the body cavity effusions. Definitive antemortem diagnosis of cholangiocarcinoma was made by histopathologic examination of a liver biopsy. Necropsy revealed multiple extrahepatic metastases, including the serosal surfaces of the intestine and spleen, diaphragm, omentum, pleural surfaces, and lung.

Hepatocellular carcinoma, or hepatoma, has been reported in several horses, most of which were less than 3 years of age.[86–88,191] Hepatocellular carcinomata are usually solitary and multilobulated. Extrahepatic metastasis occurs transperitoneally and hematogenously to lungs. Microscopically, the neoplastic cells resemble hepatocytes and retain cord arrangement. However, the normal liver architecture is lost, and sometimes the cells are difficult to differentiate. Mitotic figures are uncommon. Possible etiologic factors in other species include heredity, parasitism, chemical and plant carcinogens, and viral hepatitis.[190]

Reported clinical signs of hepatocellular carcinomain horses include depression, anorexia, weight loss, intermittent diarrhea, and abdominal distention.[86–88,191] Abnormal laboratory findings included absolute erythrocytosis, persistent hypoglycemia, sanguineous peritoneal effusion, and increased serum SDH, GGT, ALP, and indirect reacting bilirubin. In one case, absolute erythrocytosis was attributed to erythropoietin secretion by the carcinoma (inappropriate secondary erythrocytosis).[88] The same horse had a high serum concentration ofα-fetoprotein, a globulin normally synthesized only by embryonic fetal liver cells that is commonly detectable in human patients with hepatocellular carcinoma.

Two case reports describe malignant *hepatoblastoma* in young horses.[59,194] Hepatoblastoma is an embryonic tumor of the liver with a wide range of histologic patterns that include epithelial and mesenchymal elements. Clinical signs included emaciation, pyrexia, and pleural effusion. Laboratory abnormalities included mature neutrophilia, erythrocytosis with normal erythropoietin levels, hypergammaglobulinemia, hyperfibrinogenemia, and increased serum activity of GGT and ASP. In one of the cases, transabdominal ultrasonography identified abnormal hepatic architecture. Increased serum α-fetoprotein may serve as a tumor marker but cannot be used alone to confirm a diagnosis.[194]

A *mixed hamartoma* was described in the liver of a late-term aborted equine fetus.[189] Histologically, the lesion appeared to be a proliferation of large hepatocyte-like cells with eccentric nuclei and voluminous cytoplasm; abnormal bile ducts; and fibroblastic fibrocystic interstitial tissue, with complete lack of structural organization.

14.3.2.9 Hepatic Amyloidosis

Amyloidosis refers to a group of diseases characterized by the extracellular deposition of a proteinaceous fibril substance, *amyloid*, in the tissues. Amyloid deposits are composed of nonbranching fibrils in β-pleated sheet conformation formed by the proteolytic cleavage of precursor proteins by the mononuclear phagocyte system. Depending on the organ of deposition, amyloid distorts normal tissue architecture and may lead to functional impairment. In horses the liver and spleen are the most common organs involved in systemic amyloidosis.[195] Two forms of systemic amyloidosis exist and are distinguished by the type of precursor protein and 988
subsequent protein fibril. The precursor in *reactive* or *secondary* systemic amyloidosis is serum amyloid 989
protein AA, an acute phase protein produced by hepatocytes in response to chronic infection or inflammation. The AA fibrils are identified in tissues by green birefringence in polarized light, after staining with Congo red, which is lost after treatment with potassium permanganate. Most often in horses, hepatic amyloid deposits are AA fibrils andhave been associated with severe parasitism or chronic infection and inflammation.[195,196] One horse in which the liver was the principal organ of amyloid deposition presented for evaluation of chronic weight loss.[196] An antemortem diagnosis of hepatic amyloidosis was not made; however, laboratory abnormalities included severe hypoalbuminemia, polyclonal gammopathy, and large numbers of ascarid and strongylid eggs in the feces. Histopathologically, the liver revealed extracellular deposits of AA amyloid periportally and adjacent to the sinusoids in the space of Disse. The amyloid deposits were accompanied by hepatocytic atrophy and a mild mononuclear cell infiltrate.

Systemic primary, immunocytic, or *idiopathic amyloidosis* is caused by the deposition of amyloid light chain fibrils. The precursor proteins in primary amyloidosis are the variable region of immunoglobulin light chains. The amyloid fibrils also stain with Congo red, but staining is retained after potassium permanganate treatment. *Local immunocytic amyloidosis*, with deposition in the upper respiratory mucosa or skin, is more common in horses than systemic primary amyloidosis. However, at least one reported case describes systemic primary amyloidosis ina 14-year-old Thoroughbred mare with chronic weight loss and cutaneous nodules.[197] Amyloid deposits were identified in the liver, myocardium, spleen, gastrointestinal mucosa, pulmonary interstitium, pancreas, and arterial walls.

14.3.2.10 Chronic Hypoxemia

Right-sided heart failure causes the pressure in the caudal vena cava to rise. Retrograde pressure increases in the hepatic central veins cause hypoxemia and pressure necrosis of the adjacent hepatocytes. Chronic passive congestion may cause fatty change, atrophy, and fibrosis.

14.3.3 CONGENITAL ABNORMALITIES

14.3.3.1 Portosystemic Shunt

Portosystemic shunts may be intrahepatic or extrahepatic, congenital or acquired. Few reports have documented congenital extrahepatic portosystemic shunts in foals.[90,198] The vascular shunts allow blood within the portal system to bypass the liver and drain into the systemic circulation, directly or indirectly, via the caudal vena cava or the azygous vein. In the reported cases, age at presentation varied between 2 and 6 months. Vague intermittent neurologic signs of blindness, ataxia, and severe depression were consistent with

HE. Growth appeared to be stunted. These clinical signs are caused by the altered hepatic blood flow and hepatic insufficiency following hepatocellular atrophy.

Laboratory abnormalities in the reported cases varied, but the most consistent findings included a decreased BUN, prolonged BSP half-life, and increased blood ammonia concentration. Liver biopsies revealed hepatocellular atrophy and necrosis, fibrosis, and biliary hyperplasia.[90] Antemortem diagnosis was confirmed by operative mesenteric portography or nuclear scintigraphy (see Diagnostic Imaging of the Liver). Surgical correction was attempted unsuccessfully in one case.[199] Necropsy examination revealed small, firm livers. The location of the shunt(s) in reported cases varied, but all were extrahepatic, involving a direct connection between the mesenteric veins and the caudal vena cava. Alzheimer's type II astrocytes were visible in the brain in one case and were consistent with HE.[198]

Acquired portosystemic shunts are rare in horses. One report describes an 11-year-old Thoroughbred withHE. The horse subsequently was determined to have a functional portosystemic shunt following vascular thrombosis of the portal vein.[199]

14.3.3.2 Biliary Atresia

At least two cases of extrahepatic biliary atresia in foals have been documented.[200,201] An antemortem diagnosis was not possible in either case; however, veterinary attention was sought at 4 weeks of age in both foals for anorexia, depression, lethargy, poor weight gain, colic, polydipsia, polyuria, pyrexia, and icterus. Laboratory evaluation of one of the foals was consistent with biliary obstruction, as indicated by a mildly increased serum activity of SDH and greatly increased activity of GGT and conjugated bilirubin. Necropsy of both foals revealed a large, firm liver. Although not specifically documented in one case, the entrance to the bile duct and the main bile duct were absent in the other foal.[200] Histologically, both livers appeared similar and consistent with extrahepatic biliary atresia. Bile canaliculi were present and distended, with associated degenerative hepatocytes. Biliary proliferation was extensive and surrounded by fibrous tissue with interdispersed islands of hepatocytes. No bile was in the proliferative bile ducts, and the portal triad ducts were absent.

In horses, extrahepatic biliary atresia is speculated to be congenital. In human beings, other causes suggested include neonatal sclerosing cholangiohepatitis, excretion of a biliary toxin, deficit of bile flow in utero, or lumen destruction caused by ductal vascular insufficiency.[201] Despite the cause, extrahepatic biliary atresia 989
induces intrahepatic biliary hypertrophy, an abortive attempt to establish continuity. The biliary hypertrophy 990
displaces hepatocytes, causing periportal and perilobular hepatocellular degeneration, fibrous replacement, and ultimately loss of normal hepatic architecture. Although an antemortem diagnosis was not possible in either equine case, hepatobiliary scintigraphy (see Diagnostic Imaging of the Liver) successfully detected total biliary obstruction in a neonatal lamb with biliary atresia.[91]

14.3.3.3 Serous Cysts

Serous hepatic cysts have been reported and usually are an incidental finding at necropsy.[14]

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15 CHAPTER 15 EQUINE OPHTHALMOLOGY

David A. Wilkie

15.1 Examination

The equine eye presents particular challenges for diagnostic and therapeutic approaches. However, the basic principles of a complete ophthalmic examination hold true. Box 15-1 lists the equipment required for a routine ophthalmic examination. One should perform the initial ophthalmic examination in a well-lighted environment and before tranquilization. The examiner assesses facial, orbital, and eyelid symmetry; looks for ocular discharge (serous, mucoid, mucopurulent) or blepharospasm; and evaluates the cranial nerves, specifically cranial nerves II through VII. A complete ophthalmic examination includes assessment of pupillary light and menace response, maze testing, globe position and mobility, sensation of ocular and adnexal structures, and eyelid position and function. To evaluate direct and consensual pupillary light responses accurately often requires a bright focal light source (3.5-V halogen) and a darkened examination area.

Further examination or therapy may require some form of tranquilization, regional nerve blocks, and topical anesthesia. Detomidine (Dormosedan, Pfizer Animal Health, Exton, Pennsylvania) administered intravenously at 0.01 to 0.02 mg/kg or xylazine (Rompun, Haver-Lockhart, Shawnee, Kansas) at 0.5 to 1.0 mg/kg combined with butorphanol tartrate (Torbugesic, Bristol Laboratories, Inc., Evansville, Indiana) at 0.01 mg/kg administered intravenously provide a synergistic analgesic effect and facilitate examination, sample collection, nasolacrimal irrigation, lavage tube placement, and minor surgical procedures. If one intends to determine intraocular pressure (IOP), one should note that intravenous administration of xylazine significantly lowers the IOP.[1]

Sensory innervation of the globe and adnexa is from the trigeminal nerve (cranial nerve V), and motor innervation is from the facial nerve (cranial nerve VII). The ophthalmic nerves blocked most frequently are the auriculopalpebral branch of cranial nerve VII and the supraorbital (frontal) branch of cranial nerve V. Blocking of these nerves provides akinesia and anesthesia of the superior eyelid, respectively. One can palpate the auriculopalpebral nerve as it courses over the zygomatic arch in the area of the temporofrontal suture. Using a 25-gauge, ⅝-inch needle, one blocks the nerve by injecting 3 to 5 ml of mepivacaine (Carbocaine, Winthrop Laboratories, New York) over the zygomatic arch in this area. One blocks the supraorbital nerve as it emerges from the supraorbital foramen of the frontal bone by palpating the foramen, inserting a 25-gauge, ⅝-inch needle into the foramen, and injecting 2.0 ml of mepivacaine; one infuses another 2 to 3 ml subcutaneously while removing the needle. Additional sensory nerves one occasionally blocks include the infratrochlear, lacrimal, and zygomatic branches of cranial nerve V.[2] Alternatively, one can use local infiltration of anesthetic to provide anesthesia of a specific area.

One now can perform a complete ophthalmic examination (see Box 15-1). One should obtain samples for bacterial or fungal culture or evaluation of tear production before instilling any topical solution or ointment on the eye. One measures aqueous production of tears using commercially available Schirmer tear test strips, with normal wetting being 20 mm or greater in 30 seconds. Using a bright focal light source, one now examines the conjunctiva, nictitans, cornea, anterior chamber, iris, pupil, and lens. One evaluates the ocular media (cornea, aqueous humor, lens, and vitreous) for clarity and transparency; assesses the position and size of the lens, shape 995
996 and mobility of the pupil, and appearance of the corpora nigra; and examines the eye for irregularities, vascularization, and pigmentation. Although one can examine the whole anterior segment using a Finoff transilluminator with or without magnification, a new handheld monocular slitlamp that attaches to an otoscope or

ophthalmoscope handle is available (HSL-10, Heine, United States). The handheld lamp provides magnification and an appreciation for depth and three-dimensional anatomy. Fluorescein staining of the cornea detects the presence of corneal ulceration, and the appearance of fluorescein at the nares indicates a patent nasolacrimal system. Following instillation of topical anesthesia (proparacaine 0.5%, Alcaine, Alcon Laboratories, Fort Worth, Texas), one can cannulate the nasolacrimal puncta using a 3.5F urinary catheter, and irrigate the nasolacrimal system. Alternatively, one can irrigate the nasolacrimal system in retrograde fashion, cannulating the duct using a 5F catheter at the nasal opening. Using thumb forceps, one can grasp the nictitans and examine the palpebral and bulbar surfaces for foreign bodies, mass lesions, or other abnormalities. One collects conjunctival and corneal cells for cytologic study using a Kimura spatula following topical anesthesia. If available, one can use the Tonopen (Mentor O&O, Norwell, Massachusetts) to determine ocular pressure.

15.1.1

BOX 15-1 EQUIPMENT FOR BASIC EQUINEOPHTHALMIC EXAMINATION

Bright, focal light source: 3.5-V halogen light with Finoff illuminator

Direct ophthalmoscope

Indirect 20-D 5× lens

Handheld slitlamp: HSL-10 attaches to direct ophthal-moscope handle (Heine, United States)

Magnifying loupe, 2×-4×

Dressing forceps

Open-ended urinary catheter (3.5 French) for nasolacrimal catheterization

No. 5 French catheter for cannulation of nasolacrimal duct at nares

Sterile fluorescein strips

Schirmer tear test strips

Sterile culture swabs

Kimura spatula for obtaining cytologic sample

Glass slides

Sterile eyewash

Proparacaine 0.5% (Alcaine): topical anesthetic

Tropicamide 1% (Mydriacyl): short-acting dilating agent

Sedation: Xylazine (Rompun)

Butorphanol tartrate (Torbugesic)

Mepivacaine (Carbocaine): local nerve blocks

Modified from Wilkie DA: Ophthalmic procedures and surgery in the standing horse, *Vet Clin North Am Equine Pract* 7:535-547, 1991.

To examine the posterior segments of the eye (optic nerve, retinal blood vessels, and tapetal and nontapetal fundus), one should perform direct and indirect ophthalmoscopy. A new monocular indirect ophthalmoscope, the PanOptic ophthalmoscope (Welch Allyn) allows those more familiar with the technique of direct examination to perform indirect ophthalmoscopy. One evaluates the size and color of the optic nerve, number and size of retinal blood vessels, pigmentation of the nontapetal fundus, and the presence or absence of hypo- or hyperreflective changes of the tapetal fundus.

15.2 Ocular Ultrasonography

15.2.1 TECHNIQUE

One can perform ocular ultrasonography directly through the cornea or the eyelids or by using an offset device. The optimal transducer for ocular ultrasonography is the 10-MHz probe, but one can use a 7.5-MHz probe. Sedation, auriculopalpebral nerve block, and topical anesthesia generally are required. Sterile, water-soluble lubricating jelly works well as a contact material, provided one irrigates the cornea afterward.

15.2.2 NORMAL ANATOMY

Unless one uses an offset device, the cornea and a portion of the anterior chamber are lost in the near artifact. The anterior and posterior lens capsules are visible as an echodense line at the 12 and 6 o'clock positions, whereas the remainder of the lens is anechoic. The iris, corpora nigra and ciliary body are often visible. The vitreous body is normally anechoic. The posterior eye wall is visible as a concave echodensity with the optic nerve head visisble as an echodense area at the posterior pole with an anechoic optic nerve posteriorly. One can visualize the orbital contents best using a 7.5-MHz transducer to evaluate the extraocular muscle cone, optic nerve, and associated structures.

15.2.3 INDICATIONS

Ocular ultrasound is indicated for evaluation of intraocular contents when one or more of the ocular transmitting media are opaque, including opacification of the cornea, aqueous and vitreous humors, and the lens. The most common indications for ocular ultrasound are in eyes with a cataract to evaluate for a retinal detachment, following traumatic hyphema to assess posterior segment damage, or in eyes with severe corneal opacification. In addition, one can evaluate the orbit in instances of exophthalmos or orbital trauma. 996

997

15.3 Eyelids

15.3.1 CONGENITAL

Diseases of the equine eyelid are common. Congenital lesions include coloboma, agenesis, dermoid, and entropion.[3,4] Of these, entropion is the only frequently occurring disease. Entropion is inward rolling of the eyelid margin that results in facial hairs contacting the cornea, leading to irritation, conjunctivitis, and possibly secondary corneal ulceration. Causes include premature or dehydrated foals with enophthalmos, ocular pain

resulting in spastic entropion, and primary conformational problems. Manual reduction and frequent topical ocular lubrication with artificial tear ointment is the initial treatment of choice and may be the only treatment required in some foals, especially if the entropion follows dehydration. If the entropion is severe or does not respond to manipulation, one may place two or three vertical temporary everting mattress sutures to position the eyelid correctly.[3] One must take care to avoid overcorrection and the inability to close the eyelids during blinking. Suture placement is at and perpendicular to the eyelid margin, tacking this to the periocular skin over the orbital rim. Monofilament, 3–0 to 5–0 nylon suture is preferrable; one removes the sutures after 10 to 14 days. One must treat associated corneal disease to prevent secondary infection and subsequent scar formation. Entropion requiring more aggressive surgical intervention in the form of skin excision is rare. Severe or recurrent entropion is correctable by excising an elliptic portion of eyelid skin in the affected area and reapposing the skin edges (Hotz-Celsus procedure).

15.3.2 ACQUIRED

Common acquired conditions involving the equine eyelid include trauma and neoplasia.

15.3.2.1 Trauma

Eyelid trauma includes contusions and lacerations.[5] Commonly associated with eyelid trauma are corneal abrasions or lacerations, anterior uveitis, and if the trauma involves the medial canthus, nasolacrimal system damage. Eyelid contusions often result in blepharoedema and hemorrhage. Although blepharoedema does not require therapy, one can hasten recovery by using systemic flunixin meglumine (Banamine, Schering Corp., Kenilworth, New Jersey), cold compresses in the acute phase, and warm compresses beginning the day following the injury.

Eyelid lacerations are more serious and usually require immediate therapy. The vascular supply to the eyelid is extensive, and many apparent avascular segments of eyelid recover following repair. If possible, primary wound closure is preferrable. One must remove all debris from the wound before closure, disinfect the eyelid surface and adjacent tissues with a 1:25 to 1:50 dilution of povidone-iodine solution, avoid excessive tissue debridement, and under no circumstances amputate a pedicle of eyelid. Loss of eyelid margin, whether through trauma or iatrogenic, results in severe, chronic corneal irritation, vascularization, ulceration, and fibrosis. In addition, reconstructing an eyelid margin from the adjacent skin following amputation of the normal eyelid margin is difficult.

One should suture lacerated eyelids using a two-layer closure, ensuring accurate anatomic apposition of the wound edges and eyelid margin. One can perform minor surgical repairs using sedation and local nerve blocks, but the horse may require general anesthesia if the injury is severe. One sutures the deeper, conjunctival layer first using 4-0 to 6-0 absorbable suture in a horizontal mattress pattern beginning away from and working toward the eyelid margin, taking care to avoid penetrating the conjunctiva so that the suture does not contact the cornea. One then closes the skin with 4–0 to 6–0 nonabsorbable suture. The eyelid margin is the most important part of wound closure and is closed first to ensure accurate apposition. The author prefers to use a cruciate suture pattern at the eyelid margin and a simple interrupted pattern for the remainder of the skin closure. Failure to perform a two-layer closure or achieve precise apposition of the eyelid margin may result in ulcerative keratitis or other secondary complications.[6] Postoperative therapy includes administration antibiotics for 5 to 7 days for systemic effect, tetanus toxoid, warm moist compresses, and flunixin meglumine if inflammation and swelling are a problem. Topical medication is not required for eyelid injuries unless corneal or anterior segment damage accompanies the injury. One must

evaluate eyelid function, and the eyelid must provide adequate protection to the cornea. If the blink response is impaired, one should protect the cornea with topical lubricants as often as possible. Advanced blepharoplastic techniques for repair of severe eyelid trauma with resulting loss of tissue are beyond the scope of this chapter but are discussed in detail elsewhere.[3]

15.3.2.2 Neoplasia

The most common neoplasms of the equine eyelid are sarcoid and squamous cell carcinoma. In addition, melanoma, mast cell tumor, lymphosarcoma, basal cell carcinoma, and papilloma can affect the eyelid. The differential diagnosis for eyelid neoplasms includes parasitic diseases such as ocular habronemiasis and other causes of granulomatous skin disease. Treatment of eyelid neoplasia depends on the location, size, tumor type, age and purpose of the horse, cost, surgical skill, and equipment available. Treatment modalities 997
include surgical excision, radiation therapy, chemotherapy, hyperthermia, immunotherapy, cryosurgery, or a 998
combination of these. The aim of therapy is to eliminate or halt progression of the tumor while maintaining eyelid function and preserving the eye and vision. Complete excision with primary closure is the optimal treatment but often cannot be achieved because of limitations on availability of tissue for reconstruction and the extensive and aggressive nature of many eyelid neoplasms. If one cannot appose eyelid margins following tumor excision, more involved blepharoplastic techniques, such as advancement skin flaps, may be indicated (Figure 15-1).

Figure 15-1 Squamous cell carcinoma involving the medial canthus and inferior eyelid of 19-year-old Appaloosa.

15.3.2.2.1

Squamous Cell Carcinoma

Squamous cell carcinoma involving the eyelid is usually erosive and ulcerative.[7] The medial canthus is the most common site of origin (see Figure 15-1). Invasion of adjacent soft and bony orbital tissue is possible, if untreated. Treatment of ocular squamous cell carcinoma is discussed in detail under Conjunctiva and Third Eyelid.

15.3.2.2.2

Sarcoid

Periocular sarcoid is the second most common eyelid neoplasm of the horse. The average age of an affected horse is 4 years, and smooth and warty sarcoids occur. Orbital invasion and bony involvement are possible. Surgical excision is associated with frequent recurrence and often is combined with cryosurgery, hyperthermia, chemotherapy, or irradiation to ensure complete tumor destruction. Of all treatment modalities, radiation is the most expensive and yields the best overall success.[8]

Intralesional immunotherapy with cell wall extracts of bacille Calmette-Guérin (BCG) in oil has been used effectively in repeated injections 3 weeks apart. A total of four doses usually is required for complete tumor regression with the overall response reported to be 69%, provided the injection was intralesional.[8] Injection of cell wall extracts of BCG is associated with local inflammation and tumor necrosis. During this period of inflammation, one must protect the globe to prevent corneal desiccation or ulceration. The use of live BCG organisms or whole killed organisms has been associated with anaphylactic reaction and death and is discouraged. Adverse systemic reactions to cell wall extracts of BCG are rare, but premedication with systemic corticosteroids is advised.

Intralesional chemotherapy with cisplatin in an oil-water emulsion has been reported to result in regression of equine sarcoid.[9] A series of four injections, spaced 2 weeks apart, with a mean dose of cisplatin of 0.97 mg/cm^3 of tumor mass resulted in tumor regression in all treated horses. In addition, 87% of horses treated for sarcoids were relapse free at a 1-year follow-up.

15.4

Conjunctiva And Third Eyelid

The conjunctiva is divided into bulbar and palpebral surfaces. The conjunctiva merges with the cornea at the limbus, with the eyelid at the eyelid margin, and the bulbar and palpebral conjunctiva join to form the fornix. The conjunctiva has normal resident bacterial and fungal populations. The most common isolates from the equine conjunctiva vary according to geography, season of the year, and investigator.[10–12] In general, isolates of gram-positive organisms—*Corynebacterium, Bacillus, Staphylococcus,* and *Streptomyces*—are more common than gram-negative organisms from normal equine conjunctiva.[11] Fungi are isolated more commonly in the summer and autumn and have been reported to be present in 95% of normal horse conjunctiva samples.[12] In addition, fungi are isolated more frequently from stabled horses, which likely relates to the immediate environmental and husbandry conditions.[11]

Congenital diseases of the equine conjunctiva are uncommon, conjunctival dermoid being the most common congenital abnormality. A dermoid is a congenital tumor of skin and associated glands, hair, and hair follicles that can affect the cornea and adjacent conjunctiva. One should remove a dermoid that is irritating the globe or adnexa surgically by superficial keratectomy/conjunctivectomy.

Unlike the small animal patient, infectious conjunctivitis is rare in the horse. Organisms reported to result in infectious conjunctivitis in the horse include equine herpesvirus type 2,[13] *Mycoplasma* species, *Chlamydia, Moraxella equi,* and mycotic and viral agents.[3]

Foreign bodies and trauma are a common cause of conjunctival irritation and damage and often are associated with concurrent eyelid and corneal injuries. One directs treatment to eliminate the cause, treat any conjunctival or associated eyelid or corneal damage, and prevent secondary infection. One must perform a complete ophthalmic 998
examination in all animals with evidence of ocular trauma. One should consider conjunctival foreign bodies in 999
any horse with a recurrent corneal erosion, ocular pain, or conjunctivitis. One should examine the superior and inferior fornices and the bulbar and palpebral surface of the nictitans following topical administration of 0.5% proparacaine. Magnification is essential to detect foreign bodies. In addition, irrigation of the nasolacrimal system may yield foreign material associated with chronic conjunctivitis or dacryocystitis.

Severe chemosis with conjunctival prolapse and exposure may result from self-trauma, especially associated with being recumbent for a prolonged time. Treatment includes systemic antiinflammatory drugs, artificial tear ointment to protect exposed conjunctiva, keeping the head elevated, and in severe cases, repositioning the conjunctiva and a temporary tarsorraphy until the swelling resolves.

15.4.1 PARASITES

Common parasites of the equine conjunctiva include *Thelazia lacrymalis, Habronema* species, and *Onchocerca cervicalis*.[14] In a necropsy sample the prevalence of *Thelazia* in the conjunctival sac of horses is highest in young animals, with 43% of 1- to 4-year-old horses being affected.[15] In most instances, infection is not associated with clinical signs, but a chronic conjunctivitis, seromucoid discharge, and conjunctival nodules can occur.[3] The adult parasite may be visible on ophthalmic examination of the cornea and conjunctival fornix, and larvae may be visible on examination of centrifuged samples of nasolacrimal washes. Treatment includes removal of the adult worms, irrigation of the nasolacrimal system, topical corticosteroids to control inflammation, and fly control. The topical organophosphates echothiophate, 0.03% to 0.06% every 12 hours, or isoflurophate, 0.025% every 24 hours for 7 days, have been reported to kill the parasite.[16] Systemic treatment with fenbendazole at 10 mg/kg orally every 24 hours for 5 days also is reported to *Thelazia* effectively.[17]

Ocular manifestations of equine cutaneous habronemiasis result when the house or stable fly deposits the larvae of the *Habronema* spp. on or around the eye.[3,18,19] The resulting granuloma is associated with inflammation, intense pruritus, and epiphora. Yellow, caseous granules are notable throughout the granulation tissue. Histologically, eosinophils and mast cells predominate. Diagnosis is based on clinical signs, location, season of the year, and histologic findings. Clinical appearance can simulate squamous cell carcinoma. Histologically, the differential diagnosis is equine cutaneous mastocytosis.[3,19] Ocular lesions are most common at the medial canthus and may involve the skin, nasolacrimal duct, third eyelid, or conjunctiva. Treatment of equine cutaneous habronemiasis varies, and no single treatment is routinely successful.[18] Surgical excision, cryosurgery, local and systemic corticosteroid therapy, systemic ivermectin (single dose, 0.2 mg/kg intramuscularly), various larvicidal treatments in the form of topical and systemic organophosphates, and various topical formulations containing ronnel or metrifonate with a dimethyl sulfoxide vehicle are used[3,18,19] (Table 15-1). Dimethyl sulfoxide serves as a vehicle and has antiinflammatory effects, possibly related to its ability to detoxify hydroxyl radicals generated by neutrophils. The aim of therapy is to kill the larvae while

controlling the resulting inflammation. In addition, fly control is an essential part of an overall management program.

TABLE 15-1 Topical Preparations Used to Treat Equine Cutaneous Habronemiasis

INGREDIENTS	AMOUNT
Ronnel solution (33%)[12]	60 ml
Thiabendazole (33%)[12]	120 g
9-Fluoroprednisolone acetate[12]	60 mg
Dimethyl phthalate (24%)[12]	30 ml
Dimethyl sulfoxide[12]	500 ml
Nitrofurantoin ointment[13]	225 g
Ronnel solution[13]	20 ml
Dimethyl sulfoxide[13]	20 ml
Dexamethasone solution[13]	20 ml
Nitrofurazone ointment (0.2%)[14]	135 g
Trichlorfon (12.3%)[14]	30 ml
Dexamethasone (2 mg/ml) [14]	30 ml
Dimethyl sulfoxide[14]	30 ml

The blood-sucking midge *Culicoides nubeculosus* transmits *O. cervicalis* microfilariae. After transmission, the immature microfilariae migrate through lymphatics and can travel to ocular and adnexal tissues. The prevalence of equine onchocerciasis increases with age, with an overall prevalence of 50% to 60%.[14,20–22] The prevalence of onchocerciasis on histologic examination of the lateral bulbar conjunctiva is 10.8%. The presence of conjunctival microfilaria does not correlate with clinical abnormalities. Ocular onchocerciasis has been proposed as a cause of keratitis, conjunctivitis, equine recurrent uveitis, and depigmentation of the temporal bulbar conjunctiva.[22] The pathogenesis is thought to involve the death of the microfilariae, release of antigens, and development of hypersensitivity in susceptible horses. Treatment is indicated in those horses with active conjunctivitis, keratitis, or uveitis attributable to onchocerciasis and includes antiinflammatory agents such as topical corticosteroids and systemic flunixin meglumine, topical echothiophate iodide 0.025%, and systemic diethylcarbamazine, levamisole, or ivermectin[3] (Table 15-2).

15.4.2 NEOPLASIA

Neoplasms affecting the conjunctiva and third eyelid include squamous cell carcinoma (SCC), lymphosarcoma, 999
melanocytoma, mastocytoma, hemangioma, and angiosarcoma.[3,23] Diagnosis and characterization of adnexal 1000
neoplasms is based on history, signalment, location, appearance, and histopathologic examination. One can perform routine surgical biopsy with sedation, local nerve blocks, and topical anesthesia. Most adnexal

neoplasms are benign or locally invasive, the exception being ocular angiosarcoma, which is reported to have a high incidence of metastasis.[24] Treatment of adnexal neoplasms varies according to tumor type, location, and size; use of the animal; treatment modalities available; and cost. The aim of treatment is to eliminate the tumor, restore normal anatomy, and maintain function of the eye and associated structures.

TABLE 15-2 Systemic Treatment of Ocular Onchocerciasis

DRUG	DOSE
Diethylcarbamazine	4.4–6.6 mg/kg/day orally for 21 days
Levamisole	1.1 mg/kg/day orally for 7 days
Ivermectin	0.2–0.5 mg/kg intramuscularly

SCC is the most common tumor of the equine eye and adnexa (Figure 15-2). Involvement can be unilateral or bilateral, with the third eyelid and medial canthus affected most frequently.[16,25] Involvement of the corneal limbus, sclera, conjunctiva, and other adnexal structures also has been reported.[16,25,26]

The mean age of onset for SCC is 9.8 years for all horses compared with 3.8 years for ocular sarcoidosis.[26] The incidence of ocular SCC is higher in draft-breed horses and Appaloosas than in light horses,[27,28] and sexually intact males and females have a decreased incidence.[27] Adnexal hypopigmentation and increased exposure to actinic radiation[26,27] are hypothesized to predispose horses to develop ocular SCC.

Figure 15-2 Squamous cell carcinoma affecting the temporal conjunctiva, limbus, and cornea.

Ocular SCC is malignant and locally invasive with the potential to metastasize.[22,29] Metastases to local and cranial mediastinal lymph nodes, parotid salivary glands, and the thoracic cavity occur in 10% to 15% of patients.[28]

Treatment of ocular SCC varies and must be designed for the individual patient. Surgical excision,[26] radiofrequency hyperthermia,[30] cryotherapy,[31] immunotherapy,[31] radiation therapy,[26,29–31] intralesional chemotherapy,[9,32] laser surgery,[7] or a combination of these,[30,31] have been used with success. Location, size, and depth of the tumor, visual status, financial commitment, purpose of the animal, and the presence or absence of metastatic disease influence the choice of therapy.

If possible, complete surgical excision is curative and is the preferred therapy. Difficulty arises with surgical excision when preservation of the globe and ocular and adnexal anatomy and function are required. Complete excision of the third eyelid is possible and often can be performed using sedation and local nerve blocks. To avoid possible herniation of the orbital fat, one should appose the bulbar and palpebral margins of the third eyelid conjunctiva following excision using 5–0 to 6–0 absorbable suture in a simple continuous pattern.

Most SCCs are radiosensitive and can be treated successfully with sources of β- or γ-radiation. The major disadvantage of less expensive modes of radiation therapy, such as strontium 90, a source of β-radiation, is the size limit of tumor amenable to treatment. With ^{90}Sr, for example, half the β-radiation produced is lost after passage through 1 mm of soft tissue. Thus one should limit treatment to lesions less than 2 mm deep, such as corneal, scleral, or conjunctival SCCs. Radiation is most appropriate as an adjunct therapy following surgical removal of a corneal or conjunctival SCC. When used appropriately, ^{90}Sr can achieve a nonrecurrence rate for SCC of 87.5% after 2 years.[33]

The use of interstitial radiotherapy for periocular SCC has been reported.[30,33] Radioactive gold seeds (^{198}Au) were used to impart a medium-energy γ-ray (0.41 MeV) with a half-life of 93.3 hours for a total recommended dose of 5000 rad.[30] Success rates of 80% at 1 year and 70% at 2 years are reported.[33] Disadvantages of radioactive implants are substantial cost, limited availability, risks associated with human exposure, and the licensing restrictions on their handling.[30] Secondary complications of interstitial radiation therapy include temporary corneal opacity, local necrosis, hair loss, damage to normal structures, and local depigmentation.[30,33] Although no retinal or lens changes have been reported following interstitial radiation treatment, one should consider the possibility of photoreceptor damage and cataractogenesis following radiation therapy.

Radiofrequency hyperthermia involves the passage of a radiofrequency (2 MHz) electric current between two 1000
electrodes. Tissue between these probes offers resistance, resulting in thermal energy being transferred to the 1001
tissue. Tissue temperature increases to 50° C in a 1-cm^2 area,[30] with malignant cells exhibiting a greater sensitivity to thermal energy than normal cells. One should not use topical radiofrequency hyperthermia as the only mode of treatment for tumors extending deeper than 3 mm or greater than 4 to 5 cm in diameter. Superficial eyelid SCCs or corneal and conjunctival SCCs are most appropriate for treatment using radiofrequency hyperthermia. One should avoid overlapping the fields of exposure, especially in corneal tumors, because this creates a risk of excessive necrosis of normal tissue.[34]

Cryosurgery using nitrous oxide (80°C) or liquid nitrogen (185°C) in a single freeze– or double freeze–thaw cycle results in tissue destruction. Cryodestruction is most often indicated for treatment of eyelid SCCs.

Associated local depigmentation of skin and hair may occur in the treated area. One should use thermocouples, and tissue temperatures of −25° C are optimal.

Intralesional chemotherapy with cisplatin or bleomycin in an oil-water emulsion has been reported to result in regression of equine SCC. Treatment is the same as previously described for eyelid sarcoidosis. Of those horses with adnexal SCC treated with intralesional cisplatin, 65% to 93% were relapse free at 1 year.[9,32]

Regardless of the treatment used, frequent and continued follow-up examinations result in the greatest long-term success in managing ocular and periocular SCC. Recurrence may happen at the initial site of involvement and must be distinguished from granulation tissue associated with the previous treatment. In addition, one must monitor other sites and the opposite eye for the development of new SCC lesions.

15.5 Nasolacrimal System

The nasolacrimal system consists of the superior and inferior puncta and canaliculi, nasolacrimal sac, nasolacrimal duct, and nasal punctum located on the floor of the nasal vestibule.[35] The nasolacrimal system serves to carry tears from the medial canthus of the eye to the nasal vestibule. Abnormalities of the nasolacrimal system result in epiphora, an overflow of tears, following an impairment in drainage of tears. Epiphora can be serous, mucoid, or mucopurulent and must be differentiated from reflex lacrimation resulting from ocular irritation or inflammation. Examination of the nasolacrimal system includes placement of fluorescein stain in the conjunctival cul-de-sac and observing its appearance at the nasal opening. One can irrigate the nasolacrimal system antegrade from the eyelid punctum or retrograde from the nasal punctum. One applies topical anesthetic (0.5% proparacaine) to the eye and places a 3.5F open-ended feline urinary catheter in the superior punctum. One uses sterile eyewash or saline (10 to 20 ml) to irrigate the system, first observing fluid passing out of the inferior punctum and subsequently, the nasal punctum. If one performs retrograde irrigation from the nasal punctum, one uses a 5F or 6F urinary catheter and increases the volume of irrigating solution. One can perform radiographic imaging of the nasolacrimal duct, dacryocystorhinography, using contrast material injected into the duct from the superior eyelid punctum.[3,35] Lateral and oblique radiographic views are best, and useful contrast materials include 60% barium sulfate (Novapaque, Picker Corp., Cleveland, Ohio) and 31% diatrizoate meglumine and diatrizoate sodium (Renovist II, Squibb Diagnostics). Indications for dacryocystorhinography include chronic epiphora, inability to irrigate the nasolacrimal duct, suspicion of a nasolacrimal foreign body, or evaluation of the nasolacrimal duct for congenital or acquired abnormalities.

Abnormalities of the nasolacrimal system can be congenital or acquired. In the horse the most common congenital abnormality is atresia of the distal portion of the nasolacrimal duct and the nasal punctum, which results in mucoid epiphora at 3 to 4 months of age.[3,36] Additional abnormalities include atresia of an eyelid punctum, abnormal placement of an eyelid punctum, and multiple nasal openings.[3,23] Whether these conditions are inherited is unknown. To correct an atretic nasal opening, one passes a catheter from the inferior eyelid punctum to the level of the atresia. One palpates the end of the cathete in the nasal vestibule and makes an incision through the nasal mucosa overlying the catheter, exposing the catheter. One then brings the tubing out the nasal opening and, along with the portion of tubing from the inferior punctum, sutures it to the skin of the face. One leaves the tubing in place for 3 to 4 weeks and administers topical broad-spectrum ophthalmic antibiotic solution during this time.

Acquired conditions of the nasolacrimal system include dacryocystitis, foreign body obstruction, trauma, and involvement secondarily in neoplastic or inflammatory conditions of the medial canthus. Dacryocystitis appears as a mucopurulent ocular discharge without associated ocular inflammation. Dacryocystitis and foreign body obstructions are treated by nasolacrimal irrigation, as previously described, and bacterial and fungal culture with

or without the aid of radiographic evaluation. One administers topical broad-spectrum ophthalmic antibiotic solution following irrigation. One also can use topical corticosteroids or systemic nonsteroidal antiinflammatory drugs (NSAIDs) to help control swelling and inflammation. Traumatic or other damage to the nasolacrimal duct is best repaired at the time of injury, and placement of a tube in the nasolacrimal duct for 3 to 4 weeks to maintain 1001
patency while healing occurs is recommended. Surgical repair of the nasolacrimal duct is a referral procedure and 1002
requires general anesthesia. If the nasolacrimal duct cannot be made patent, treatment is symptomatic therapy for the epiphora or surgical trephination (conjunctivorhinotomy) to create a new outflow pathway for the tears.

15.6 Cornea

15.6.1 ANATOMY AND PHYSIOLOGY

The cornea consists of the epithelium, stroma, Descemet's membrane, and endothelium. The epithelium is 7 to 15 cells thick and is replaced every 7 to 10 days. The corneal endothelium is a monolayer of cells with little to no regenerative capacity. Damage to the endothelium is therefore of great significance, for complete repair is often not possible and results in permanent corneal edema. The cornea is transparent and avascular and is supplied with sensory nerves from the ophthalmic branch of the trigeminal nerve. The epithelium and anterior stroma are innervated richly with sensory nerves, whereas the middle and inner cornea is less well supplied. Nutrition and waste removal are carried out by the tear film, aqueous humor, and diffusion to and from the scleral and conjunctival blood vessels. The cornea is maintained in a state of deturgescence by the mechanical barrier and pump mechanisms of the epithelium and endothelium. Interference with these barriers results in corneal edema, with endothelial damage being the more significant. Chronic irritation of the cornea results in superficial vascularization, whereas inflammation of the anterior uvea results in deep corneal vascularization. Corneal pigmentation often follows vascularization. Cellular infiltration of the cornea occurs in neoplastic, infectious, and inflammatory disease. All of these processes can occur alone or in combination, resulting in an alteration of corneal transparency. In addition, scar formation and noncellular infiltrates such as mineral and phospholipid deposits (corneal degeneration) alter corneal transparency. Establishing the cause of these changes and, if possible, eliminating the cause are essential.

15.6.2 ULCERATIVE DISEASES

Corneal ulceration is perhaps the most frustrating and potentially devastating disease of the equine eye. Of all species commonly treated in veterinary ophthalmology, the cornea of the horse is the slowest to heal, is the most likely to become infected, and has the poorest prognosis for outcome. In addition, the size and temperament of the animal makes frequent treatment difficult for owner and veterinarian. In most instances, corneal ulceration results from an initial trauma, but secondary infection is common, especially in those eyes treated with topical corticosteroids following ulceration.

15.6.2.1 Clinical Signs and Diagnosis

A corneal ulcer is a break in the corneal epithelium. Clinically, ulceration results in lacrimation, blepharospasm, photophobia, conjunctival hyperemia, corneal edema, and possibly miosis and aqueous flare. The diagnosis of a corneal ulcer is based on these clinical signs and fluorescein staining of the cornea. The underlying stroma retains the fluorescein stain and appears green. One should submit bacterial and fungal culture samples from all corneal ulcers in the horse and should obtain culture samples from the margin of the ulcer, before instilling any therapeutic or diagnostic agents in the eye. Once one obtains a culture samples

and has stained the cornea with fluorescein, one applies topical anesthetic and obtains a scraping from the ulcer for cytologic examination. One places the cells on a glass slide and stains them to examine them for bacteria, fungal hyphae, and cell type. Gram's and Giemsa stains work well for examination. The presence of gram-negative rods indicates the possibility of an infection with *Pseudomonas* sp. The presence of fungal hyphae is pathognomonic for mycotic keratitis, with *Aspergillus* spp. and *Fusarium* spp. being the most frequent corneal pathogens. Mixed bacterial and fungal infections are common.

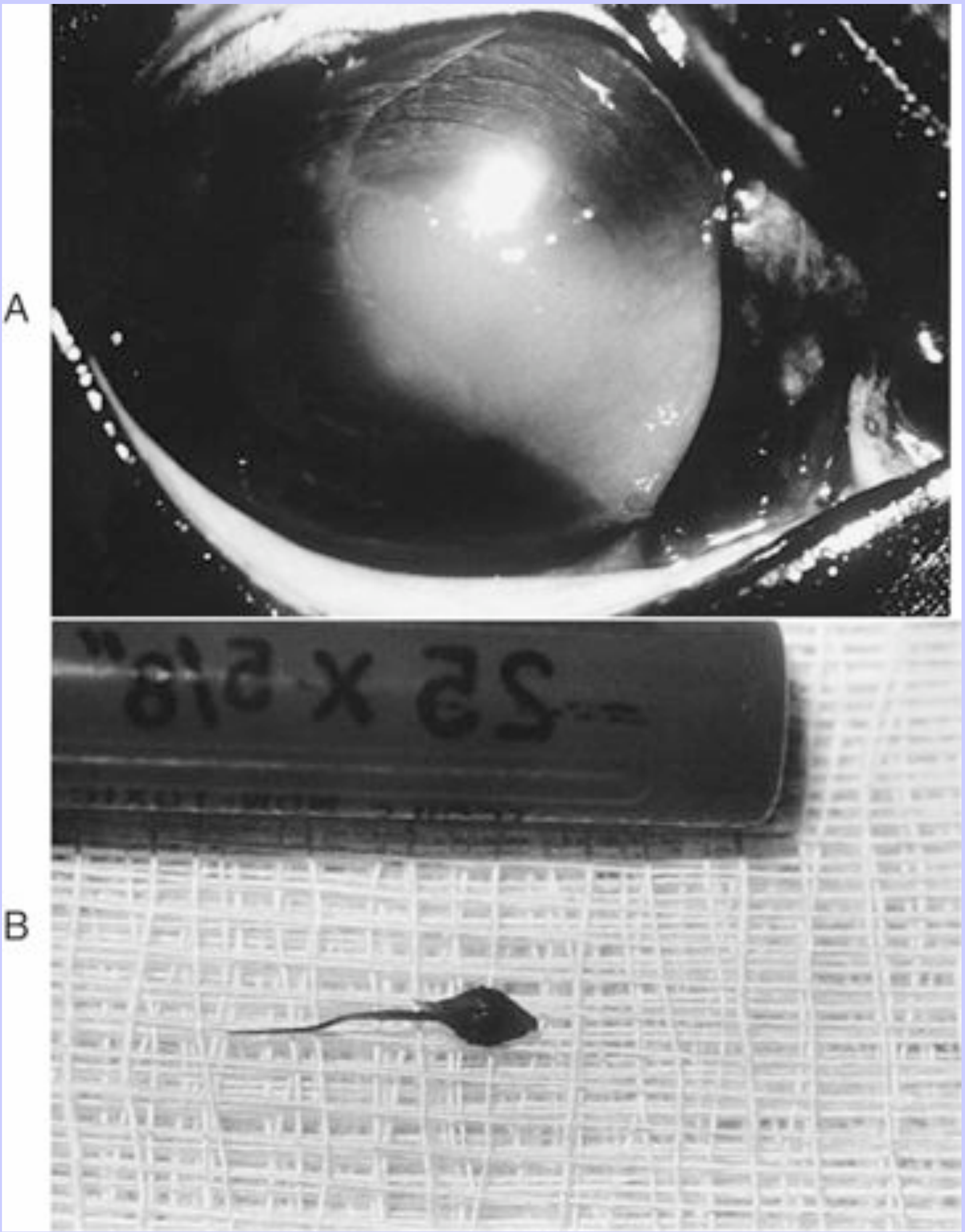
One should characterize a corneal ulcer according to size, depth, and the presence or absence of cellular infiltration. In addition, one examines the anterior chamber for anterior uveitis. With all corneal ulcers, one must attempt to establish the cause of the ulceration and eliminate it (Figure 15-3). One examines the palpebral conjunctiva and bulbar surface of the nictitans for the presence of a foreign body, evaluates the blink response and tear film, and obtains a complete history regarding trauma and previous medication. One must not administer topical corticosteroids in the presence of a corneal ulcer, and a history of previous topical corticosteroid therapy increases the likelihood of infectious, especially fungal, keratitis.

Some specific types of corneal ulcers in the horse include indolent ulcers,[37] ulcers with eosinophilic cellular infiltrate,[38] collagenase and mycotic ulcers, and possibly viral ulcerative keratitis.[3,23]

15.6.2.2 Routine Treatment

Treatment of an uncomplicated corneal ulcer involves controlling pain and inflammation, eliminating or preventing infection, and preventing secondary complications (Table 15-3). Healing occurs by migration and mitosis of the adjacent epithelial cells and depending on the size of the ulcer should be complete in 2 to 6 days for an uncomplicated corneal ulcer. Complicated corneal ulcers are those that fail to heal in an appropriate time, are secondarily infected, have an ongoing source of irritation or reulceration, have a 1002
collagenase component, are associated with corneal vascularization, or are worsening despite appropriate 1003
treatment.

Figure 15-3 **A,** Chronic, superficial corneal ulcer. The shape and orientation of the corneal ulcer suggest a foreign body on the bulbar surface of the nictitans. **B,** Foreign body removed after examination of the bulbar surface of the nictitans.



If one observes miosis, one can admininster topical atropine 1% to dilate the pupil, decrease the pain of anterior uveitis, and prevent posterior synechiae formation. One uses topical atropine as needed to dilate the pupil, but treatment frequency should not exceed 4 times daily. All topical ophthalmic medications are

absorbed and have systemic effects, and topical atropine can result in colic following ileus. During the time one administers topical atropine, one should evaluate intestinal motility by auscultation, and the owner should observe for fresh fecal material in the stall each day. Systemic flunixin meglumine is indicated to suppress inflammation and control pain associated with a corneal ulcer, which in combination with topical atropine helps dilate the pupil.

Topical broad-spectrum antibiotics such as neomycin-bacitracin-polymyxin B (Neosporin, Burroughs Wellcome Co., Triangle Park, North Carolina; AK-Spore, Akorn, Metairie, Louisiana) are the initial antibiotics of choice. Ointments are preferred for their ease of administration and prolonged corneal contact time. Frequency of administration varies according to severity of the disease. When one uses topical antibiotics prophylactically for an uncomplicated corneal ulcer treatment, administration 3 or 4 times daily is sufficient, but in severe infectious keratitis, therapy might be hourly. If one suspects infection with *Pseudomonas* sp., topical gentamicin, polymyxin B, tobramycin, or preferably ciprofloxacin is indicated every 2 to 4 hours. Although gram-positive organisms predominate initially in equine infectious keratitis, intensive topical antimicrobial therapy results in a significant shift to gram-negative organisms and a change in susceptibility patterns.[39] Additional treatment varies according to the type and severity of the ulcer (see Complicated Ulcerative Diseases).

One should not administer topical corticosteroids to a horse with a corneal ulcer.

15.6.3 COMPLICATED ULCERATIVE DISEASES

Complicated corneal ulcers are those that require treatment over and above routine ulcer management, are infected, exhibit chronicity or recurrence, are in imminent danger of perforation, or have a collagenase component (Figure 15-4). Secondary infection of a corneal ulcer is suggested by increasing corneal edema, interstitial keratitis associated with an increase in stromal inflammatory cells, corneal vascularization, purulent discharge, severe anterior uveitis, and stromal necrosis and liquefaction.

In many instances, complicated corneal ulcers require frequent and prolonged therapy, and some form of medication delivery system is indicated to ensure adequate treatment. Use of subpalpebral and nasolacrimal medication delivery systems has been described previously.[40] Placement of a through-and-through subpalpebral lavage system is described subsequently.

Many complicated corneal ulcers require a combination of surgical and medical therapy to improve the likelihood of a successful outcome.

15.6.3.1 Indolent Corneal Ulceration

Indolent corneal ulcers are by definition chronic and superficial.[37,41] Indolent ulcers often have a rim of loose or detached epithelium at the margin, focal corneal edema, moderate discomfort, and elicit minimal corneal vascularization. As with all chronic corneal ulceration, one must examine the eyelids, conjunctiva, and third eyelid thoroughly for the presence of foreign bodies, ectopic cilia, or other abnormality that might result in persistent ulceration.

The cause of indolent ulcers is suspected to be a failure in the attachment of the corneal epithelium to the 1003
underlying basement membrane. This attachment normally develops following epithelial migration and 1004
mitosis in the repair of a corneal ulcer. A basement membrane abnormality is suspected to be the underlying problem.

TABLE 15-3 Commonly Used Ophthalmic Medications

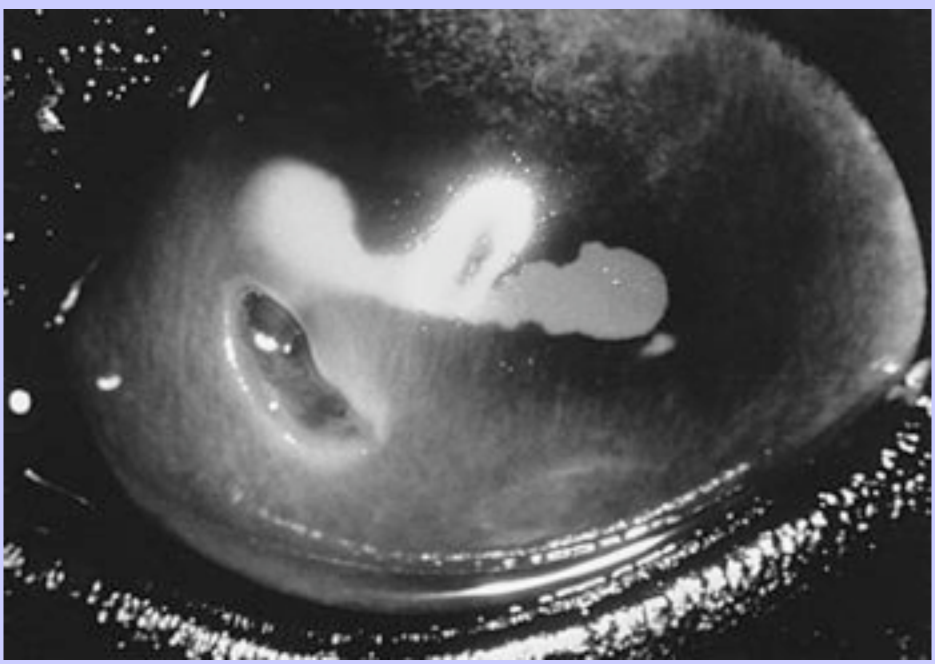
ROUTE TOPICAL	CATEGORY		DRUG	INDICATION	DOSE[*]
	Antibiotics		Gentamicin	Corneal ulceration	q2–6h
			Neomycin-bacitracin-polymyxin B	Corneal ulceration	q2–6h
			Tobramycin 0.3% (Tobrex)	Corneal ulceration	q2–6h
	Antifungals		Natamycin (Natacyn)	Corneal ulceration with suspected fungal keratitis	q2–4h
			Miconazole 1% IV, topical	Corneal ulceration with suspected fungal keratitis	q2–4h
	Antiinflammatories				
		Corticosteroids	1.0% Prednisolone acetate (Econopred Plus)		1–6/day
			0.1% Dexamethasone solution (Decadron)		1–6/day
			0.05% Dexamethasone ointment (Decadron)		1–6/day
		Nonsteroidal	0.03% Flurbiprofen (Ocufen)		4/day
			1.0% Suprofen (Profenal)		4/day
	Parsympatholytics		Tropicamide 1%	Diagnostic agent to dilate the pupil	Single dose
			Atropine 1%	Therapeutic agent to dilate the pupil long term	1–4/day
SUBCONJUNCTIVAL	(Do not exceed a volume of 1.0 ml)				
	Antiinflammatories				

	Corticosteroids	Dexamethasone acetate		10–15 mg
		Betamethasone		5–15 mg
SYSTEMIC				
	Antiinflammatories	Flunixin meglumine	Extra-or intraocular inflammation	1.1 mg/kg PO, IM, IV b.i.d.
		Phenylbutazone	Extra-or intraocular inflammation	2–4 mg/kg PO b.i.d.
		Aspirin	Extra-or intraocular inflammation	25 mg/kg PO, IV s.i.d/b.i.d.

* *PO,* Orally; *IM,* intramuscularly; *IV,* intravenously.

Treatment of indolent corneal ulcers includes removal of abnormal, loose epithelium and facilitation of epithelial attachment. Following sedation, auriculopalpebral nerve block, and topical anesthesia, one debrides the loose epithelial margins of the indolent ulcer to the point of normal attachment using a cotton swab or cilia forceps. One then performs a superficial linear keratotomy using a 25-gauge needle. Using the beveled edge of the needle, one places a series of superficial, parallel horizontal and vertical grooves in the cornea 0.5 to 1.0 mm apart that extend through the basement membrane and need be only deep enough to be visualized.

Figure 15-4 Deep corneal ulcer and descemetocele with associated focal corneal edema and miosis.



These areas serve as attachment sites for the migrating epithelium, shortening the healing time. One administers topical, broad-spectrum antibiotics every 6 hours and, if miosis is present, uses atropine 1% as needed to dilate the pupil. Systemic NSAIDs improve patient comfort. Use of a soft contact lens, 24 to 36 mm in diameter (OpTECH, Inc., Englewood, Colorado), may help protect the corneal epithelium from the shearing forces of the eyelid and third eyelid during healing but is not essential. Topical hyperosmotics have been advocated for indolent corneal ulceration but are of no benefit in the author's opinion, and topical corticosteroids are contraindicated. One can use a superficial keratectomy or a conjunctival pedicle graft or both for indolent ulcers that fail to respond to debridement and superficial linear keratotomy. 1005

15.6.3.2 Superficial Punctate Keratitis

Superficial punctate keratitis is characterized by multifocal, lacelike epithelial to subepithelial corneal opacities, some of which may be flourescein-positive, with mild corneal edema. Blepharospasm may be evident, but many horses are asymptomatic. The cause is unknown, but viral agents, specifically equine herpesvirus type 1, onchocerciasis, and immune-mediated causes have been proposed.[13,44,45]

Treatment recommendations vary and include topical antibiotic and corticosteroid application 3 to 4 times daily, topical cyclosporin A,[46] topical antiviral therapy, or a combination of these. Although the condition may respond to topical corticosteroids, recurrence is common, and one must exercise extreme caution when using topical corticosteroids. Topical ophthalmic antiviral agents (idoxuridine and trifluridine) or cyclosporin are administered every 6 to 8 hours and are the treatment of choice for most ophthalmologists.

15.6.3.3 Eosinophilic Keratoconjunctivitis

Eosinophilic keratitis may present with blepharospasm, chemosis, and conjunctivitis, but the hallmark feature is a yellow-white caseous plaque adhering to the limbal cornea with perilesional edema and corneal ulceration.[38,47] One also may find similar caseous material adhering to areas of ulcerated conjunctiva. Cytologically, the caseous material is predominantly eosinophils with a few mast cells.[38] The lesion may be unilateral or bilateral, and more than one lesion may be present within an eye. The condition appears seasonally, presenting in the spring and summer. The diagnosis is based on clinical signs, season of the year, and cytologic examination.

Untreated, eosinophilic keratoconjunctivitis is a chronic disease with healing by vascularization and scarring over several months. During this time secondary infection and acute exacerbation is possible. Treatment with topical corticosteroids has been described as reducing clinical signs, but mean time to resolution was 64 days, which is not significantly greater than without treatment.[38] In addition, secondary bacterial or fungal infectious keratitis is a risk with the use of topical corticosteroids. Topical lodoxamide 0.1% (Alomide, Alcon Laboratories) every 6 to 8 hours is the treatment of choice, along with fly control using fly masks. Superficial keratectomy also may be of benefit in resolving eosinophilic keratoconjunctivitis.

15.6.3.4 Mycotic Keratitis

One must consider all chronic corneal ulcers and ulcers that have been treated with topical corticosteroids as mycotic ulcers until proved otherwise. Keratomycosis is more common in the summer months and in warm climates.[48] Mycotic ulcers often have multifocal areas of cellular infiltrate and colonies of fungal organisms

(Figure 15-5) that appear as white lesions deep in the corneal stroma adjacent to the ulcer and often appear 7 to 10 days following initial ulceration. These infiltrates are often fluorescein-negative. Diagnosis of mycotic keratitis is based on history, clinical signs, cytologic examination, culture, and if possible, histopathologic examination of a biopsy sample (Figure 15-6). Failure to see fungal elements on cytologic examination or culture does not rule out mycotic keratitis. The most common isolate for eyes with keratomycosis is *Aspergillus* sp.[48] *Fusarium* spp., *Penicillium* spp., and *Candida albicans* also are associated with keratomycosis. If in doubt, treating the horse for fungal infection is appropriate.

In eyes in which mycotic keratitis is suspected or documented, topical antifungals are indicated in addition to routine ulcer management. The only approved ophthalmic antifungal is natamycin 5% suspension (Natacyn, Alcon Laboratories), but it is often cost-prohibitive. Although not approved for ophthalmic use, the 1005
imidazole antibiotics (miconazole, ketoconazole) may have the best efficacy for the treatment of 1006
keratomycosis.[48] Miconazole 1% can be compounded as a solution or ointment and administered every 2 to 4 hours topically for mycotic keratitis. An itraconazole–dimethyl sulfoxide ointment also has been used topically to treat keratomycosis.[49] In addition, dilute povidone-iodine solution is fungicidal and can be applied to the cornea with a cotton swab once every 1 to 2 days. Iodine is irritating to corneal and conjunctival tissues and should be irrigated from the conjunctival cul-de-sac following application. All topical antifungal medications have limited ability to penetrate intact corneal epithelium, although miconazole is better than most. If the lesion is fluorescein-negative, debriding the epithelium before medicating may be necessary (see Corneal Stromal Abscess). One must continue administering topical antifungal medication for a minimum of 3 to 4 weeks along with concurrent routine topical and systemic corneal ulcer management. Dermal antifungal preparations are not appropriate for topical use in the eye, but one may administer human vaginal antifungal preparations as a last resort.

Figure 15-5 Mycotic keratitis. Deep corneal vascularization, stromal edema, and cellular infiltration resulted from infection with *Aspergillus* sp.

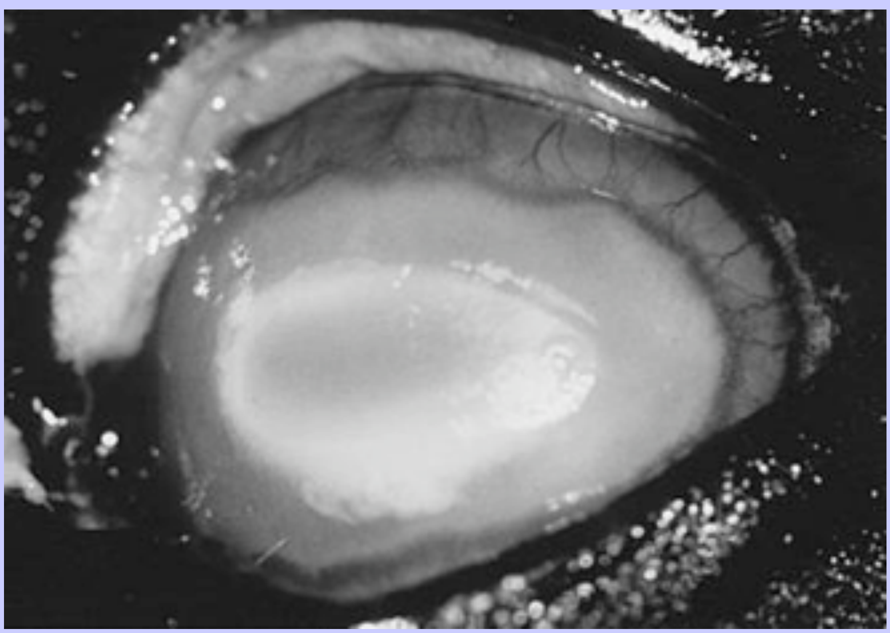
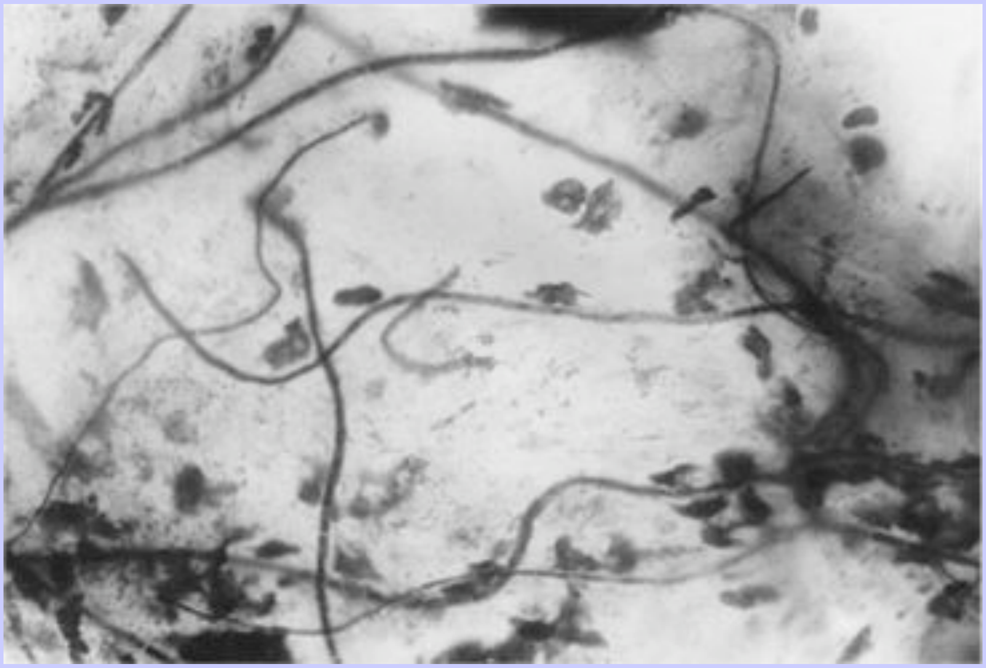


Figure 15-6 Corneal cytologic sample from horse with a combined infection of *Aspergillus* sp. and *Streptococcus* sp. Filamentous hyphae and bacteria are present in the sample.



Recently, use of the systemic antimycotic agent fluconazole has been evaluated.[50] In the author's opinion, oral administration of fluconazole (Diflucan, Pfizer Roerig, New York) at 1 mg/kg every 12 hours for 14 days and then every 24 hours for 7 days is associated with significant improvement and resolution of early keratomycosis and corneal stromal abscessation following fungal infection.

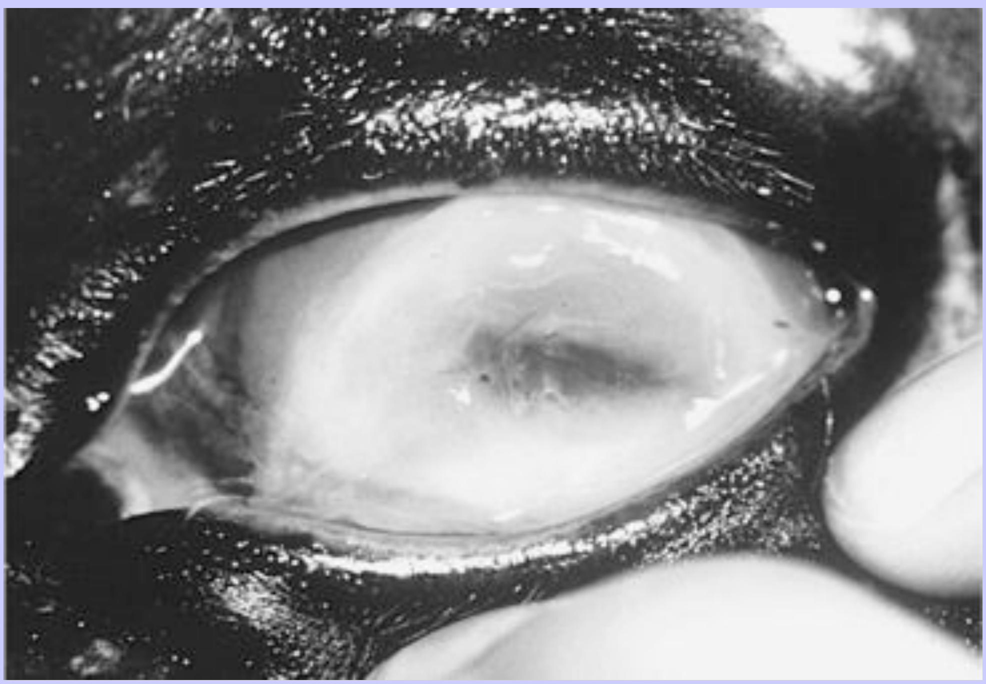
Many mycotic corneal ulcers are managed best using a combination of surgical and medical management.[23] Surgical debridement in the form of a partial or full-thickness keratectomy may help to remove much or all of the infected corneal stroma, respectively; is performed under general anesthesia; and requires microsurgical instrumentation and magnification. For a partial-thickness keratectomy, one may manage the resulting ulcer medically or, more appropriately, may suture a conjunctival pedicle graft[3,51,52] to the ulcer to aid in healing. The corneal vascularization provided by a conjunctival graft facilitates healing. One can perform a penetrating keratoplasty with a corneal transplant for deeper lesions[53] (see Corneal Surgery). Most mycotic corneal ulcers/abscesses ultimately vascularize and scar to some degree regardless of the treatment method. Topical corticosteroids have little if any effect on corneal scar formation and are not indicated, even following resolution of keratomycosis, because the possibility of sequestered organisms exists with subsequent exacerbation.

15.6.3.5

Collagenase Ulcers

Keratomalacia, or corneal melting, results from host-derived collagenase (from neutrophils and keratocytes) and bacterial enzymes such as those produced by *Pseudomonas aeruginosa*.[54] Collagenase ulcers progress rapidly and can result in corneal perforation within 24 hours (Figure 15-7). One should obtain samples for bacterial and fungal culture and cytologic examination. Cytologic examination must include Gram's staining to examine for the presence of gram-negative rods suggesting *Pseudomonas* spp. The topical antibiotics of choice for *Pseudomonas* are tobramycin 0.3% or ciprofloxacin (Ciloxan, Alcon Laboratories)[39,54] every 2 hours. One can administer a topical anticollagenase such as acetylcysteine (Mucomyst, Mead Johnson Pharmaceutical, Evansville, Indiana) diluted to a 5% to 8% solution with artificial tears or autologous serum
every 4 hours to minimize collagenase activity. Topical tetracycline every 4 to 6 hours also may be of benefit 1006
because it inhibits metalloproteinases that are activated in keratomalacia. In addition, debridement and a 1007
conjunctival pedicle graft or penetrating keratoplasty often are indicated to repair the defect, aid in healing, and prevent corneal perforation.

Figure 15-7 Severe melting caused by collagenase ulcer following infection with *Pseudomonas aeruginosa*. Corneal malacia surrounds a large descemetocele.



15.6.3.6

Descemetocele

Descemet's membrane is the basement membrane of the endothelium and the last barrier to corneal perforation. Descemet's membrane does not stain with fluorescein and appears as a clearing in the center of an otherwise edematous corneal ulcer (see Figure 15-4). As with all corneal ulcers in the horse, routine

diagnostic procedures for a descemetocele include culture and cytologic examination. A descemetocele is a surgical emergency and likely requires referral to a veterinary ophthalmologist. Surgical management is designed to provide support to the weakened cornea and blood vessels and fibroblasts to repair the damage. Support is achieved with a conjunctival graft or a lamellar or penetrating keratoplasty (see Corneal Surgery). Routine treatment for a corneal ulcer follows surgery and is best delivered through a subpalpebral lavage system to avoid manipulation of the eyelids and corneal graft. The conjunctival graft may be debrided 6 to 8 weeks after surgery in an attempt to minimize scar formation by severing the blood supply to the graft tissue.

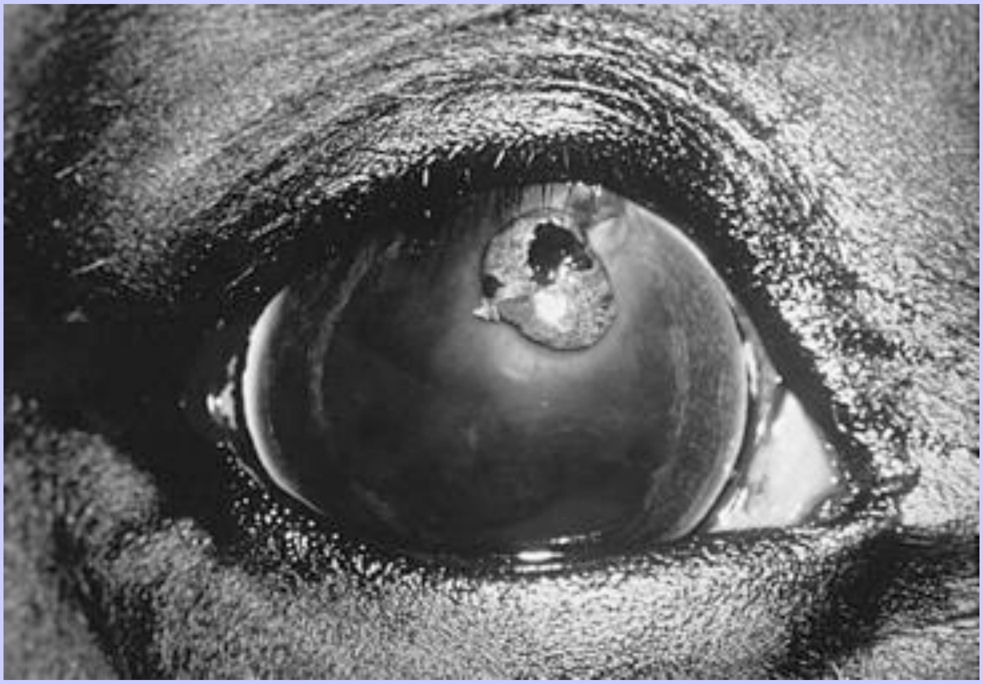
15.6.3.7

Corneal Perforation and Laceration

Causes of corneal perforation include rupture of a deep corneal ulcer or descemetocele and sharp and blunt trauma. Depending on the cause of the perforation and severity of the damage, treatment involves primary repair, an intraocular prosthesis, or enucleation. If the horse is to be referred for consultation and treatment, one can prevent self-trauma during transportation by using cradles or other protective devices and sedation as required. The prognosis following a penetrating injury varies depending on the cause, size of the wound, location, depth of penetration, intraocular damage, and the presence or absence of infection or retained foreign objects. In general, perforating corneal wounds in the horse have a grave prognosis for vision and a guarded prognosis for cosmesis. Such wounds always are associated with secondary anterior uveitis, and an iris prolapse is usually present (Figure 15-8). Sequelae to a corneal perforation include corneal scar, anterior and posterior synechiae, cataract, glaucoma, retinal detachment, phthisis bulbi, blindness, and loss of the eye.

Primary repair of a corneal perforation is a referral procedure. Treatment includes repairing the rent, reestablishing the anterior chamber, preventing infection, and decreasing inflammation and pain. Removal of a penetrating object is best done at the time of repair. One administers tetanus toxoid before repair. One should culture the aqueous humor, along with any retained foreign objects, for aerobic bacteria and fungi. If iris tissue protrudes but appears viable and minimally contaminated, one replaces it; however, one amputates contaminated, nonviable iris using electrocautery to minimize hemorrhage. If electrocautery is not available, one can perform sharp excision and control the bleeding. Intracamerally administered epinephrine, 1:10,000, can help control intraoperative bleeding and also facilitates pupil dilation. One can use a viscoelastic agent to tamponade vessels in addition to maintaining the anterior chamber and manipulating tissues. If the lens capsule has ruptured secondary to trauma, one should remove the lens; lens protein is antigenic and may stimulate severe anterior uveitis. If one can appose wound margins, one repairs the cornea with partial-thickness, simple interrupted or continuous 7–0 to 8–0 absorbable sutures. One reforms the anterior chamber with a balanced salt solution or, if unavailable, lactated Ringer's solution. One can use conjunctival grafts to promote rapid corneal healing and vascularization and to provide support. If a portion of cornea is missing or the wound edges cannot be apposed, a corneal-scleral transposition or other type of graft procedure is required. Following surgery, one administers topical broad-spectrum antibiotics every 2 to 4 hours, uses topical atropine as needed, and administers systemic antibiotics and flunixin meglumine. Use of a subpalpebral lavage delivery system is indicated.

Figure 15-8 Corneal perforation with associated hyphema and iris prolapse.



Blunt trauma, whether contusive, penetrating, or perforating, generally results in more severe ocular damage than injury from a sharp object[5] (Figure 15-9). In contrast to sharp perforating injuries, blunt trauma results in a rapid increase in IOP, an explosive rupture from the inside outward, and the expulsion of the intraocular 1007
contents. The resulting rent in the fibrous tunic is often large and irregular, and portions of the cornea or 1008
sclera may be lost. The typical wound is one that originates at the limbus, extending forward into the cornea and posterior into the sclera. If the posterior portion of the eye ruptures, the horse may show hyphema and decreased IOP and may require ocular ultrasound for accurate diagnosis.

Figure 15-9 Corneal perforation following blunt trauma. The lesion is explosive and expulsive with loss of intraocular contents. Enucleation is the treatment of choice.

Repair of these explosive ruptures is difficult, and the treatment of choice may be enucleation. If cosmetic repair is important, one may use an intraocular silicone prosthesis in some patients, provided enough tissue is left to close the fibrous tunic.[3] One should perform this procedure as soon as possible following injury. If the injury is chronic and atrophy of the globe has occurred, placement of an intraocular prosthesis is not possible. The only cosmetic alternative in these horses is an orbital prosthesis, which is expensive, time-consuming, and requires frequent maintenance on the part of the owner.[3]

15.6.4 TECHNIQUE FOR PLACEMENT OF A SUBPALPEBRAL LAVAGE DELIVERY SYSTEM

A subpalpebral lavage delivery system works well to facilitate administration of topical medication in a horse with ocular pain or a fractious temperament. One can place the system using sedation, auriculopalpebral and supraorbital nerve blocks, topical anesthesia, and appropriate equipment (Box 15-2). The through-and-through subpalpebral lavage technique described is preferred over other subpalpebral lavage or nasolacrimal delivery systems.[40] This technique results in less irritation and fewer complications, and the system is less likely to become displaced. Recently, a technique for inferomedial placement of a single-entry subpalpebral lavage tube has been described.[55]

15.6.4.1

BOX 15-2 EQUIPMENT REQUIRED FOR PLACEMENT OF A SUBPALPEBRAL LAVAGE DELIVERY SYSTEM

Sedation

- Xylazine, 100 mg/ml
- Butorphenol, 10 mg/ml

Nerve blocks

- Carbocaine 2%
- 25-guage needle

Topical anesthetic

- Proparacaine 1%

Hubless 12-guage needle

Silastic tubing (0.065-mm outer diameter)

1-inch tape

2–0 Vetafil suture

Tom cat urinary catheter, open ended

Sterile injection cap

Tongue depressor

From Wilkie DA: Ophthalmic procedures and surgery in the standing horse, *Vet Clin North Am Equine Pract* 7:535-547, 1991.

Once one sedates the horse and performs the nerve blocks, one elevates the superior eyelid and directs a 12-gauge hubless needle superotemporally from the conjunctival surface. One must take care to avoid traumatizing the cornea and always must direct the needle from the conjunctiva out to skin to minimize the possibility of ocular trauma. One places the needle as far temporal and into the conjunctival fornix as possible while remaining anterior to the bony orbital rim. One then passes the needle through the superior eyelid and out the skin and passes the Silastic lavage tubing through the needle and removes the needle. Silastic tubing is preferred because of its low tissue reaction and pliability. One then repeats the procedure in the superonasal portion of the eyelid, again directing the needle outward from the conjunctiva and as far from the temporal hole as possible. One must perform the technique with a hubless needle to allow both needle passes to be directed away from the eye. One then passes the tubing through the needle and removes the needle. One ties a knot in

the medial portion of the tubing as it emerges from the skin of the superior eyelid, places enough throws so that the tube does not migrate back through the eyelid, and makes the throws tight enough to occlude the lumen.

One approximates the amount of tubing on the inside of the eyelid adjacent to the palpebral conjunctiva. One pulls the tubing nasally and makes two or three small holes in that portion of the tubing midway between the 1008
two eyelid puncture wounds, taking care to avoid severing the tube. One then pulls the tube back into place. 1009

One sutures the tubing to the skin of the lateral canthus and to the forehead to prevent migration of the tubing. One can attach the tube to the halter or, in those animals that are extremely difficult to medicate, to the mane on the affected side. One inserts an open-ended feline urinary catheter in the end of the Silastic tubing and attaches an injection cap, which one then tapes to a tongue depressor to provide support, and one attaches the entire apparatus to the halter, taking care to avoid kinking the tubing. One then should test the system using sterile eyewash. One observes the irrigating solution dripping over the cornea and running down the face to be certain tube placement is correct. One also takes note of the volume of solution required to reach the eye. This indicates the volume of air required to deliver the medication to the eye.

Medication used with a subpalpebral lavage system must be in solution, not ointment. The volume of medication used at each treatment time should be 0.1 ml, and one then must use air to deliver this medication to the eye. The use of irrigating solution to deliver the medication dilutes the medication. One should not exceed 0.1 ml because this medication will be absorbed systemically and excessive volume simply overflows onto the face. A 25-gauge needle works well for injecting medication and preserving the injection cap. Once daily one should irrigate the system with sterile eyewash to ensure its patency and to rinse away mucous buildup.

Complications from a subpalpebral lavage system include trauma during tube placement, improper placement of the delivery holes, irritation to the cornea from the tubing, and migration of the tubing. Observation of the medication reaching the cornea always is essential. Improper placement or migration of the tube may result in delivery of medication to the subcutaneous tissues and severe irritation. If corneal trauma occurs, one must remove the tube and replace it or find an alternative means of medication.

15.6.5 CORNEAL SURGERY

Surgery in the form of a superficial keratectomy, conjunctival graft, or corneal graft may be indicated in addition to aggressive medical therapy for many complicated corneal ulcers.[56] One must perform corneal surgery using general anesthesia to optimize the outcome.

A superficial keratectomy provides a biopsy for histopathologic examination, debrides and debulks damaged and infected corneal stroma, and provides a bed for transplanting and suturing a conjunctival or corneal graft. One uses a No. 64 Beaver blade or Martinez corneal dissector first to outline the area to be excised and then to undermine and remove the affected area of cornea. In general, one can remove 50% to 70% of the corneal thickness, but excision of 50% or more is an indication to reinforce the remaining cornea with a conjunctival or corneal lamellar graft. The normal equine cornea is 1.0 to 1.5 mm thick and is thinnest centrally. The cornea increases in thickness with disease because of edema, cellular infiltration, vascularization, and scar formation.

Conjunctival grafts include advancement, hood, bridge, pedicle, and complete conjunctival grafts.[51,52,56] The author prefers to perform a conjunctival pedicle graft for most focal corneal ulcers and uses the other types for more extensive lesions.[52] Before placement of a conjunctival graft, debridement of the affected cornea by superficial keratectomy is indicated. One mobilizes the bulbar conjunctiva, leaving an intact blood supply. One should attempt to appose conjunctival and corneal epithelium at the edge of the graft and to place 7–0 to 8–0

Vicryl (polyglactin 910) sutures two thirds deep into healthy cornea. A continuous suture pattern, simple or double, works best and is associated with less graft retraction and dehiscence than a simple interrupted suture pattern for equine conjunctival grafts. Conjunctival grafts adhere to the underlying exposed corneal stroma, providing the blood supply and cells required to repair and rebuild the damaged cornea. Opacification of the graft site occurs but is generally less than what would have occurred without surgery. One can minimize opacification by trimming the graft and severing its blood supply 6 to 8 weeks following the initial surgery.

Autologous and homologous lamellar corneal grafts, corneal-scleral transposition, and penetrating keratoplasty are more involved techniques reserved for the most severe corneal ulcers or for perforating corneal lesions. These are described elsewhere and are referral procedures.[56]

Nictitating membrane or third eyelid flaps are of little or no benefit for managing complicated and infected equine corneal disease and are contraindicated in many instances.

15.6.6 NONULCERATIVE DISEASES

Nonulcerative corneal diseases include corneal scar, corneal edema, stromal abscessation, cellular and noncellular infiltrates, and nonulcerative keratouveitis.[57]

Corneal scars are not painful, follow a history of corneal disease, and are associated with corneal vascularization. Topical corticosteroid therapy does not decrease corneal scarring significantly but does predispose the eye to infection and decreases healing in the event of a corneal ulcer. Tattooing of a corneal scar for ocular cosmesis is described[3,44] but not advised.

Cellular infiltration in the corneal stroma includes neoplastic and inflammatory cells. The most common 1009
corneal neoplasia is SCC (see Conjunctiva and Third Eyelid; Figure 15-2). Corneal lymphosarcoma also occurs. 1010
Inflammatory cellular infiltration (neutrophils, lymphocytes, plasma cells, eosinophils) occurs in infectious keratitis (bacterial, mycotic) usually and is associated with corneal ulceration. However, a small, focal corneal ulcer can become infected and reepithelialize, resulting in a corneal stromal abscess. In addition, inflammatory corneal infiltration occurs with equine ocular onchocerciasis and nonulcerative keratouveitis.

Noncellular corneal infiltration or corneal degeneration is a sequela of previous corneal or limbal inflammation. Accumulations of mineral and phospholipids may remain within the corneal stroma following inflammation and appear as crystalline infiltration. Such accumulations are rare, generally are not painful, and do not require treatment. In some instances, they predispose the eye to corneal ulceration and can be removed with a superficial keratectomy.[3]

15.6.6.1 Corneal Edema

Corneal edema, if not associated with a corneal ulcer (fluorescein-negative), results from corneal endothelial damage. Blunt trauma to the globe can result in displacement of the corneal endothelium from the posterior surface of the cornea, which results in full-thickness, diffuse corneal edema and which may gravitate to the ventral cornea with time. In addition, diseases of the anterior uvea and aqueous humor, anterior uveitis and glaucoma, and anterior lens luxation can interfere with endothelial cell function, resulting in diffuse corneal edema. Although no specific treatment exists for corneal edema of endothelial origin, hyperosmotic agents such as sodium chloride 5% (Muro-128, Bausch & Lomb, Rochester, New York) applied topically every 4 to 6 hours may decrease the severity of the edema and help prevent rupture of corneal bullae. In the author's

opinion, this has only minimal efficacy. With time, the corneal endothelium may reattach or adjacent endothelial cells may hypertrophy, resulting in a decrease in corneal edema. During the period that the edema is present, the cornea is compromised and at risk of ulceration. One therefore should avoid topical corticosteroids.

15.6.6.2 Nonulcerative Keratouveitis

A condition consisting of interstitial keratitis with peripheral corneal vascularization, edema, and cellular infiltrate and anterior uveitis (miosis, aqueous flare, iritis) has been described in five horses.[57] The pathogenesis of nonulcerative keratouveitis is unknown, but anterior uveitis has been suggested to follow interstitial keratitis and that an immune-mediated mechanism is involved. No infectious or neoplastic process is apparent. Histologically, nonulcerative keratouveitis is characterized by vascularization, fibroblasts, and inflammatory cells, predominantly lymphocytes. No infectious agents are visible on light or transmission electron microscopy.

Treatment is similar to that for equine recurrent uveitis and involves suppression of inflammation and control of ocular pain. Topical and subconjunctival corticosteroids, topical NSAIDs, topical cyclosporin A,[46] topical mydriatic cycloplegics, and systemic NSAIDs effectively control the signs, but some level of long-term maintenance therapy usually is required.

The differential diagnoses for nonulcerative keratouveitis include corneal stromal abscess, equine ocular onchocerciasis, and neoplasia.

15.6.6.3 Corneal Stromal Abscess

The usual cause of corneal stromal abscess formation is a focal, superficial corneal ulcer that allows opportunistic infection of the corneal stroma and then heals, trapping the microorganism. Topical antibiotic-corticosteroid therapy in eyes with corneal epithelial cell loss may predispose to stromal abscess formation.[3,58,59] The lesion results in discomfort apparent as photophobia, blepharospasm, and excessive lacrimation. A chronic, yellow-white, corneal stromal infiltrate with associated corneal edema and deep corneal vascularization is present.[58,59] Anterior uveitis may be present. Fluorescein staining of the cornea is often negative. Diagnosis is based on clinical signs. One should obtain a corneal scraping from the lesion, following removal of the overlying corneal epithelium, and should be submitted for cytologic examination, bacterial culture and sensitivity, and fungal culture. Gram-positive cocci (*Streptococcus* and *Staphylococcus* spp.) are the predominant organisms recovered,[58] but fungal stromal abscesses also occur. Differential diagnosis includes nonulcerative keratouveitis.

If the overlying epithelium is intact, one should remove it before initiating treatment to facilitate penetration of antibiotics. Ciprofloxacin is the topical antibiotic of choice because it penetrates intact corneal epithelium; its use is recommended every 4 hours pending culture and sensitivity results. In addition, some authors recommend subconjunctival injections of antibiotics.[3,58] If one suspects a mycotic stromal abscess, oral administration of fluconazole (Diflucan, Pfizer Roerig, New York) at 1 mg/kg every 12 hours for 14 days and then every 24 hours for 7 days is indicated.[50] Alternately, one can use an itraconazole-dimethyl sulfoxide ointment topically, but this may be associated with significant irritation.[49]

If anterior uveitis is present, one administers topical atropine 1% as needed to dilate the pupil, up to a maximum of 4 times a day. Systemic flunixin meglumine helps reduce ocular pain and secondary complications associated with inflammation. Topical corticosteroids are contraindicated. Resolution of the 1010
abscess requires 2 to 8 weeks, is associated with corneal vascularization, and results in corneal scar 1011
formation. In addition, the associated anterior uveitis may result in secondary intraocular complications.

A superficial keratectomy and conjunctival pedicle graft,[59] posterior lamellar keratoplasty,[60] or penetrating keratoplasty[53,61] is also appropriate and may result in a more rapid resolution and reduced corneal opacification than medical management alone. If one performs a keratectomy, one should submit the excised corneal stroma for culture and cytologic or histopathologic examination, for testing of the corneal stoma is more diagnostic than preoperative samples.[59]

15.6.6.4 Equine Ocular Onchocerciasis

Onchcocerca cervicalis is a common equine parasite and has been implicated in conjunctivitis, peripheral keratitis, anterior uveitis, and peripapillary chorioretinitis. Corneal lesions result from *Onchcocerca* microfilariae that migrate to and die in the corneal stroma and that may incite an inflammatory response. The lesion begins peripherally at the temporal limbus but can extend axially. Interstitial keratitis, manifested as corneal edema, vascularization, and subepithelial focal corneal opacities, occurs and may be associated with temporal bulbar conjunctival vitiligo, conjunctivitis, and anterior uveitis. Diagnosis is based on history, clinical signs, and the presence of eosinophils on cytologic examination and microfilariae on conjunctival biopsy.[62] One can demonstrate microfilariae cytologically through a biopsy of temporal bulbar conjunctiva, placing the sample on a slide with warm saline and examining for the presence of motile microfilariae. One must interpret the presence of microfilariae along with the clinical signs because one can find microfilariae in many horses without associated ocular disease.

Treatment includes suppression of ocular inflammation and microfilaricidal therapy (see Table 15-2). Topical corticosteroids and systemic phenylbutazone or flunixin meglumine control inflammation (see Table 15-3). If anterior uveitis is present, one can use topical atropine 1% as needed to dilate the pupil. Pretreatment with antiinflammatory medications for 2 to 3 days before administration of microfilaricides is indicated to minimize the ocular response to the dying microfilaria.

15.6.6.5 Band Keratopathy

Single or multiple linear streaks affecting one or both eyes in the horse have been described.[63] Histologically, these are areas of thinning of Descemet's membrane and may be congenital. The principle differential is a corneal stria following chronic glaucoma and enlargement of the globe with stretching of Descemet's membrane. Ophthalmic examination should reveal evidence of prior inflammation or glaucoma, and one should assess vision and determine IOP.

15.7 Uvea

15.7.1 ANATOMY

The uvea is the middle, vascular tunic of the eye and comprises the anterior portion, the iris and ciliary body, and the posterior choroid. Histologically, the uvea contains blood vessels, pigment cells, smooth muscle, and in the horse, a noncellular, fibrous tapetum in the choroid. The uvea is the primary site of the blood-ocular barrier, which has importance in the formation of the aqueous humor, serves as a barrier to blood-borne materials, and is an immunologic barrier to the internal components of the eye. Inflammation disrupts this barrier (uveitis). Smooth muscles in the iris and ciliary body, under autonomic control, regulate pupil size and accommodate for near and far vision, respectively. In uveitis, spasm of these muscles results in constriction of the pupil and pain, seen clinically as miosis and photophobia. The ciliary body is the source of aqueous humor, which supplies nutrition to the cornea and lens. Aqueous humor is produced by active and passive processes; its production is decreased by inflammation and by pharmacologic agents used in glaucoma therapy. The choroid supplies nutrition to the retina and also serves as a heat sink to protect the photoreceptors from the heat generated by light striking the retina. The tapetum, contained within the superior choroid, reflects light back across the retina, thereby maximizing the use of available light.

15.7.2 ABNORMALITIES OF THE PUPIL

The pupil of the horse is horizontally elliptic with the dorsal and ventral margins having pigmented prominences termed *corpora nigra*. The pupillary light reflex in the horse is similar to that of other species with a direct and a consensual response. The afferent fibers of the pupillary light reflex are carried in cranial nerve II, and the efferent parasympathetic fibers are carried in cranial nerve III. Abnormalities in pupil response to a light stimulus result from afferent lesions involving the retina or optic nerve. Afferent abnormalities are associated with loss of vision in the affected eye. Efferent lesions are rare but include damage to cranial nerve III with an associated ventrolateral strabismus, abnormalities of the iris itself as with synechiae, and glaucoma. One also must differentiate excited horses with sympathetic override, weak light source, and improper examination technique from an abnormal pupillary light response.

Sympathetic nerve fibers supply the dilator muscles of the iris. These fibers travel in the spinal cord, emerge in
the ventral nerve roots at first and second thoracic nerves, course through the thorax, run in association with the
internal carotid artery, synapse at the cranial cervical sympathetic ganglion, and travel to the eye with the ocular 1011
arteries. Damage to the sympathetic fibers results in Horner's syndrome. Clinical signs include ptosis, miosis, 1012
enophthalmos, protrusion of the third eyelid, and sweating on the affected side of the face and neck. One directs
treatment toward the primary disease, if possible. In some horses the clinical signs of Horner's syndrome
resolve without treatment.

15.7.3 UVEAL CYSTS

Cysts of the anterior uvea (iris, corpora nigra, and ciliary body) have been reported in the horse.[64,65] The cysts are usually heavily pigmented and may not transilluminate, making a uveal melanoma the most likely differential diagnosis. Ocular ultrasonography reveals these as cystic structures and confirms the diagnosis.

Treatment is not required unless the cysts are numerous or impair sight. One can collapse the cyst or aspirated it by paracentesis or, if available, disrupt it using laser energy delivered transcorneally.[65] One can deliver diode laser energy to the cyst through the cornea using an indirect ophthalmoscope and a 20-D condensing lens. One performs the procedure on the standing horse and photocoagulates the cyst wall, shrinking the cyst and preventing recurrence.

15.7.4
ANTERIOR SEGMENT DYSGENESIS

Anterior segment dysgenesis has been described in the Rocky Mountain horse, Kentucky Saddle horse, and Mountain Pleasure horse breeds. The abnormalities associated with this syndrome include macropalpebral fissure, megalocornea, ciliary body and peripheral retinal cysts, congenital miosis, iris stromal hypoplasia, iridocorneal angle abnormalities, cataract, retinal dysplasia, and retinal detachment.[66] The abnormalities appear linked to coat color with the highest prevalence of abnormalities associated with chocolate coat and white mane and tail.

15.7.5
UVEITIS

Uveitis is an inflammation of the iris and ciliary body (anterior) and choroid (posterior). Uveitis is a common manifestation of many ocular and systemic infectious and noninfectious diseases, and as such, an attempt to ascertain the cause is essential. Differentiating uveitis from other ophthalmic diseases resulting in a red and painful eye, such as glaucoma, corneal ulceration, and conjunctivitis is also essential. Uveitis is reported as the leading cause of blindness in horses throughout the world.[44,67]

15.7.5.1
Clinical Signs

Acute inflammation of the anterior uvea results in spasm of the iris and ciliary muscles, which is apparent clinically as miosis and photophobia, and a breakdown in the blood-ocular barrier (Box 15-3). The primary mediators of intraocular inflammation are prostaglandins. Eyelids and conjunctiva may be swollen and hyperemic in the acute phase of anterior uveitis. Protein and cells leak into the anterior chamber, resulting in aqueous flare, hypopyon, and keratitic precipitates. Chronic inflammatory changes in the aqueous humor can result in secondary corneal endothelial and lens changes (e.g., corneal edema and cataract) and adhesions between the iris and adjacent lens or cornea, termed *synechiae* (Figure 15-10). Inflammation of the choroid, because of its close association with the retina, usually also involves the retina and is termed *chorioretinitis*. Chorioretinitis is diagnosed by direct or indirect ophthalmoscopy and results in retinal and subretinal transudate and exudate, retinal vascular changes, hemorrhage, retinal detachment, and retinal degeneration. Choroidal depigmentation and areas of repigmentation or pigment clumping may be present (Figure 15-11). Other chronic changes resulting from intraocular inflammation include secondary glaucoma, blindness, and phthisis bulbi. The development of intraocular changes following uveitis is related directly to the duration and severity of the acute episode and is exacerbated by recurrent episodes of inflammation.

15.7.5.1.1
BOX 15-3 SIGNS OF ACUTE AND CHRONIC UVEITIS

15.7.5.1.1.1
Acute

Miosis

Photophobia

Blepharospasm

Eyelid swelling

Aqueous flare

Corneal edema

Chorioretinitis

Keratitic precipitates

Hypopyon

Hypotony

15.7.5.1.1.2
Chronic

Posterior synechiae

Cataract

Pigmentation of anterior lens capsule

Atrophy of corpora nigra

Glaucoma

Peripapillary depigmentation

Phthisis bulbi

Blindness

Lens luxation

15.7.5.2
Causes

The causes of anterior and posterior uveitis include primary ophthalmic and systemic diseases.[23] Blunt or 1012
penetrating ocular trauma, intraocular neoplasia (primary or secondary), corneal ulceration, parasitic 1013
infiltration, and numerous systemic infectious diseases with associated septicemia, toxemia, immune-complex disease, and viremia are associated with uveitis. Of the systemic infectious diseases implicated in equine uveitis, infections of *Streptococcus equi* (strangles) and *Leptosprira interrogans* serogroup *pomona*[68,69] and gram-negative sepsis occur most commonly. In addition to a complete ophthalmic examination, evaluating the entire animal through a complete physical examination and appropriate laboratory testing is essential. A complete ophthalmic evaluation must include fluorescein staining of the cornea; penlight examination to evaluate pupil size, aqueous humor content, lens position and transparency; and direct and consensual pupillary light responses. Direct (with or without indirect) ophthalmoscopic

examination of the fundus is also essential. Failure to perform these routine diagnostic tests results in misdiagnosis and incorrect therapy and may result in a failure to consider and examine for systemic disease, thereby placing the health of the animal in jeopardy.

Figure 15-10 Numerous posterior synechiae and a cataract occur following chronic anterior uveitis. The corpora nigra are adhered to the anterior lens and result in distortion of the pupil margin.

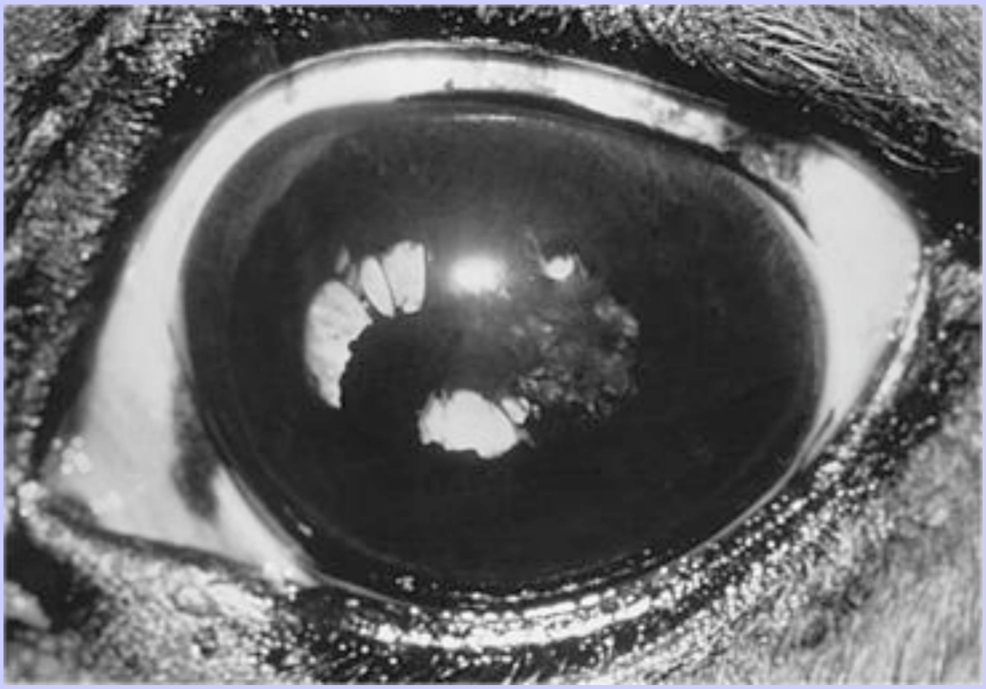
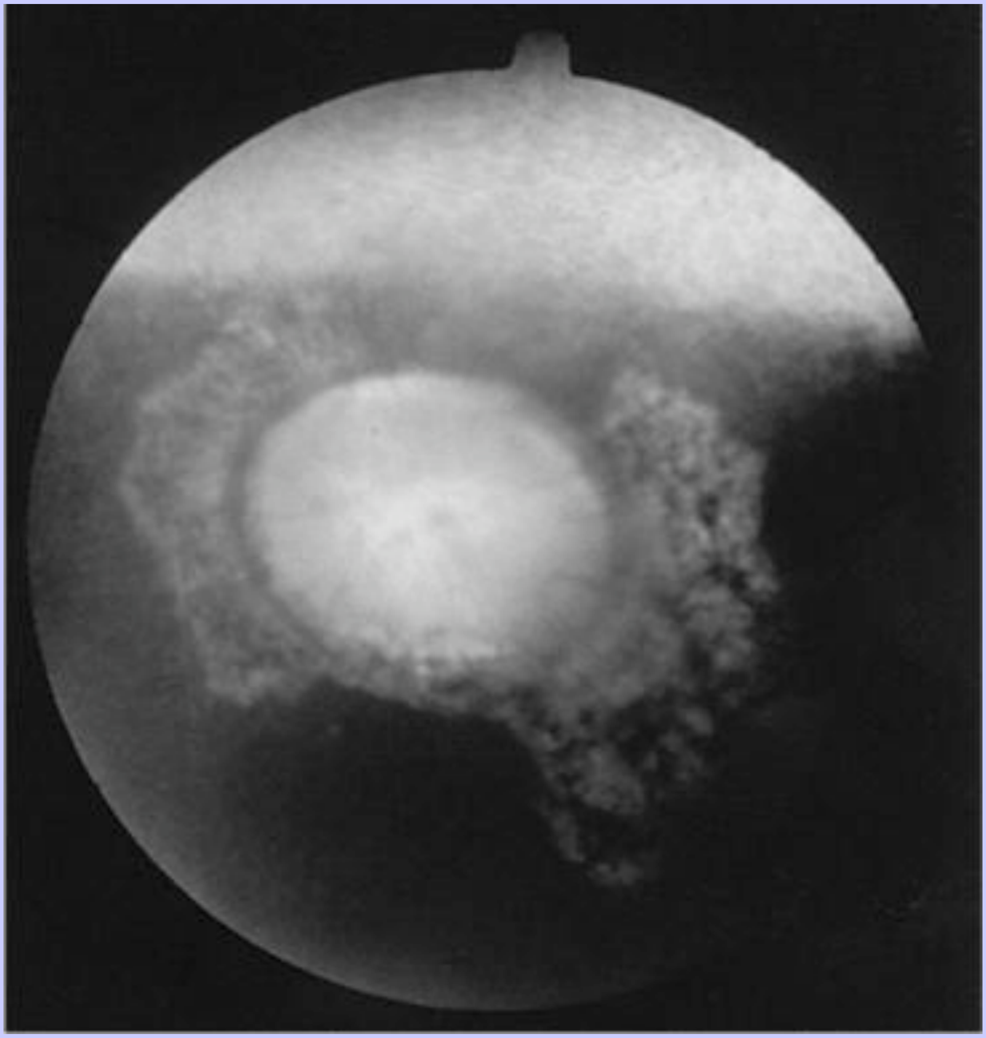


Figure 15-11 Peripapillary depigmentation, a so-called butterfly lesion, associated with the chorioretinitis of equine recurrent uveitis.



Primary ophthalmic diseases that result in anterior uveitis include corneal ulceration, lens-induced uveitis, intraocular neoplasia and direct ocular trauma. Corneal ulceration results in secondary anterior uveitis through a reflex pathway involving the ophthalmic branch of cranial nerve V. Fluorescein staining of the cornea therefore is indicated in eyes with anterior uveitis. The lens is considered to be an immune-privileged site and as such is capable of stimulating an inflammatory reaction. Lens protein is exposed by traumatic rupture of the lens capsule or during the degenerative process of a hypermature cataract undergoing liquefaction and leakage. Either of these processes can lead to anterior uveitis with rupture of the lens resulting in the more severe inflammation and, frequently, secondary glaucoma. Uveitis resulting from direct ocular trauma may be associated with rupture of the fibrous tunic of the eye, hyphema, lens luxation, corneal endothelial damage, retinal detachment, or proptosis. A complete ophthalmic examination is essential. If the anterior and posterior segments of the eye are not completely visible, then an ocular ultrasound examination is indicated, specifically to evaluate the position of the lens and retina and to look for changes in the

echogenicity of the vitreous humor. In addition to a complete ophthalmic evaluation, one must examine the entire animal for signs of trauma to other body systems. Fractures and soft tissue damage of the head and neck, thoracic and abdominal trauma, fractures of the limbs, and neurologic changes involving the central nervous system are potential complications associated with ocular trauma.

Systemic diseases resulting in anterior and posterior uveitis are numerous and include bacteremia, septicemia, disseminated mycoses, and the syndrome of equine recurrent uveitis. Infection with *Borrelia burgdorferi* has been reported to result in arthritis and panuveitis in a pony.[70] Foals with neonatal septicemia are especially prone to anterior and posterior uveitis. The uveitis in these foals is usually sterile. Systemic antimicrobial, topical and systemic antiinflammatory, and topical atropine 1% therapy are the treatments of 1013
choice. Copious fibrin production may result from anterior uveitis, especially in young foals, and can result 1014
in posterior synechia and opacification of the lens capsule. Intracameral tissue plasminogen activator, 25 to 75 μg administered in the first few days after fibrin formation, can result in complete lysis and resolution.

15.7.5.3 Equine Recurrent Uveitis

Equine recurrent uveitis (ERU; moon blindness, periodic ophthalmia) is the most common cause of blindness in horses, with Appaloosas at increased risk compared with non-Appaloosas.[69] ERU is an immune-mediated disease with numerous initiating or exacerbating factors[3,68,71] (Box 15-4). The clinical signs of ERU are similar to those of other causes of uveitis, the distinguishing feature being its recurrent nature. The frequency and severity of each recurrence vary greatly. Even in times of clinical quiescence, inflammation is believed to continue at a subclinical level, resulting in further intraocular damage. Diagnosis is based on history and clinical examination. Although serologic evaluation for *Leptospira* species, *Brucella* species, or *Toxoplasma* and conjunctival biopsies to evaluate for the presence of *Onchocerca cervicalis* have been advocated,[3] the results of these tests rarely alter individual therapy, in part because of the chronicity of the disease and because the inciting cause may have begun years before development of uveitis.

One directs treatment of the individual horse toward suppressing inflammation, controlling pain, and preventing sequelae. In situations in which the prevalence of ERU in a barn exceeds that normally expected, serologic testing and examination to attempt to determine the cause is indicated. Routine vaccination for *Leptospira* species is controversial and may be contraindicated in horses with active inflammation.

The prognosis for ERU varies according to the severity of uveitis, duration of episodes, response to treatment, and frequency of recurrence. Each episode results in some degree of intraocular damage, and damage to the corneal endothelium, lens, and retina is cumulative because repair of these tissues is difficult. In addition, damage often occurs at a subclinical level during the so-called inactive periods. One should consider horses with evidence of previous uveitis, corneal edema, cataract, synechiae, pigment on the anterior lens capsule, atrophy of the corpora nigra, and degeneration of retina and optic nerve as ophthalmologically unsound. One must consider all animals that have had an episode of acute uveitis at risk to develop chronic recurrent uveitis. All animals with chronic recurrent uveitis have a guarded prognosis for long-term maintenance of normal vision, with blindness often being the end result.

15.7.5.3.1 BOX 15-4 CAUSES IMPLICATED IN EQUINE RECURRENT UVEITIS

Leptospira interrogans serovar *pomona*

Onchocerca

Toxoplasma

Virus infection: adenovirus, influenza

Brucella

Streptococcus

Borrelia burgdorferi

Trauma

Other

15.7.5.4

Treatment

The treatment of any uveitis, acute or chronic, involves specific therapy determined by the cause of the uveitis and nonspecific therapy designed to decrease inflammation, pain, and prevent further intraocular damage. The initiation of specific therapy depends on correctly identifying the cause, whether a primary ophthalmic disease or a systemic problem. Failure to control intraocular inflammation may lead to severe secondary ophthalmic complications such as glaucoma, synechiae, cataract, retinal detachment, phthisis bulbi, and blindness.

Nonspecific therapy includes topical and systemic corticosteroids and NSAIDs to decrease inflammation and atropine to dilate the pupil and decrease the pain of ciliary muscle spasm. Selection of medication, frequency of treatment, and route of administration depend on the cause and severity of the uveitis and whether the uveitis is anterior, posterior, or both.

Topical medications for treating anterior uveitis include atropine, corticosteroids, cyclosporin A, and NSAIDs. One administers topical atropine 1% as needed to dilate the pupil, but generally not more than 4 times a day. One should monitor intestinal motility because topical atropine can result in ileus and colic. Topical corticosteroids are useful, provided corneal ulceration is not present as determined by fluorescein stain. Prednisolone acetate 1% ophthalmic is the corticosteroid of choice because it achieves the highest intraocular levels. Alternatively, one can use dexamethasone 0.1% ophthalmic solution or 0.05% ointment. Frequency of administration varies according to severity, with initial frequency being every 4 to 6 hours and subsequently decreased as the eye responds to therapy. One should continue topical corticosteroid therapy for several weeks after resolution of clinical signs. In addition, subconjunctival corticosteroids have been advocated in severe cases of anterior uveitis[3,44] (see Table 15-3). Topical NSAIDs are available, but little 1014
information exists regarding their efficacy for treating ERU. The author's experience suggests some efficacy, 1015
but significantly less clinical response than with topical corticosteroids. One can use topical NSAIDs in horses with anterior uveitis and associated corneal ulceration with less risk than corticosteroids. Topical corticosteroids and NSAIDs have a synergistic effect and can be used in combination. Topical antibiotics are not required unless a corneal ulcer is present; corticosteroids then are contraindicated. Topical cyclosporin A 0.2% ointment (Optimmune, Schering-Plough, Kenilworth, New Jersey) may be efficacious for treating active uveitis and as a long-term treatment to decrease the frequency and severity of subsequent recurrences.[46] Unfortunately, although cyclosporine is the drug of choice, it has limited ability to penetrate the intact cornea and reach target tissues.

One uses systemic NSAIDs with topical therapy. Flunixin meglumine is the systemic NSAID of choice for acute ocular inflammation and can be used safely in the presence of corneal ulcer. Alternatively, one can use phenylbutazone or aspirin. Chronic oral administration of aspirin at 25 mg/kg every 24 hours has been advocated to decrease the frequency and severity of recurrent episodes of uveitis.[3] One should place horses with active uveitis in a darkened stall to decrease discomfort and limit exercise.

Recently, surgical management of ERU has been advocated. Surgeries include intravitreal placement of a sustained-release cylosporine-impregnated device[72–74] or vitrectomy.[75] Implantation of a 4-μg/day intravitreal cyclosporin A implant decreased the frequency and severity of recurrent uveitis episodes in 23 horses with a mean follow-up of 1 to 29 months (mean 10.5 ± SD 7.6 months).[72,74] Of the treated horses, 18 of 23 (78.2%) had sight at the time of follow-up. Complications of the intravitreal cyclosporin A implant include glaucoma and retinal detachment, both of which may relate to ERU. Compared with intravitreal cyclosporin A implantation, pars plana vitrectomy is a more invasive surgery, requiring more instrumentation and expertise and is associated with significant postoperative vision-threatening complications. Pars plana vitrectomy does appear, however, to decrease the severity and frequency of ERU episodes.

15.7.6 GLAUCOMA

Glaucoma is an elevation of IOP to a level incompatible with the health of the eye. Equine glaucoma is secondary in virtually all cases, typically resulting from ERU.[76] The reported normal IOP in the horse varies according to the technique used and whether sedation is used, with sedation resulting in a decrease in IOP. One should perform an auriculopalpebral nerve block before determining IOP in the horse. Normal values are reported to range from 20 to 30 mm Hg. Measurement of IOP in the horse presents a challenge in that indentation tonometers, such as the Schiøtz, are not well suited for use in the horse. Applanation tonometers, especially portable devices such as the Tonopen, are best suited for determining equine IOP. In the author's practice, determination of IOP is a routine part of a complete ophthalmic examination. If one plans to determine IOP in the horse, one should remember that intravenous administration of xylazine significantly lowers the IOP.[3]

Although congenital glaucoma (megaloglobus) has been reported in the horse, secondary glaucoma resulting from anterior uveitis, especially ERU, is the most common cause of equine glaucoma.[76] The clinical signs of glaucoma in the horse are often more subtle than in other species. Acute glaucoma may result in pain (photophobia, blepharospasm, lacrimation), corneal edema, linear corneal opacities (striae), mydriasis (provided uveitis or posterior synechiae are not present), and decreased menace response. The linear opacities in equine glaucoma often extend from limbus to limbus and are areas of thinning of Descemet's membrane.[77] Similar linear opacities also occur in normal horses, have been termed *band keratopathy,* and are not significant. In chronic glaucoma, corneal edema and corneal striae may be visible. Enlargement of the globe (buphthalmos), retinal degeneration, and blindness also occur.

Unfortunately, most horses with glaucomatous eyes are presented late in the disease, resulting in a poor response to therapy. Medical treatment of acute glaucoma involves topical agents such as timolol maleate 0.5% (Timoptic, Merck & Co., Inc., West Point, Pennsylvania), dorzolamide 2% (Trusopt, Merck), and the combination of these two drugs (Cosopt, Merck).[78,79] One administers these drugs alone or in combination 2 to 3 times daily in an effort to reduce IOP by increasing the outflow and decreasing production of aqueous humor. Of all the topical glaucoma agents, timolol maleate and dorzolamide are most effective in the horse, and the combination is more effective than administration of the two drugs concomitantly.[78] In the horse, because of

the large percentage of uveoscleral outflow, careful use of topical atropine may actually reduce IOP in some eyes.[80] Use of atropine is controversial, however. Topical corticosteroids, systemic flunixin meglumine, or combined therapy is indicated to control any concomitant inflammation or pain. Failure of topical therapy to control acute glaucoma indicates the need for surgical intervention.

Surgery for the acute glaucoma patient includes transscleral cyclocryosurgery or cyclophotocoagulation using a Nd:YAG or diode laser.[76,81–84] Both procedures are designed to reduce the production of aqueous humor and decrease IOP. Cyclophotocoagulation has fewer intraocular side effects than cyclocryosurgery and is the 1015
surgical treatment of choice. In a retrospective evaluation of 27 horses treated by transscleral diode laser 1016
cyclophotocoagulation, adjunct medical therapy was required for 90% of horses and vision was maintained in 64% with a mean follow-up of 33 months (7 to 69 months).[76] Surgical filtering procedures, designed to provide an alternative outflow pathway for aqueous humor, are generally unsuccessful in the horse.[5]

Treatment of chronic glaucoma is surgical and is designed to reduce discomfort. Enucleation or evisceration with insertion of an intraocular silicone prosthesis is the procedure of choice.[85]

15.7.7 HYPHEMA

Hyphema is blood in the anterior chamber of the eye (see Figure 15-8). As is true of bleeding into other body cavities, blood in the anterior chamber of the eye does not usually clot. One must keep in mind that not all hyphema results from ocular trauma. One must consider systemic diseases that result in clotting disorders or intraocular inflammation.

If the hyphema is complete and precludes the evaluation of intraocular structures, ocular ultrasound is indicated to assess the lens position, retina, and posterior eye wall. The greatest resolution in ocular ultrasound is achieved with a 10-MHz or, if unavailable, a 7.5-MHz probe. One places the probe directly on the cornea or images through the eyelid or an offset device. Provided no other intraocular damage is evident, hyphema will resolve, often without significant sequelae. If associated intraocular damage has occurred, potential sequelae include cataract, posterior synechiae, glaucoma, retinal detachment, and blindness (Figure 15-12).

Figure 15-12 Ocular ultrasonography using a 7.5-MHz probe. A retinal detachment is visible with the retina remaining attached at the optic nerve and ciliary body.

When hyphema results from a traumatic event, concurrent anterior uveitis is usually present. Although hyphema usually does not require therapy, the associated anterior uveitis does. As previously discussed, one treats anterior uveitis with topical atropine and systemic flunixin meglumine. If no corneal ulcer is associated with the hyphema, one also may administer topical corticosteroids. Resolution of hyphema may require 7 to 21 days; to decrease the incidence of rebleeding, the horse should not be exercised during this time. Surgical intervention to remove the hyphema rarely, if ever, is indicated. In instances in which lysis of a clot is beneficial, intracameral injection of tissue plasminogen activator (25 μg) (Activase, Genentech, Inc., South San Francisco, California) may be indicated.[86]

15.8 Lens

The lens originates from surface ectoderm and is a clear, avascular, biconvex structure suspended posterior to the iris by lenticular zonules arising from the ciliary body. The lens depends on the aqueous and vitreous humors to supply nutrition and remove waste products. One should examine the lens in a darkened environment following dilation of the pupil with tropicamide 1.0% (Mydriacyl, Alcon Laboratories). Using a bright focal light source, one evaluates the lens for size, shape, location, and transparency. In addition, a new handheld, monocular slitlamp that attaches to an otoscope or ophthalmoscope handle is available (HSL-10) and provides magnification and an

appreciation for depth and three-dimensional anatomy of the lens and anterior segment. Abnormalities of the lens include congenital malformations, cataract, and luxation.

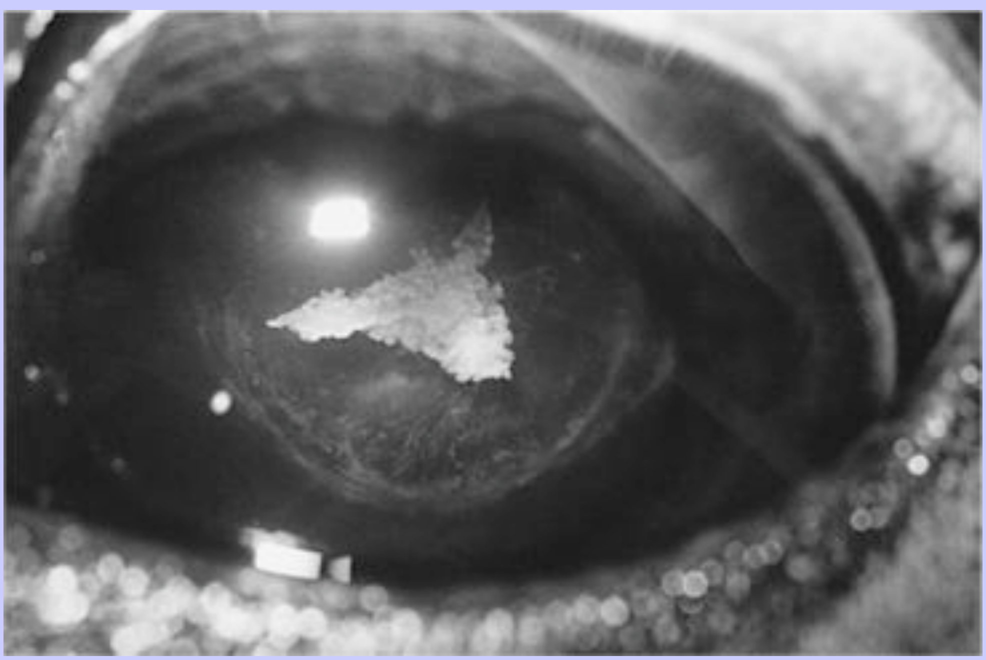
15.8.1 CONGENITAL

Congenital disorders of the lens, other than cataract, are rare. Abnormalities of size (aphakia, microphakia), shape (coloboma, spherophakia), and location (luxation) generally are associated with other ocular abnormalities such as microphthalmos, retinal dysplasia, or retinal detachment. These abnormalities can be uni- or bilateral and may or may not be inherited. Treatment usually is not required except for cataract and luxation. One should discourage breeding of affected animals.

15.8.2 CATARACTS

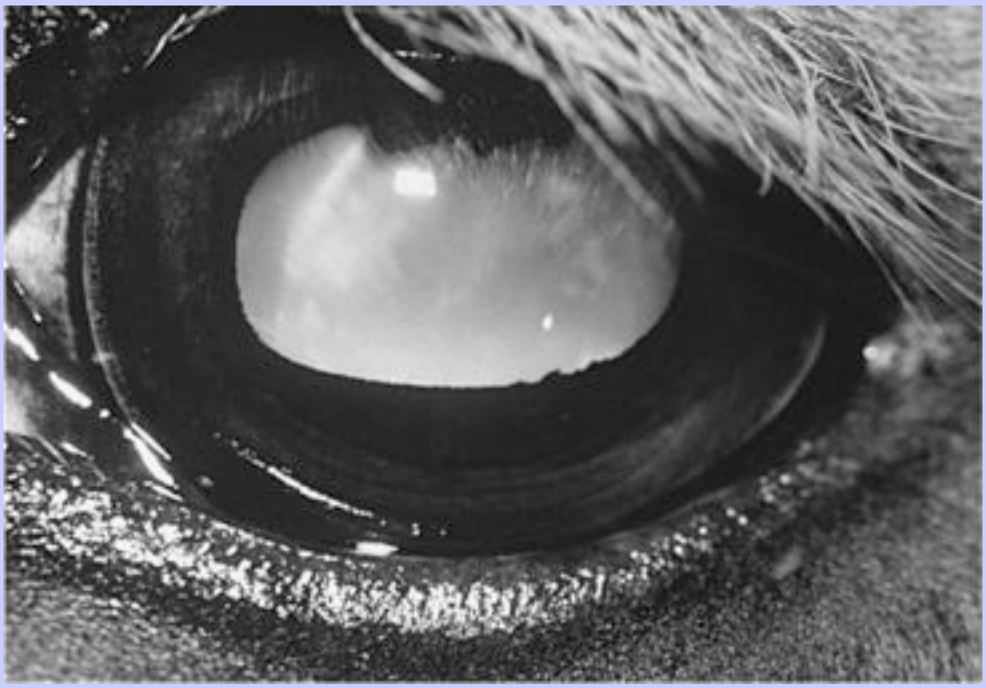
A cataract is any opacity, of any size, involving the lens or its capsule (Figures 15-13 and 15-14). Cataracts can be uni- or bilateral and are classified according to location and degree of lens affected (Box 15-5).
Classification helps determine the cause and likelihood of progression and helps one follow progression. These 1016
terms are only descriptive and are not mutually exclusive. For example, one such description might be an 1017
"anterior, cortical, axial, incipient cataract." In general, anterior, cortical, and equatorial cataracts are more likely to progress than are nuclear and posterior cataracts. In addition, cataracts are classified based on age of onset and, if possible, cause. Classification by age of onset includes congenital, juvenile, adult, and senile cataracts. Congenital cataracts are the most common congenital ocular anomaly in the horse, can be unilateral or bilateral, and may affect only the nuclear portion of the lens.[87] Juvenile and adult cataracts, although acquired, are still heritable.

Figure 15-13 An incipient posterior cortical cataract.



Although many causes of cataracts exist in other species, most causes have not been documented in the horse. Potential causes of cataract formation include inherited, inflammatory, traumatic, metabolic, toxic, and nutritional abnormalities. Suspected inherited cataracts have been reported in several breeds of horses (Belgian, Morgan) and are thought to occur in other breeds but are difficult to prove because of the small number of offspring produced. One must remember that inherited cataracts are not necessarily congenital and that not all congenital cataracts are inherited. In general, if one cannot determine a cause of a cataract and intraocular inflammation is not evident, one should discourage breeding of the animal. The most common cause of cataract formation in the horse is ERU.

Figure 15-14 A mature cataract.



15.8.2.1 BOX 15-5 CHARACTERIZATION OF CATARACTS

15.8.2.1.1 ### Location

Capsular

Subcapsular

Cortical

Equatorial

Axial

Nuclear

Anterior/posterior

15.8.2.1.2 Severity

Incipient: earliest form; small vacuoles

Immature: most of lens affected; fundus visible

Mature: entire lens affected; fundus not visible

Hypermature: liquifying lens fibers; leaking through lens capsule

Morgagnian: entire cortex liquified; nucleus settles ventrally

Treatment of cataracts, if required, is surgical. The decision on whether to treat depends on the severity and cause of the cataract and the presence or absence of concomitant ocular disease. Cataracts that are unilateral or not severe enough to interfere significantly with vision generally do not require treatment. Cataracts resulting from intraocular inflammation, such as those associated with ERU, may not be amenable to surgery. However, with the availabilty of a sustained-release cylosporine-impregnated device,[72–74] one can perform cataract surgery along with a partial vitrectomy and implantation of a cyclosporin A device. Treatment of cataracts associated with abnormalities of the retina, such as retinal detachment or degeneration, is not indicated. If the cataracts are bilateral, interfere with vision, and do not result from ERU or other intraocular inflammation, then one can surgically remove the lens and its anterior capsule. Before cataract surgery, one should evaluate retinal anatomy and function using ocular ultrasound and electroretinography. One should operate on foals with congenital cataracts early (<6 months) to avoid possible deprivation amblyopia. Surgery is performed best using phacoemulsification.[88–90] Controversy exists regarding unilateral cataract extraction in horses with a 1017
contralateral normal eye. Because artificial lens implants are unavailable for horses, cataract extraction results 1018
in a significant degree of postoperative hyperopia (farsightedness).[44] Although significant disparity in postoperative vision between eyes must result, one study suggests that such horses perform better following cataract surgery than as unilaterally blind horses before cataract extraction.[88] Cataract surgery is a referral procedure.

15.8.3 LUXATION

Lens luxation and subluxation can be congenital or acquired. Acquired lens luxation results from glaucoma and buphthalmos, trauma, or most frequently, uveitis, especially ERU. Once luxated, the lens can be displaced anteriorly or posteriorly. A posteriorly luxated lens results in liquefaction of the vitreous from lens movement. The lens settles inferiorly, may or may not attach to the retina, and generally does not result in significant problems or require therapy. Anterior lens luxation results in anterior uveitis, trauma to the corneal endothelium resulting in diffuse corneal edema, and obstruction of aqueous humor circulation resulting in secondary glaucoma. Treatment of anterior lens luxation is surgical removal using an intracapsular extraction technique, provided the eye is visual. This is a referral procedure. In a blind, painful eye, enucleation or intrascleral prosthesis is the treatment of choice.

15.9 Fundus

One can examine the equine fundus best in a darkened environment following mydriasis. Direct and indirect ophthalmoscopy are useful for examining the equine fundus, although direct ophthalmoscopy is preferable for examining the optic nerve and retinal blood vessels. Examination should include evaluation of the tapetal and nontapetal fundus, retina, retinal blood vessels, and optic nerve. Variations in the normal equine fundus are common, and familiarity with these variations is essential.[16,44,87,91] The horse has a paurangiotic retina (partially vascularized) with 30 to 60 small retinal vessels radiating from the margin of the optic disk. These vessels are visible for a distance of one to two disk diameters. The remainder of the equine retina is avascular, being supplied from the underlying choroidal blood vessels. The optic disk is situated in the nontapetal fundus, is oval, and is salmon-pink. The fundus is divided into tapetal and nontapetal regions. The tapetum is situated in the dorsal fundus and is responsible for the characteristic yellow-green of this portion of the fundus. Variations in tapetal color are related to coat color and include yellow, orange, and blue-green.[16,87] The tapetum of the horse is fibrous and is penetrated by small choroidal vessels that appear as dark dots in the tapetum, termed the *stars of Winslow*. The ventral, nontapetal fundus is generally dark brown or black but can appear lighter or nonpigmented depending on coat color.[16,87]

Abnormalities on fundic examination include changes in size, shape, and color of the optic nerve and retinal vessels, elevation or depression of the optic nerve, hemorrhage, changes in tapetal reflectivity (hyper- and hyporeflective), and changes in pigmentation. Hyperreflection abnormalities often indicate thinning or loss of retinal tissue. Hyporeflective changes can indicate an increase in tissue thickness resulting from cellular infiltrates, edema, or folding of the retina, as in retinal dysplasia. Inflammation can result in depigmentation and pigment clumping in the nontapetal fundus and hyperpigmentation in the tapetal fundus. Equine motor neuron disease has been described to result in a mosaic pattern of yellow-brown pigmentation in the tapetal fundus accompanied by a horizontal band of pigment at the tapetal-nontapetal junction.[92]

15.10 Retina

15.10.1 CONGENITAL STATIONARY NIGHT BLINDNESS

Equine night blindness is a bilateral, congenital, nonprogressive retinal disease of Appaloosa horses. The degree of visual disturbance varies among horses, with mildly affected horses exhibiting signs only in dark conditions, whereas severely affected horses are totally blind in the dark, exhibit apprehension in daylight, and may have a bilateral dorsomedial strabismus and nystagmus.[93] The diagnosis is based on history, breed of horse, clinical signs, and maze testing in illuminated and darkened environments, but one must confirm it by electroretinogram because the fundic examination in affected horses is normal. Electroretinography demonstrates an almost purely negative waveform in the scotopic (dark-adapted) response, characteristic of equine night blindness.[93,94] The results of the electroretinogram indicate an abnormality in signal transmission from the photoreceptor cells to the inner retina.[93] Although the inheritance of equine night blindness in the Appaloosa is not defined completely, the disease is suspected to be an autosomal recessive disease. No treatment is available.

15.10.2 CHORIORETINITIS

Inflammation of the retina and choroid in the horse most often results from ERU and may be associated with concomitant optic neuritis. In addition, the vascular nature of the choroid results in susceptibility to blood-borne disease, as with bacteremia, septicemia, and viremia, and most often occurs in foals with pneumonia, strangles 1018
(Streptococcus equi), and other severe infectious diseases. Inflammation of the anterior portions of the eye, 1019
anterior uveitis, often is associated with chorioretinitis. Treatment of chorioretinitis includes systemic therapy for the primary infectious disease, if present, and flunixin meglumine to decrease inflammation. Topical treatment is indicated only with anterior uveal involvement. The sequelae of chorioretinitis include retinal degeneration, retinal detachment, and optic nerve atrophy.

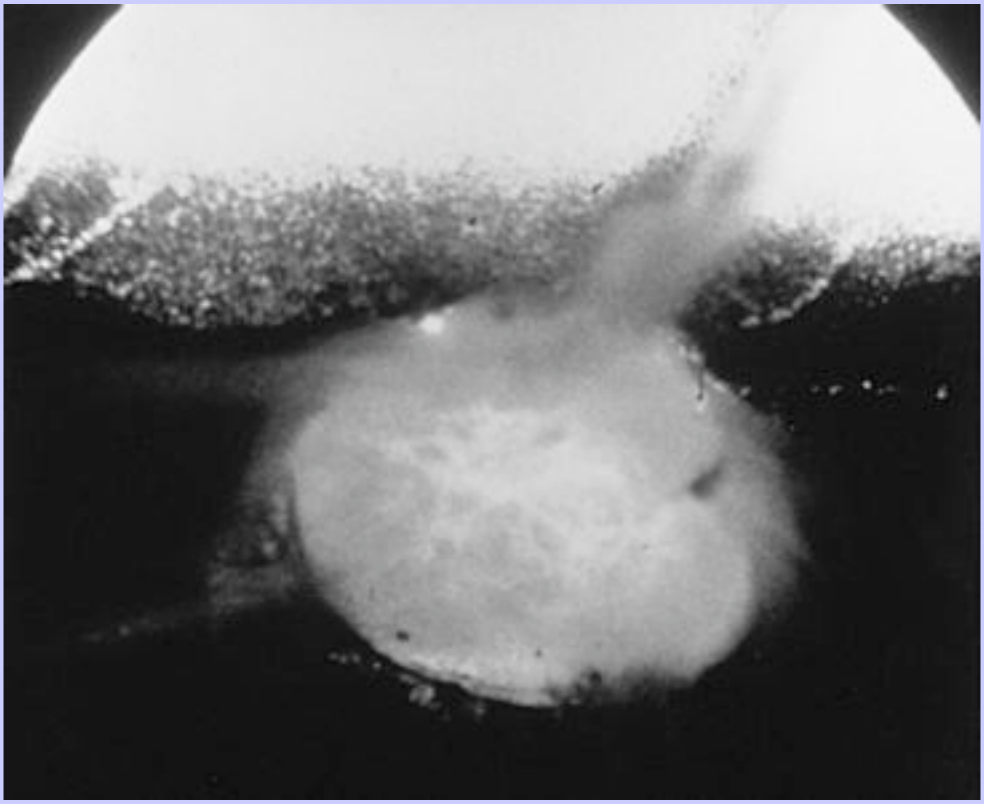
15.10.3 RETINAL DETACHMENT

Retinal detachment can be congenital or acquired, partial or complete (Figure 15-15). Because the equine retina depends almost totally on the underlying choroid for its blood supply, detachment results in rapid and severe retinal degeneration. Common causes of retinal detachment in the horse include an inherited abnormality (associated with retinal dysplasia), ERU, and trauma. Congenital retinal detachment often is associated with other ocular abnormalities. Hyphema may be associated with traumatic detachment, requiring ocular ultrasonography for definitive diagnosis. Treatment is limited to controlling the inciting disease and use of systemic antiinflammatory drugs.

15.10.4 PHOTIC HEAD SHAKING

A condition of head shaking induced by exposure to light and eliminated by blindfolding, darkened environment, and contact lenses has been reported in the horse.[95,96] The problem usually occurs in the spring and summer, is exacerbated by exercise, and may be accompanied by sneezing, snorting, and nasal rubbing. Differential diagnoses for photic head shaking include middle ear disorders, ear mites, guttural pouch mycosis, other ocular disorders, and nasal and dental disease. Ophthalmic examination in these animals is normal. An optic-trigeminal response is hypothesized to occur with optic stimulation, resulting in referred stimulation in areas innervated by the trigeminal nerve.[95]

Figure 15-15 Gray folds of retina are visible overlying and adjacent to the optic nerve, indicating retinal detachment.



Treatment using cyproheptadine at 0.3 mg/kg (Sidmark Laboratories Inc., East Hanover, New Jersey) orally every 12 hours has been effective in several horses.[95,96] Cyproheptadine is an antihistamine (histamine$_1$ blocker) and a serotonin antagonist and is hypothesized to work in photic head shaking by moderating the trigeminal nerve sensation, having a central effect on melatonin, or through anticholinergic activity.[95]

15.10.5 EQUINE MOTOR NEURON DISEASE

Equine motor neuron disease results from a dietary deficiency in the antioxidant vitamin E.[97,98] Ceroid-lipofuscin subsequently accumulates in the retinal pigment epithelium.[97] With ophthalmoscopy, this appears as irregular, sometimes linear accumulations of pigment in the tapetal and nontapetal retina (Figure 15-16, *A* and *B*). The ophthalmoscopic findings, along with appropriate musculoskeletal signs, highly suggest a diagnosis of equine motor neuron disease.

15.11 Optic Nerve

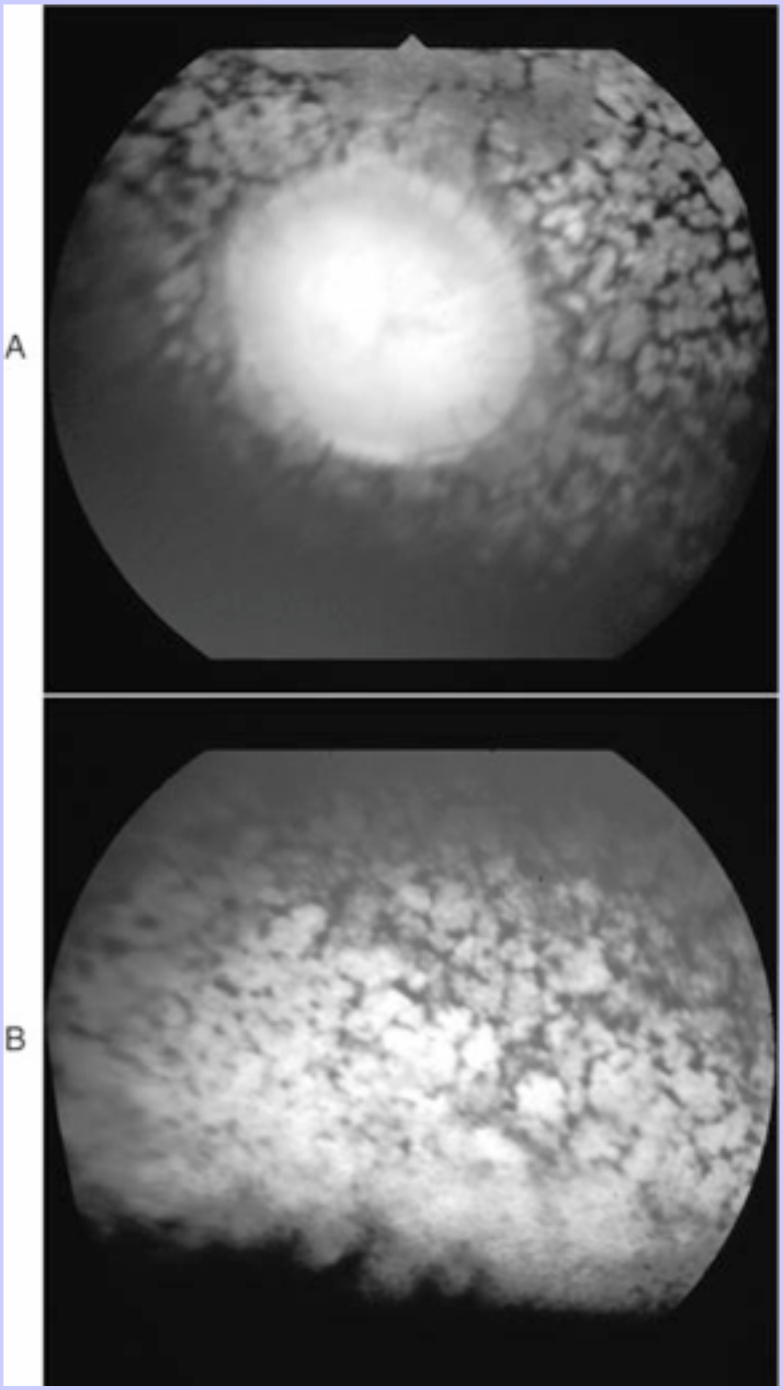
The equine optic nerve is oval, salmon-pink, and located in the nontapetal region of the fundus. Arterioles and venules (30 to 60) extend a short distance from the optic nerve into the surrounding peripapillary retina. On direct

ophthalmoscopic examination the margin of the optic nerve is sharp and well-defined, as are the retinal blood vessels. Often the nerve fiber layer of the retina is visible as linear white streaks radiating outward from the optic nerve. Abnormalities of the optic nerve may appear as indistinct margins, pallor, vascular attenuation, peripapillary depigmentation, edema, hemorrhage, or proliferative changes.

15.11.1 OPTIC ATROPHY

Trauma to the head of the horse has been associated with acute uni- or bilateral blindness resulting from optic nerve damage. The pupil in the affected eye is dilated, but the remainder of the ophthalmic examination may be normal initially. Occasionally, retinal hemorrhage and papilledema are present. Fundic examination 3 to 4 weeks after the traumatic episode reveals optic nerve pallor and absence of retinal blood vessels, indicating atrophy (Figure 15-17). The cause of this lesion is hypothesized to be stretching of the optic nerve with 1019
subsequent rupture of the optic nerve axons[99] or trauma from bony fractures adjacent to the optic nerve. A 1020
small number of these horses may benefit from systemic antiinflammatory therapy in the acute phase. The prognosis, however, is guarded and treatment is usually unrewarding. Optic nerve atrophy also is associated with ERU and results from chronic glaucoma.

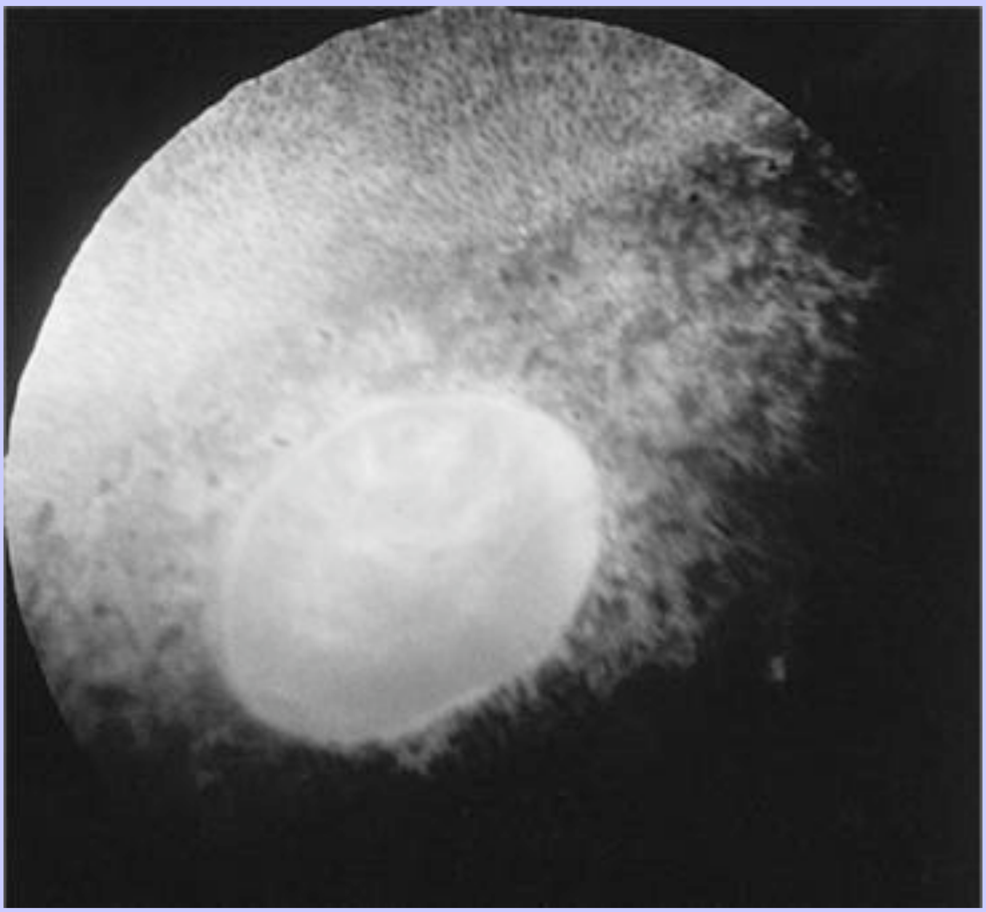
Figure 15-16 **A,** The optic nerve and peripapillary region of horse affected with equine motor neuron disease. The linear accumulations of pigment are ceroid-lipofuscin in the retinal pigment epithelium. **B,** Similar lesions are visible in the dorsal tapetal retina.



15.11.2 ISCHEMIC OPTIC NEUROPATHY

Ischemic optic neuropathy and resulting blindness is described following treatment of guttural pouch mycosis by arterial occlusion. The horses are acutely blind with a fixed and dilated pupil on the affected side immediately following surgery. Blindness is thought to result from ischemia and infarction of the optic nerve and its associated fibers. The initial fundic examination is normal, with abnormalities first seen 3 days following surgery. Indistinct optic disk margins suggesting papilledema or papillitis and raised, white lesions involving the optic nerve are the initial changes.

Figure 15-17 Severe optic nerve atrophy associated with head trauma. The optic nerve is pale, with loss of peripapillary retinal blood vessels. The eye is not visual.



15.11.3 OPTIC NERVE HYPOPLASIA

Horses affected with optic nerve hypoplasia have visual impairment or blindness and slow to absent pupillary light reflexes.[16,87] The abnormality is congenital and can be uni- or bilateral. Optic nerve hypoplasia may be

associated with other ocular abnormalities, including cataracts, retinal detachment, and microphthalmos.[87] The optic disk appears small and pale with fewer retinal vessels than normal. Clinically, optic nerve hypoplasia can appear similar to acquired optic nerve atrophy. One can make a definitive diagnosis on histologic examination. No treatment is indicated, and although the definitive cause is not known, owners should not use the affected horse and possibly the sire and dam for breeding.

Other abnormalities of the optic nerve include bilateral optic neuropathy and blindness following severe hemorrhage[101] proliferative optic neuropathy as in aged horses,[102] neoplasia of the optic nerve,[103] and optic neuritis, which may be associated with ERU. 1020

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15.12 Orbit

The horse has a complete bony orbital rim composed of the frontal, lacrimal, zygomatic, and temporal bones. The caudolateral and ventral walls of the equine orbit are fasciae. Abnormalities of the equine orbit most often result from trauma. The zygomatic arch and supraorbital process of the frontal bone and the medial orbital wall are most susceptible to injury. Damage to these areas can involve the supraorbital foramen and its associated nerve or the osseous portion of the nasolacrimal system, respectively.

The diagnosis of an orbital fracture is based on history, clinical signs, and radiographs. Radiographic evaluation of the equine orbit is technically difficult and often unrewarding. Oblique views, highlighting the area of greatest concern, usually are required. One must take care to evaluate the paranasal sinuses—the frontal, maxillary, and sphenopalatine—especially when subcutaneous emphysema is present.

Traumatic fractures of the orbit often are associated with concomitant injury of the globe and adnexa. Complete ophthalmic examination is essential and must include assessment of facial symmetry, vision (menace response, maze testing), pupillary light reflex, fluorescein staining for the presence of a corneal ulcer, examination of the anterior chamber for hyphema and anterior uveitis, fundic examination evaluating the retina and optic nerve, assessment of globe and eyelid mobility, and nasolacrimal irrigation. Head trauma in the horse has been reported to result in acute blindness with fixed, dilated pupils resulting from optic nerve compression, stretching, or avulsion. In those eyes in which ocular damage precludes examination of the posterior segment, ocular ultrasound examination is advisable.

Blunt trauma to the orbit can result in a breakdown of the fibrous orbital septum and subsequent herniation of orbital fat. Clinically, the condition is a nonpainful swelling that appears at the time of, or following, orbital trauma and is treated best surgically, removing or replacing the herniated portion of the orbital fat pad and attempting to repair the rent in the septum.

Traumatic proptosis is rare in the horse because of the complete bony orbital rim. If the globe protrudes, it indicates severe head trauma, and thorough physical and neurologic examinations are required. Provided the optic nerve and extraocular muscles are intact, one should replace an exophthalmic globe, a procedure that requires general anesthesia. Once one replaces the globe, one performs a temporary tarsorrhaphy to protect the eye until the swelling subsides. Systemic antibiotics and antiinflammatory drugs are indicated to control postoperative complications.

Treatment of orbital trauma in the acute phase includes systemic antiinflammatory therapy, systemic antibiotics, especially if a paranasal sinus is involved, and cold compresses. The antiinflammatory drug of choice is systemic flunixin meglumine. Local therapy is required to manage corneal exposure, ulceration, and anterior uveitis. If eyelid movement is impaired because of neurologic dysfunction or swelling of the eyelids, one must protect the

cornea from exposure and desiccation using a topical, sterile ophthalmic lubricant applied as frequently as possible, or in the more severe cases, by using a temporary tarsorrhaphy. Topical broad-spectrum antibiotics are indicated in horses with corneal ulceration, and atropine relieves the discomfort associated with anterior uveitis.

Although facial and orbital fractures in horses often heal without surgery, they may do so in a manner that results in deformity and interferes with the normal function of the eye and adnexa. Therefore surgical correction may be indicated, especially in those fractures that are displaced. One must consider fractures that extend into a paranasal sinus as open fractures, because these sinuses contain resident bacterial and fungal flora. Early repair is associated with a more favorable cosmetic result, for skull fractures consolidate rapidly and the resulting fibrous callus may interfere with surgical reduction. Generous skin flaps in the surgical approach are advised because fractures are often more extensive than initially thought. One should avoid excessive periosteal dissection because periosteum provides stability and blood supply to the damaged area.

Orbital neoplasia is rare and may result in exophthalmos, strabismus, and vision and pupil abnormalities. Skull radiographs, orbital ultrasound, cytologic examination of a fine-needle aspirate, and histopathologic examination are useful in confirming the diagnosis. SCC, lymphosarcoma, adenocarcinoma, medulloepithelioma, melanoma, and tumors of neuroendocrine origin have been described.[104] Differential diagnoses include trauma and orbital abscess and cellulitis. Because most horses are presented late in the course of disease, orbital exenteration is often the treatment of choice. If one suspects an orbital neurendocrine tumor, one must take care during enucleation/exenteration because extensive intraoperative hemorrhage is frequent.

A technique for radical enucleation/exenteration with partial orbital rim resection, mesh skin expansion and second intention healing has been described recently for extensive periocular tumors in horses.[105] This technique is indicated when skin is insufficient to complete a standard primary closure following surgical resection.

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16 CHAPTER 16 DISORDERS OF THE REPRODUCTIVE SYSTEM

Grant S. Frazer

16.1 16.1—Reproductive Anatomy and Physiology of the Nonpregnant Mare

Daniel C. Sharp

Michael B. Porter

Equine reproductive physiology deviates from other domestic and companion animal models, often providing the open-minded practitioner and researcher a lifetime of challenges or frustrations, depending on their approach to them. This chapter presents the authors' current understanding of equine reproductive physiology set against a backdrop of accepted dogma for other species as often as appropriate.

16.1.1 Seasonal Reproductive Cycle

A dominant and often troublesome feature of equine reproduction is the adherence to an annual rhythm of reproductive competence and incompetence. The annual reproductive cycle of mares reflects an evolutionary strategy to maximize survival of foals by programming the breeding season attuned to gestation length. Thus mares, with approximately 11 months gestation, begin to breed in the midspring so as to time parturition in midspring. Therefore the mechanisms best able to regulate timing of the annual reproductive cycle are long-term and highly reliable. Nutritional and environmental temperature changes do not fit that requirement well. The four main phases of the annual reproductive rhythm are discussed next.

16.1.1.1 ANESTRUS

Anestrus usually occurs during the shortest days of winter and is characterized by reproductive incompetence in most mares. Measurements of hypothalamic gonadotropin-releasing hormone (Gn-RH)[1–4] indicate a low hypothalamic Gn-RH content and secretion rate during anestrus. Pituitary gonadotropin secretion consequently is reduced, with low or undetectable circulating concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[5–7] Although pituitary stores of FSH appear to be adequate throughout anestrus,[8] this is not the case with LH. The messenger RNA (mRNA) encoding LH subunit production becomes undetectable in pituitary tissue during anestrus.[9,10] Thus pituitary gonadotropins (FSH and LH) are low during anestrus because of suppression of the releasing signal from the hypothalamus (Gn-RH), plus inactivation of the gene encoding LH synthesis in the pituitary. The result is, not surprisingly, loss of function of the ovaries. Ovarian activity, including follicular development and hormone production, is minimal, with few follicles greaterthan 5 mm in diameter and undetectable circulating concentrations of estradiol and progesterone.

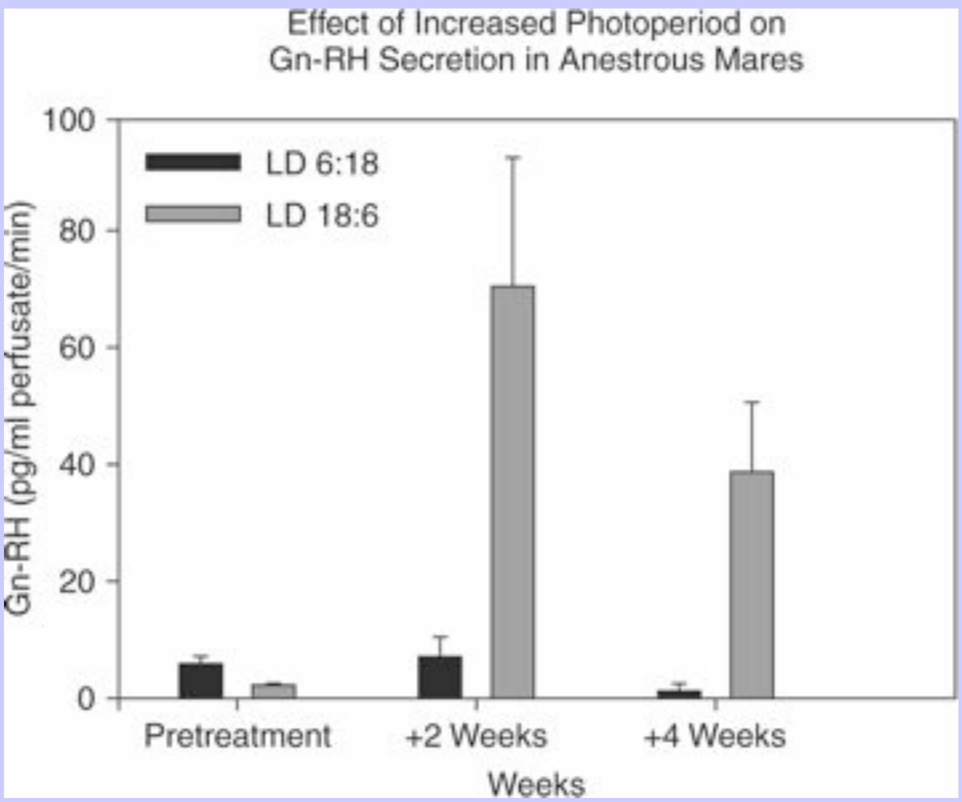
As might be expected from this reduction in circulating hormones, sexual receptivity in mares during anestrus tends to be reduced or lacking altogether. Given the lack of circulating hormones, the passivity of mares in response to teasing not surprisingly is highest in winter.[6] 1025

16.1.1.2 VERNAL (SPRINGTIME) TRANSITION

The vernal transition of the annual reproductive cycle, which begins sometime around the first of the calendar year, is the most troublesome commercially. The first event in transition—likely the initiating one—is an increase in hypothalamic Gn-RH observable within 1 week after the winter solstice[2] or 2 weeks after exposure to an artificially increased photoperiod several weeks before the solstice (Figure 16.1-1).[11] Whether the increase in hypothalamic Gn-RH represents a response to increasing day length, refractoriness to short day length, or some other factor remains unresolved.

Hart, Squires, Imel, et al.[8] reported that the increased Gn-RH secretion shortly after the winter solstice leadsto increased FSH secretion from available pituitary stores, but that LH levels remain low. Sherman, Wolfe, Farmerie, et al.[9] explained this phenomenon, stating that the mRNA encoding LH subunit synthesis was undetectable in the winter and early spring. The third major event during the vernal transition, after hypothalamic Gn-RH secretion and subsequent release of pituitary FSH stores, is follicular development. During vernal transition, the size and number of manually detectable follicles increases.[12,13] The follicular population changes from only two or three small follicles (5 to 10 mm in diameter) to six or more midsize follicles (15 to 25 mm), with the diameter of the largest follicle exceeding 35 to40 mm.[12] A major problem early in the breeding season is that many of these large vernal transition folliclesdo not ovulate. In ponies, an average of 3.5 ± 0.7 large (>30 mm) anovulatory follicles develop in sequence before the first ovulation of the year.[6] These anovulatory vernal transition follicles are the cause of high reproductive inefficiency during the springtime, and one cannot possibly discern, by palpation or ultrasound, whether they will be ovulatory. Consequently, many mares are sent to the stallion inappropriately, adding to the frustration and expense of breeding. Furthermore, mares with chronic failure of uterine clearance likely suffer most from such an inappropriate breeding, and the penalty to the breeder is often greater expense aimed at resolving uterine contamination.

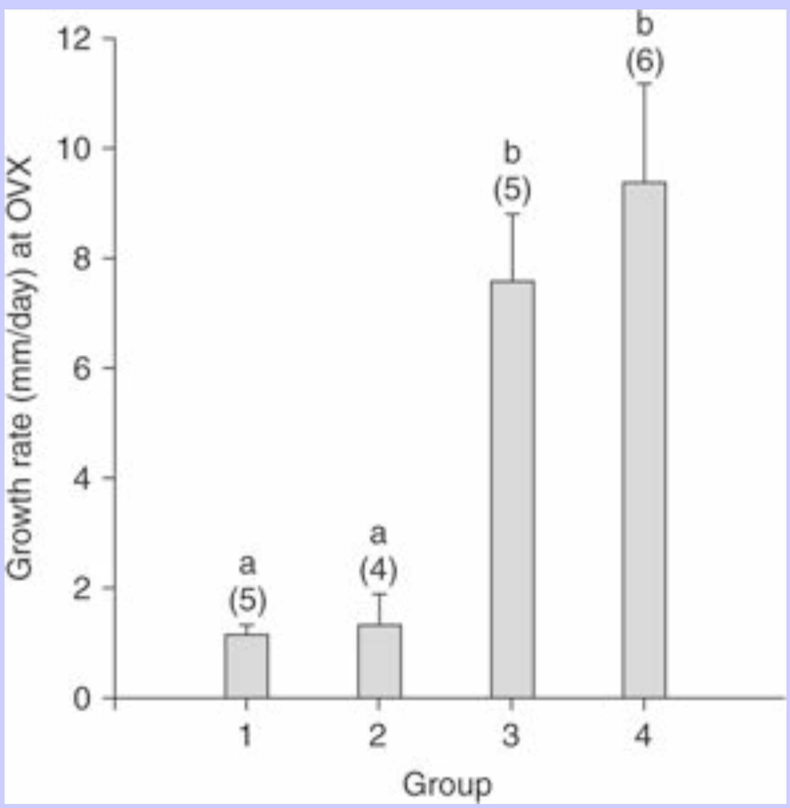
Figure 16.1-1 Gonadotropin-releasing hormone (Gn-RH) secretion (picograms per milliliter per minute, LS means ± SEM) as measured with push-pull perfusion of the pituitary in mares exposed to short photoperiod (LD 6:18, *n* = 4) andin mares exposed to long photoperiod (LD 18:6, *n* = 4) for4 weeks. In the bars marked *pretreatment*, all mares hadbeen exposed to LD 6:18. Subsequently, four mares were exposed to LD 18:6, and Gn-RH secretion was measured at2-week intervals. Time of year the experiment was begun was December 1. Gn-RH was highly significantly elevated in mares after only 2 weeks of photostimulation.



The first several vernal transition follicles are different from fully functional follicles during the breeding season. Although they attain a diameter comparable with preovulatory follicles of the breeding season (>30 mm), they do so much more slowly. Careful tracking of follicles reveals that the first and second large follicle of the year grow at a rate of only 1 to 2 mm per day(Figure 16.1-2), compared with growth rates of about 10 mm per day for follicles that go on to ovulate.[14] Therefore one may assess the potential functional status of springtime follicles by frequently monitoring their growth.

The slower growth rate of vernal transition follicles reflects other metabolic deficiencies, including poor vascularity compared with ovulatory follicles and significantly smaller granulosa cell content.[15] The poor 1026
granulosa cell investment of early vernal transition follicles is significant in that the granulosa cell layer 1027
contributes substantially to the steroidogenic capabilities of the follicle. Thus steroid production by the early vernal transition follicles is limited. The follicles do not produce estradiol, as monitored by peripheral blood, follicular fluid, or in vitro production rate. Estradiol concentration is low or undetectable in peripheral plasma until 5 or6 days before the first ovulation of the year.[7,14,16] One can measure circulating estradiol to discriminate between nonfunctional and functional (ovulatory) follicles.

Figure 16.1-2 Growth rate (millimeters per day) of follicles during vernal transition. Groups represent mares at first, second, third, and fourth follicle of the year. (From Tucker KE, Cleaver BD, Sharp DC: Does resumption of follicular estradiol synthesis during vernal transition in mares involve a shift in steroidogenic pathways? *Biol Reprod* (suppl 1) 48:188, 1993.



When one of the sequential vernal transitional follicles acquires steroidogenic capability, the circulating estradiol increases significantly 5 to 6 days before the first ovulation of the year. The average date for the first ovulation of the year is consistent from year to year, and variance among mares in a population is low. Data collected from as far north as 43 degrees north latitude to as far south as18 degrees north latitude have

indicated an onset of the breeding season (date of first ovulation of the year) to be around the first week of April in mares and the second week of May for pony mares that have not been exposed to artificial lighting. Once the first ovulation of the year has occurred, vernal transition is completed and regular polyestrous cycles occur throughout the late spring and summer in unbred mares.

Renewed LH synthesis and secretion, also manifested by a large surge 5 to 6 days before the first ovulation of the year, is associated with the rise in estradiol. Sharp, Grubaugh, Davis, et al.[17] and Cleaver and Sharp[18] have demonstrated that administration of estrogen advances this secretion of LH in mares during early vernal transition, and Sharp, Wolfe, Cleaver, et al.[10] further demonstrated that estrogen administration to ovariectomized mares at the same time of year stimulated the mRNA encoding for the LH α and β subunits. In other words, estrogen administration was associated with activation of the LH gene. However, as Freedman, Garcia, and Ginther[5] and Peltier, Robinson, and Sharp[7] have demonstrated, the increase in peripheral LH occurs at approximately the same time in mares with and without ovaries. Thus the role of estrogen in bringing about the resurgence ofLH synthesis and secretion remains unclear. Furthermore, one must resist the temptation to use estrogen to hasten LH synthesis in mares during the early vernal transition because of the negative effects of estrogen on FSH secretion. Initiating LH synthesis may be possible, only to find that it inhibits follicular development.

16.1.1.3 BREEDING SEASON

The next section discusses the breeding season in detail. The breeding season is characterized by regularly recurring cycles of estrus and diestrus throughout the summer, unless pregnancy occurs.

16.1.1.4 AUTUMNAL TRANSITION

The transition from reproductive competence in the breeding season to reproductive quiescence characteristic of anestrus is perhaps the least understood part of the annual seasonal reproductive cycle in mares. The period of reproductive decline has a much greater variance during the fall than does the period of reproductive renewal in the spring. The variance is understandable from the evolutionary aspect, because mares likely faced little pressure to reproduce in the fall, for their offspring would face more difficult survival at foaling the next year. Although this is an oversimplification, the autumnal transition may be characterized simply as the gradual loss of gonadotropic support for ovarian function, resulting in reduced follicular development. The final act of the autumnal transition may be development of a large preovulatory follicle that fails to ovulate.[19]

16.1.2 Morphologic Behavioraland Hormonal Changesof the Estrous Cycle

16.1.2.1 ESTRUS

Estrus, defined by receptivity of the mare to a stallion, is characterized by profound changes in the ovaries as follicles develop and in the uterus, cervix, and vagina as the developing follicles begin producing estrogen and by the occurrence of a prolonged surge or increase in LH.

16.1.2.1.1

Behavior

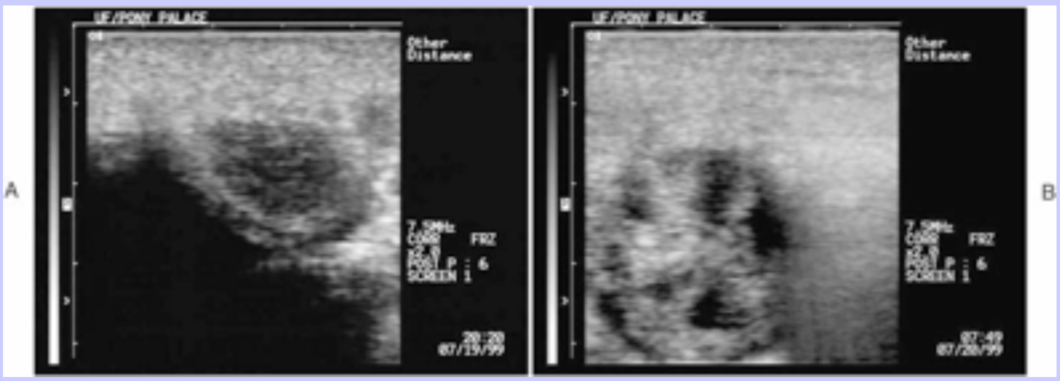
Understanding estrous behavior, as monitored by teasing with a stallion, is an important foundation to any breeding program. *Estrus*, the word coined by Walter Heape,[20] is thought to come from the Greek word *Oestridae*, a genus of the gadfly, because locomotor activity of many female ungulates increases during estrus. Estrous behavior is associated temporally with increasing estrogen concentrations peripherally but also may be associated with the concomitant decline in progesterone levels. Support for the idea that estrogen may not be essential for estrous behavior comes from observations of estrous display in mares with inactive ovaries and from ovariectomized mares during anestrus.[21] The displays of estrous mares reflect a wide range of behaviors and extents, however, and successful breeding farms often owe their success in part to careful monitoring and record keeping for each mare. Among the signs observed during estrus are elevationof the tail in the presence of the stallion, eversion of the vulvar lips to expose the clitoris ("winking"), squatting with rotation of the pelvis, urination, and remainingcalm (i.e., not moving away) in the immediate presenceof a stallion. Recording behavioral signs tends to takethe decision making 1027
out of the watching process, and potentially provides a more precise record of progress through the estrous 1028
cycle.

16.1.2.1.2

Ovarian Morphology

One can use morphologic changes during estrus to monitor the progress toward ovulation. The equine ovary is structurally different from other species in that the cortical elements containing the primordial follicles are located centrally in the ovary instead of peripherally as in all other species that have been studied so far. This central position of potential ovulatory follicles presentsa dilemma in that ovulation cannot occur at the site of follicle development. Most of the surface of the equine ovary is covered with mesothelium, which apparently does not allow the tissue remodeling required for rupture of the follicle. Instead, ovulation in the mare always occurs at a specific site, called the "ovulation fossa," that is located ventrally on the ovary. The bean shape of the equine ovary creates a recessed fossa area in which germinal epithelium is located. This inside-out structure of the mare's ovary likely contributes to some of the unusual aspects of the equine estrous cycle, including the period of prolonged estrus, the large preovulatory follicle size, and the prolonged increase in LH that precedes ovulation. A logical assumption is that the structure of the ovary led to coevolution of a hypothalamic-pituitary axis that was able to present gonadotropins over a prolonged duration. The ovarian structure requires extensive tissue remodeling for a developing follicle to reach the restricted areaof the fossa where it can ovulate. The extended gonadotropin secretion seems likely to be an important part of the tissue remodeling process that precedes rupture of the follicle. Administration of Gn-RH analog to stimulate endogenous LH release, or human chorionic gonadotropin (LH activity), results in increased levelsof tissue remodeling enzymes in the follicle within24 hours.[22] This prolonged process presents challenges to the breeder or veterinary practitioner who is attempting to schedule breeding at a time appropriate to the expected ovulation.

Figure 16.1-3 **A**, Portion of uterine horn showing edema score of 1 (relatively homogeneous pattern with little evidence of fluid). **B**, Portion of uterine horn showing edema score of 4 (marked heterogeneity with clear evidence of fluid-filled lacunae).



16.1.2.1.3

Tubular Reproductive Tract

Perhaps the two most prominent changes in the equine tubular reproductive tract are the appearance of the external portion of the cervix and the pattern of intrauterine edema as monitored with ultrasound. The vaginal portion of the cervix exhibits cyclic changes that reflect the rise and fall of estrogens and progesterone. During estrus, the cervix becomes relaxed and droops toward the ventral floor of the anterior fornix, whereas in diestrus, under the influence of progesterone, the external cervical folds become more rigid and buttonlike, or “tight and white” in the vernacular. One can appreciate these changes by visual examination with a vaginal speculum.

Changes in intrauterine edema are evident, as monitored by ultrasound imaging, in the pattern of fluid (edema) present in the endometrial folds during estrus (Figure 16.1-3). The appearance of edema often is used to indicate estrus, but an important note is that the relationship between edema and ovulation is inverse. Edema is maximal several days before ovulation and then begins to dissipate. Ovulation occurs 3 to 4 days after peak edema scores are exhibited and the edema pattern is in the process of dissipation.[23] Because estrogen stimulates edema rapidly (within 6 hours), the presence of edema appears to be a useful indicator of estrogen secretion. However, dissipation of the edema is an active process, requiring the 1028
presence of ovaries, and likely involves progesterone as well. Far more intriguing then is the question of 1029
the mechanism of dissipation of edema. What is the purpose of edema in the first place if an active process exists for its dissolution before ovulation?

16.1.2.1.4

Hormones

Estrus in mares is characterized by increasing estrogen and a prolonged elevation in LH. The prolonged elevation of LH,[24] often reaching peak concentrations after ovulation, may reflect the need for extensive tissue remodeling of the ovaries for ovulation to occur. Estradiol reaches peak concentrations of greater

than30 to 40 pg/ml 2 to 3 days before ovulation. Evidence exists for a positive feedback effect of estradiol on LH,[25] but evidence also suggests that LH rises spontaneously after corpus luteum regression and the loss of progesterone negative feedback. This raises the question of whether estrogen positive feedback is necessary for the ovulatory increase in LH or whether it merely serves to enhance the default situation of elevated LH in the absence of progesterone.

16.1.2.2 DIESTRUS

Diestrus is essentially the polar opposite of estrus from the behavioral standpoint.

16.1.2.2.1 Behavior

Mares generally exhibit little or no interest in the approach of a stallion, and this often is manifested by violent demonstrations (kicking, switching of the tail, laying the ears flat, vocalizing) in a teasing situation. Rising progesterone concentrations are thought to play a role in the sexual nonreceptivity of mares. Although not critically determined, the sexually negative behavior of mares is assumed widely to be associated with progesterone concentrations greater than 1 ng/ml. Progesterone concentrations increase quickly after ovulation, reaching 1 ng/ml by 24 to 48 hours after ovulation, which coincides with the expected onset of diestrus.

16.1.2.2.2 Ovarian Morphology

The primary feature of ovarian morphology in diestrus is the presence of a corpus luteum within the ovary. Because of the inside-out structure of the ovary, and the restriction of ovulation to the ovulation fossa, the corpus luteum remains almost entirely within the ovary and provides little evidence of its presence by transrectal palpation, unlike ruminants. The advent of ultrasound imaging has improved diagnostic capabilities by enabling practitioners to visualize the corpus luteum.

16.1.2.2.3 Hormones

Diestrus is dominated by elevated progesterone, with its negative feedback effect on LH secretion.

16.1.3 Postpartum Period

Immediately following expulsion of the fetus, the equine uterus begins the process of involution, and this continues for several days after passage of the placenta. During this time, detection of fluid within the uterine lumen by ultrasound is not abnormal. However, the uterine fluid (lochia) and debris should dissipate within 7 to 10 days after parturition in preparation for “foal heat” in the mare.[26] One should not detect fluid by ultrasound 10 to 14 days postpartum, and the uterus should be restored to nearly prepregnancy condition within 3 to 4 weeks.

Foal heat is described as the first period of sexual receptivity following parturition and usually begins 7 to 9 days after foaling.[27] The first ovulation after foaling occurs on average at 9 days after foaling. Depending on the efficiency of uterine involution, the fertility of this foal heat varies. If significant fluid is present within the uterus at foal heat, breeding the mare is not recommended. In addition, studies regarding the effect of season on first postpartum ovulation suggest that mares that foal in January and February tend to have longer intervals

from foaling to the first fertile ovulation than mares that foal in late spring.[28] After the foal heat most mares have a 30-day heat that occurs approximately 21 days after the foal heat and may represent a more fertile estrous cycle.

In addition to the effects of season, a condition known as lactational anestrous may develop immediately after foaling. In some cases the condition may persist until weaning (4 to 6 months). Determining whether the lactational anestrous results from the foal nursing or the time of year (seasonal) is important. Limited studies have been performed in which foals were removed from mares at varying time points after parturition. Research indicates that removal of the foal hastens the onset of estrus. Foal removal has been associated with elevated LH concentrations and increased ovarian follicle diameter.[29]

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16.2

16.2—Mare Breeding Soundness Examination

Nigel R. Perkins

The veterinarian may perform a breeding soundness examination of the mare for a variety of reasons, including prepurchase or prebreeding examination of a mareto confirm the absence of detectable abnormalities involving the genital tract and examination of a subfertile mare following unsuccessful breeding or tentative identification of a

potential problem associated with the genital tract. Procedures that one should perform in all breeding soundness examinations include perineal examination, rectal palpation, ultrasonography, vaginoscopy, and digital vaginal examination. One also should consider additional procedures, choosing procedures tailored to the signalment, history, and initial findings of each mare.

16.2.1 Signalment and History

History and signalment represent an important and often overlooked part of the breeding soundness examination. They can identify possible problems and guide the diagnostic approach (Table 16.2-1). Breeding history should include more detailed information from the immediate past, including information on the standard of breeding management and facilities. Obtaining historical information on the entire breeding career of the mare is also valuable, though more difficult. 1030

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TABLE 16.2-1 Details of Information to Be Gathered During History Taking

HISTORY	COMMENTS
General health	Vaccination, deworming, foot care, dental care, nutritional history, systemic diseases, management
Athletic	Level of activity; when athletic career ended
Cycling	Length of estrus, expression of estrus, interovulatory interval
Breeding	Number of cycles bred, breeds per cycle, natural or artificial insemination, fertility of stallion; when mare was last bred
Pregnancies	Number of diagnosed pregnancies and outcomes
Foaling	Any history of dystocias; how they were managed
Prior reproductive examinations	Diagnoses, treatment, management
Prior reproductive surgeries	Nature of surgery, date performed and outcome
Drug use during breeding season	Ovulation induction, estrous cycle manipulation

16.2.2 General Physical Examination

General observation of the mare while loose and being led to the stocks allows assessment of behavior and preliminary assessment of condition and conformation. One should perform a brief general physical examination to ensure the mare has no detectable condition that could interfere with her ability to conceive or carry a foal to term. One should inspect the mammary glands visually and with digital palpation for evidence of abnormalities such as mastitis, fibrosis, and neoplasia and should ensure the mare is in good body condition. In general, excessive condition does not interfere with reproductive potential, but poor condition may.

16.2.3 Restraint

One must ensure the mare is restrained adequately before examination. What constitutes adequate restraint depends on the nature of the mare, the skill and experience of the handler and veterinarian, the procedures being performed, and the physical facilities available. One should exercise particular care when performing a breeding soundness examination (BSE) on mares with little prior exposureto procedures such as transrectal palpation. Chemical restraint is an effective and safe method in most cases for BSE procedures, particularly in those procedures that are less well tolerated or more prolonged. Chemical restraint may cause some degree of relaxation of the perineal region and may alter the assessment of vulval conformationin some cases. If possible, one should examine vulval conformation before tranquilizing the mare.

16.2.4 Examination of External Genitalia

One should inspect the perineal region for indications of vulval discharge and gross abnormalities of the anogenital region. Vulval discharge or urine scalding of the inner thighs may suggest genital infection, urine pooling, or urinary incontinence. Clitoral enlargement may indicate prior exposure to androgens or progestagens or an intersex condition.

Normal vulval lips are vertical, have good tone, and are in close apposition. One should part the vulval lips gently to determine if air is readily aspirated into the vagina. If vulval apposition is poor or aspiration of air readily occurs, then the mare may be prone to pneumovagina and at increased risk of ascending vaginitis.

Vulval integrity is important in the formation of an effective seal against contamination of the genital tract with air, urine, feces, and potential pathogens.[1] One should assess vulval conformation by digital palpation adjacent to the vulval lips to locate the caudal bony pelvic brim. Pascoe's description of a Caslick index has been modified to produce a simple assessment technique for vulval conformation[2]:

- VC (vulval conformation) good: No Caslick is required. Dorsal commissure of the vulva is lessthan 4 cm above the bony pelvic brim, and vulvallips are within 10 degrees of vertical and form an effective seal.
- VC marginal: Caslick possibly is required. Dorsal commissure of the vulva is 4 to 7 cm above the bony pelvic brim, and vulval lips are within 10 to 20 degrees of vertical, and vulval lips still form an effective seal.
- VC poor: Caslick or some other corrective procedure definitely is required. Dorsal commissure of vulval is5 to 9 cm or more above the bony pelvic brim, and vulval lips are 30 degrees or more in front of vertical, and vulval seal is less than effective.

16.2.5 Transrectal Palpation and Ultrasound Examination of the Genital Tract

An absolute imperative is confirmation of the nonpregnant status of the mare before performing any procedure that could threaten a pregnancy, even if the history suggests that she is not pregnant.

Transrectal palpation of the genital tract is associated with some risk of rectal mucosal trauma and potentially
laceration. The risk increases if one uses excessive force, if palpation continues in the presence of 1031
pneumorectum, and if the mare strains excessively. Infusion of 50 ml of2% lidocaine into the rectum through a 1032
piece of flexible tubing or added to a small quantity of lubricant, may help ease straining. Administration of 50

to 100 mg propantheline bromide (Propan-B, Vetpharm Ltd, Auckland, New Zealand) by intravenous injection several minutes before the examination also may help prevent straining but may interfere with assessment of uterine tone.

TABLE 16.2-2 Application of Transrectal Palpation and Ultrasound of the Genital Tract for Assessing Various Parameters

ORGAN	STRUCTURE	DETAIL OF ASSESSMENT	TECHNIQUE
Ovary	Ovary	Size, shape, presence of ovulation fossa	Palpation (P), ultrasound (US)
		Echotexture of contents	US
		Consistency of tissue	P
	Corpus hemorrhagicum	Presence	P, US
	Corpus luteum	Presence/absence, size, shape, echotexture	US
	Follicles	Size	P, US
		Shape, wall thickness, echotexture; echotexture of contents	US
		Turgidity	P
	Uterus	Diameter, shape, tone, wall thickness, endometrial folds, luminal fluid (larger amounts)	P, US
		Wall echotexture, endometrial folds, luminal fluid volume, echotexture	US
	Cervix	Length, width, depth	P, US
		Echotexture	US
		Relaxation	P
Vagina and vestibule		Fluid accumulation	P, US

One should take a methodic approach to palpation of the genital tract. The author normally locates the uterus and palpates structures in the following sequence: right uterine horn, right ovary, right uterine horn, left uterine horn, left ovary, left uterine horn, uterine body, and cervix.

One assesses cervical size and consistency by pressing the cervix down against the pelvic floor. One can measure cervical relaxation most effectively on a sliding scale from 0% relaxed (tightly closed, tubular cervix) to 100% relaxed (indistinguishable from adjacent uterus).[3]

Purposeful assessment of anatomic structures other than the genital tract as part of the palpation examination, including pelvis, intestines, lymph nodes, and bladder, is important. One should note the presence of soft tissue or bony changes that may narrow the luminal diameter of the pelvis. Possible causes include vaginal lacerations, hematoma, abscessation, neoplasia, and pelvic fracture.

One uses the same basic approach for ultrasound examination. While holding a linear transrectal ultrasound probe, one can extend the fingertips beyond the ventral surface of the probe to assist in locating genital tract structures and to palpate uterine horns to assess uterine tone. One initially identifies the uterine body as a landmark by sweeping the probe from side to side in the anterior pelvis. One then follows the uterine body cranially to the bifurcation and sweeps the probe out toward the right uterine horn and right ovary. One examines the structures in the same order as for palpation. As one withdraws the probe into the pelvic cavity, one examines the uterine body by moving the probe from side to side.

One should use a simple recording system and should note the uterine horn diameter about one third of the distance from the uterine body. One also should note uterine tone, along with presence of dilations and fluid. One can assess endometrial folds by gently palpating the uterus between fingers and thumb and can assess dimensions and shape of each ovary to ensure that the ovulation fossa is detectable (loss of ovulation fossa can be an early indicator of distortion of the normal ovarian shape caused by neoplasia). One records all follicles larger than 15 mm in diameter individually, records smaller follicles as multiple small follicles, and records loss of turgidity for preovulatory follicles (softness being a possible indicator of impending ovulation). One should consider ultrasound examination to be a routine part of a reproductive tract examination. Ultrasound examination allows the use of electronic callipers for measuring structures and allows assessment of tissue and fluid characteristics. Table 16.2-2 gives comparative information on the application of palpation and ultrasound for assessing various genital tract structures.

16.2.6 Evaluation of the Ovaries

One should suspect abnormal ovarian enlargement if an ovary is greater than 10 cm in diameter and when one cannot attribute the increased size to recent ovulationor a follicle and enlargement persists for more than a month.[3,4] Although an enlarged ovary may be associated with pathologic processes (neoplasia,[5,6] abscess), 1032
more often enlargement has physiologic causes (persistent follicles,[7] hematoma, multiple corpora lutea). Small and inactive ovaries may follow seasonal anestrus, reproductive senescence in old mares, pituitary gland dysfunction, severe malnutrition, administration of anabolic steroids, or congenital infertility caused by sex chromosome abnormalities.[8] Sex chromosome abnormalities often are associated with hypoplasia or segmental aplasia of the tubular tract. Karyotyping may be useful to investigate such mares further. Chromosomal abnormalities alsohave been reported from subfertile mares that have foaled previously and have no detectable abnormalities of the genital tract. With this in mind, one may consider karyotyping in mares for which routine diagnostic procedures fail to explain subfertility. Diagnosis and treatment of specific ovarian disorders are discussed elsewhere in this chapter. 1033

16.2.7 Evaluation of the Uterus

Ultrasonography is useful for evaluating presence, quantity, and echotexture of uterine luminal fluid. Fluid echotexture correlates with the amount of debris or white blood cell infiltration into the fluid. Table 16.2-3 shows a grading system for recording uterine fluid echotexture.[9] As a result, the presence of an amount of grade I, II, or III fluid during any stage of the cycle indicates endometritis. Table 16.2-4 shows a grading system for recording the volume of intrauterine fluid.

Complete absence of detectable uterine luminal fluid during estrus is associated with absence of cytologic inflammation in 99% of cases.[10] Small or minute volumes of clear fluid during estrus are likely to be normal, particularlyif present during early estrus before complete cervical relaxation and maximal uterine drainage.

Large volumes of clear fluid during estrus, fluid that persists into late estrus, or smaller volumes during early diestrus suggest an increased predisposition to endometritis and reduced pregnancy rates compared with mares without fluid.[10,11] Luminal fluid visible more than 12 to 20 hours after breeding indicates mating-induced endometritis.[12,13] This condition recently has been reported in 10% to 15% of mares being bred on a commercial Thoroughbred stud.[14]

TABLE 16.2-3 Grading System for Recording Volume of Intrauterine Fluid

CLASSIFICATION	MAXIMUM FLUID DEPTH (mm)	GROSS DESCRIPTION
VS (very small)	1 to 2	Barely detectable
S (small)	1 to 5	Often focal
M (moderate)	5 to 20	Obvious fluid
L (large)	>20	Immediately apparent

TABLE 16.2-4 Classification System for Recording Uterine Fluid Echotexture

GRADE	ULTRASOUND APPEARANCE	GROSS APPEARANCE
I	White (hyperechoic)	Thick and creamy
II	Light grey	Milky
III	Black with white specks	Obvious sediment in fluid
IV	Black (anechoic)	Clear fluid

Discrete hyperechogenic reflections in the uterine lumen on ultrasonographic examination indicate air. Pneumouterus may be visible in normal mares soon after uterine treatment, insemination or breeding but should not be visible more than 48 hours after breeding or treatment. The presence of pneumouterus at times other than soon after breeding or uterine therapy indicates loss of integrity of physical barriers to contamination (vulva, vestibulovaginal sphincter, and cervix). Endometritis is discussed in detail later in this chapter.

Circumscribed, discrete collections of clear fluid within the uterine lumen indicate endometrial cysts or a conceptus. Cysts may occur on the endometrial surface or within the endometrium. Cysts may be single, discrete structures closely resembling a conceptus, or complex, compartmentalized structures with irregular borders. Cysts that protrude into or occlude the uterine lumen may reduce fertility by interfering with embryo mobility and maternal recognition of pregnancy.[15–17] Cysts within the endometrium may interfere with gland function.

Properties of an early conceptus that one can use to distinguish it from a cyst include movement between days 10 to 16 after ovulation, spherical symmetric appearance, rate of growth (cysts assumed to grow little if at all), and appearance of the embryo proper after about day 20. In addition, palpable changes in the tract occur that are consistent with early pregnancy (increased uterine tone and closed, elongated cervix) and that would not be expected to occur in a nonpregnant mare with endometrial or lymphatic cysts. Any association between cystsand reduced fertility remains unclear.[18] Large cysts or large numbers of cysts may interfere with embryonic movement, maternal recognition of pregnancy, and early placentation. Extensive glandular cystic

change also may affect uterine gland function adversely and compromise establishment or normal development in pregnancy.

Pyometra is a chronic condition with mucoid or mucopurulent material within the uterine lumen, withor without cervical occlusion. The condition does not depend on the presence of a corpus luteum as it usually does in other 1033
species and typically does not result in any systemic signs of illness. One may mistake pyometra for pregnancy 1034
on palpation because of fluid distention of the uterus, but one cannot diagnose the condition effectively with ultrasound.[19]

Adhesions and scarring involving the endometrium may result from infusion of irritating solutions into the uterus and perhaps from trauma associated with dystocia and its correction. Luminal adhesions involving the cervix have similar initiating causes. The role of chronic endometritis or iatrogenic causes such as mechanical curettage in uterine or cervical adhesions is less clear. Adhesions may reduce fertility, for example, by interfering with function of the uterotubal junction or with embryo movement and maternal recognition of pregnancy.[16]

Occlusive adhesions may result in accumulation of fluid in the proximal portions of the tubular tract, and one may detect this on palpation or ultrasound examination of the tract. More commonly, one detects adhesions during hysteroscopy.

Focal uterine lesions may respond well to surgical removal using a biopsy instrument or laser surgical equipment guided by hysteroscopy. If lesions are extensive or involve the deeper layers of the endometrium, then the prognosis may be poor and treatment may not be warranted. Cervical adhesions commonly are managed by manual disruption and then repeated application of topical ointment containing an antibiotic and a steroid in an attempt to prevent recurrence. In many cases, cervical adhesions tend to recur once treatment stops.

Ventral sacculation is associated with a loss of normal muscular contractile activity in the uterine wall with the uterine wall forming sacculations ventrally at the baseof one or both uterine horns. The condition is more common in older, multiparous mares and may indicate increased risk of endometritis caused by loss of the normal contractile mechanisms responsible for expelling uterine luminal fluid.

16.2.8 Intravaginal Procedures

16.2.8.1 HYGIENE

Good hygienic practice is essential in performing any vaginal procedure and particularly those procedures that involve penetration of the cervix. Hygiene is perhaps the simplest and most fundamental principle of good reproductive practice. One should bandage the proximal portion of the tail or enclose it in a plastic sleeve and tie it to the neck of the mare with a quick release knot or should have an assistant hold the tail out of the way. One then washes the perineal region with water and detergent and rinses thoroughly, preferably with a spray nozzle attached to a hose to deliver a constant supply of fresh clean water. One wipes just inside the vulval lips with damp cotton to remove any contaminating material; this is particularly important for mares with sloping vulval lips or if one has just performed a rectal examination.

Use of sterile, plastic sleeves for vaginal procedures is ideal. A practical alternative the author uses is to maintain a zip-lock bag of clean, plastic sleeves in a dust-free area to be used for vaginal procedures only.

One should use sterile lubricant and sterile equipment and should be meticulous about avoiding contamination of equipment or sleeves/gloves during any procedure.

16.2.8.2 VAGINAL SPECULUM EXAMINATION

One inserts a sterile, lubricated speculum into the vestibule in a slightly dorsal direction to avoid the urethral opening and pushes the speculum throughthe vulvovestibular sphincter, assessing sphincter integrity on insertion of the speculum. Single use, sterile, disposable specula are preferrable to a metal Caslick speculum for routine examinations because they reduce the risk of iatrogenic infection. In some cases, a self-retaining metal Caslick speculum may provide better visualization of the caudal vagina and vestibule.

Vaginoscopy is useful for detecting vaginal hyperemia, suppurative exudates, persistent hymen, urine pooling, varicose veins, vaginal trauma, rectovaginal defects, and cervical defects. Although one may perform vaginal speculum examination at any stage of the cycle, inspection of mares suspected of pooling urine is performed best during estrus because estrogenic relaxation of the genital tract and perineal region is maximal at this time.[20] Vaginal speculum examination is an important component of the breeding soundness examination but should not be considered a routine procedure to be performed repeatedly in normal mares. Repeated vaginoscopy may be associated with increased risk of iatrogenic vaginitis and persistent infection. As a result, the author prefers to assess cervical relaxation routinely by transrectal palpation.

16.2.8.3 MANUAL EXAMINATION OF THE VAGINAAND CERVIX

Examination of the genital tract is incomplete without manual exploration of the vagina and cervix. The major benefit in this procedure is detection of cervical defects. Once one inserts the hand into the anterior vagina, one places the forefinger into the cervical lumen and the thumb in the vaginal fornix. One then rotates the hand and palpates the entire circumference of the cervix between finger and thumb to feel for disruption in the cervix caused by lacerations or damage to submucosal layers that might interfere with the ability of the cervix to close effectively. Cervical disruption is only significant if it prevents the cervix from forming an effective seal during diestrus and pregnancy. A partial tear involving the external os of the cervix 1034
may not require treatment if the cervix can maintain an effective seal. For this reason, one should assess the 1035
severity of cervical disruption when the mare is in diestrus.[21]

One should take care while performing a manual examination of the vagina and cervix to palpate thevaginal surface for problems such as rectovaginal fistulae, vaginal lacerations, and perivaginal hematoma or abscess formation.

16.2.8.4 CULTURE OF THE UTERINE SECRETIONS AND ENDOMETRIAL CYTOLOGIC EXAMINATION

One should not perform any procedure that involves penetrating the cervix of a mare until one has confirmed the nonpregnant status of the mare by transrectal palpation alone or along with ultrasonography.

A variety of instruments and techniques have been described for culturing the genital tract. The lack of consistency in methodology almost certainly has contributed to the lack of consensus regarding the role and interpretation of genital tract cultures.

One may take culture samples from multiple sites within the genital tract, including endometrium, cervix, vagina, clitoral fossa, and clitoral sinuses. One should take samples for genital tract culture to investigate possible endometritis or as part of a BSE from the endometrial lumen and not from the vagina or cervix. One should take an endometrial culture sample before any other invasive procedure in a BSE to avoid the risk of inadvertent contamination of the uterine lumen before taking the sample. One should use a guarded culture rod to minimize the risk of contamination with material from sites other than the uterine lumen,[22] and one may use single- or double-guarded culture rods. Taking an endometrial culture from a uterine biopsy sample is also possible as long as one takes the culture sample in a sterile manner and before placing the tissue into fixative. Consensus is lacking on the optimal time of the cycle for taking endometrial culture and cytologic samples. Some support exists for performing endometrial cultures on the first or second day of estrus when uterine secretions are increasing and the flushing action of the uterus is just beginning.[23,24] Mares with endometritis have been reported to accumulate free luminal fluid in late diestrus, and this represents an alternative time for endometrial culture.[25]

One should carry the guarded culture rod into the vagina and through the cervix beside one finger and then pass it cranial to the finger into the endometrial lumen, pushing the culture tip through the guard and rolling it against the endometrial surface for 30 to 60 seconds. One withdraws the culture tip into the rod before withdrawing the culture rod to avoid contamination during removal from the genital tract.

One should transfer the sample onto final growing medium immediately after completing the culture to allow quantification of colony numbers as an indication of severity of infection. Where possible, colony growth should be quantified because heavy growth after 24to 48 hours of incubation is more meaningful than afew scattered colonies. If immediate plating is not possible, one should transfer the culture tip in a sterile manner into appropriate transport medium to transport the sample to a laboratory. Use of transport medium allows proliferation of some species and possibly reduces growth of others, so colony counts are no longer interpretable.

Interpretation of endometrial culture results is difficult. False-positive and false-negative results are common. Several recommendations to minimize the likelihoodof an erroneous interpretation follow. One should interpret culture results along with results of concurrent endometrial cytologic examination. In addition, one should consider whether the organisms recovered represent pure or mixed growth and whether growth is heavy or light. Further work is required on technique and interpretation for endometrial cultures. For example, recovery of organisms in pure culture traditionally has been given more meaning than recovery from mixed cultures. However, recent evidence suggests more cases of acute endometritis are associated with mixed bacterial growth on culture than pure growth.[26]

A wide range of organisms have been isolated from acute cases of endometritis. A combination of positive culture and inflammation on endometrial cytologic examination is sufficient to diagnose endometritis and identify the organism(s) recovered as the causative agent(s). A positive bacterial culture in the absence of any cytologic evidence of inflammation is considered likely to be caused by contamination during the culture procedure and does not indicate endometritis.

In the absence of cytologic findings, interpretationof culture results is difficult. Recovery of pure or heavy cultures of the following organisms may indicate endometritis: β-hemolytic streptococci, hemolytic *Esherichia coli*, *Pseudomonas* spp., *Klebsiella* spp., and *Candida* spp.[27,28] One should view recovery of other organisms without concurrent cytologic information with more suspicion.

Some practitioners use endometrial cytologic examination as a rapid screening test to detect endometrial inflammation in mares at the beginning of the breeding season or before breeding. Numerous techniques have been described for collecting cytologic samples. The approach used by the author is simple, rapid, and feasible in busy clinical practice. One uses a guarded culture rod with a snap-on cap (Kalayjian, Kalayjian Industries Inc., Long Beach, California) to collect a culture sample. One withdraws the swab tip into the sheath and rolls the rod against the endometrium to collect fluid and cells inthe cap. After withdrawing the 1035
rod from the mare, one can cut off the cap and tap it against a slide to make the smear from the small drop of 1036
fluid.[29] The smear is dried and stained using any commercial staining kit; for example, Diff-Quik (American Scientific Products, McGaw Park, Illinois). If no endometrial cells are visible on an initial inspection of the slide, one may not have collected the sample from the uterus and one should take another sample. Researchers agree that the presence of polymorphonuclear leukocytes (PMNs) indicates bacterial endometritis, but less consensus exists on the most appropriate methodology for quantifying PMNs. Suggestions are that more than 1 to 2 PMNs in five high-power microscope fields (400×) and more than 1 PMN per 10 endometrial cells in more than one area of the slide may be significant.[30,31] In most cases of inflammation, large numbers of PMNs exist and the diagnosis is obviousand rapid.

16.2.8.5 ENDOMETRIAL BIOPSY

Preparation for endometrial biopsy is the same as for culture or cytologic examination. Diestral samples are generally preferable to estral samples because physiologic changes in the endometrium during estrus make the slides more difficult to interpret. However, one may take samples at any stage of the cycle as long as the information on the stage of cycle is provided and the person reading the slide is experienced at assessing equine biopsy slides. In the absence of clinically detectable pathologic findings involving the uterus, a single endometrial biopsy sample is representative of the entire endometrium.[32]

One introduces the sterile, mare biopsy instrument into the uterine lumen and guides it by one hand placed in the vagina. The traditional technique then involves withdrawing the hand from the vagina and inserting it into the rectum. One then can direct the instrumentto a specific site within the uterus, generally the base of one horn. One then turns the instrument on its sideand the gently opens the jaws. One presses a portion of endometrium between the jaws using the index finger within the rectum and closes the jaws to remove the tissue sample. A simpler vaginal technique is useful for collecting biopsy samples and does not require a sample from a specific site.[33] One introduces the instrument into the uterine lumen and leaves the hand in the vagina withthe index finger in the cervical lumen. One advances thetip of the instrument about 2 to 3 cm into the uterine lumen with the jaws closed, opens the jaws, and advances the instrument another 1 to 2 cm. One then can deviate the instrument slightly to one side and close the jawsto collect a sample. Using this approach, one takes the sample from the cranial uterine body close to the bifurcation. This approach has the advantage of being quick and simple and allows the operator to return for a second sample immediately if the first sample is too small, whereas the rectovaginal technique often results in gross contamination of the instrument and vulva during the procedure so that a second sample is often not possible unless the mare is cleaned again and a second sterile instrument is available.

One removes the specimen from the biopsy basket using a small-gauge needle and transfers it to fixative solution, preferably Bouin's fixative or alternatively 10% buffered formalin. One should transfer samples placed into Bouin's solution to 10% formalin or 80% ethanol after 12 to 24 hours for optimal retention of cell detail and tissue integrity.[34] Interpretation of endometrial biopsy results based on the Kenny system is discussed later in this chapter.[35–37] Endometrial fibrosis is a permanent, degenerative change occurring

around glands (fibrotic nests) or gland branches and also adjacent to the basement membrane of the luminal epithelium. Fibrosis often forms in concentric layers around gland branches or glands, and the number of layers of fibrosis is related to the severity of the degenerative change.[35] Paired biopsy samples, taken at the initial diagnostic workup and again 4 weeks after completion of uterine therapy, appear to improve the usefulness of an endometrial biopsy as a prognostic indicator of fertility in a mare. In one study, mares classified as grade III before treatment that improved to grade II after treatment achieved a foaling rate of 40%, whereas mares that were still grade III after treatment did not foal. Any improvement is likely to be in reversible pathologic changes such as inflammation. Fibrotic changes are irreversible.[38]

16.2.8.6 UTERINE ENDOSCOPIC EXAMINATION: HYSTEROSCOPY

Hysteroscopy allows direct visual inspection of the uterine lumen through a flexible fiberoptic endoscope. Hysteroscopy is indicated when other diagnostic procedures do not detect a cause for subfertility or for further examination of a mare with a suspected uterine luminal lesion.[39,40]

Tranquilization is a useful restraint aid, for the procedure is associated with a mild level of discomfort. One prepares the perineum as for other vaginal or uterine procedures. If the endoscope is sterilized by immersion in chemicals, rinsing with sterile water is essential, especially of the biopsy channel. Failure to rinse the equipment adequately could result in deposition of irritant chemicals into the uterine lumen. One carries the sterilized endoscope into the vagina, manipulates it through the cervix, and then advances it into the uterus. One occludes the cervical lumen by inserting one or two fingers alongside the endoscope or by gently holdingthe external os of the cervix around the endoscope. One distends the uterine lumen with saline or
water infused through a flexible catheter held adjacent to the endoscope or with air delivered through the 1036
endoscope. One should assess endometrial folds while the uterus is being distended because they flatten out 1037
and become difficult to distinguish in the fully distended uterus. One then manipulates the endoscope up each uterine horn, allowing visualization of the entire endometrial surface up tothe horn tip where the oviductal papilla should be. Hysteroscopy allows direct visualization of a variety of abnormal changes, including inflammation, polyps or neoplasia, cysts, adhesions, and severe scarring or fibrotic change. Hysteroscopy also can aid treatment of a number of conditions, including guided retrieval of foreign bodies and laser surgical management of conditions such as endometrial cysts or adhesions.

Excessive distention of the uterus may cause discomfort and elevated heart rate and should be avoided.[41] Mares subjected to hysteroscopy appear to be at risk of developing endometritis subsequently and examining the mare during the subsequent estrus period may be advantageous to check for evidence of endometritis.

16.2.9 Hormonal Assays

In the nonpregnant mare, one may collect serum or plasma samples to measure a variety of reproductive hormone concentrations including progesterone, estrogens, testosterone, inhibin, and gonadotropins. Other conditions that may have effects on cyclicity and fertility include abnormalities involving the thyroid and adrenal glands and pituitary gland neoplasia.

The ovarian luteal tissue produces progesterone. Serum progesterone concentrations are low during estrus and begin to rise within 12 to 24 hours after ovulation, peaking between days 5 to 10 after ovulation.[42] One can use progesterone assays in nonpregnant mares to assistin determining the stage of the cycle and to confirm that ovulation has occurred. One also can use the assays as an indirect method of pregnancy diagnosis, but false positives are common. Progesterone is necessary for pregnancy maintenance, and primary luteal insufficiency

has been linked with pregnancy loss. Support for this association comes from anecdotal descriptions of mares maintaining pregnancy while on progesterone supplementation despite previous histories of pregnancy loss. Authors have recommended that progesterone concentration in peripheral blood of pregnant mares be greater than2.5 to 4 ng/ml for normal pregnancy maintenance.[43] However, no evidence indicates that primary luteal insufficiency is an important cause of pregnancy loss. Single samples of peripheral blood concentrations are difficult to interpret because repeated sampling of normal pregnant mares has shown that blood levels vary widely over short periods of time.[44]

Thyroid gland dysfunction has been linked to subfertility in mares. Thyroidectomy of pony mares was not shownto have any adverse effect on reproductive performance, and the diagnosis of thyroid gland dysfunction as the cause of poor reproductive function remains controversial.[45] Horses with pituitary gland hypertrophy or hyperplasia (Equine Cushing's like disease), typically exhibit variable degrees of hirsuitism with a range of other systemic signs.[46] Diagnosis and treatment of this disorder is discussed in detail elsewhere in this text.

Ovarian neoplasia, particularly granulosa theca cell tumors, may be associated with increased production of testosterone and inhibin by the affected ovary. Serum inhibin concentration is elevated in approximately90% of mares with a granulosa theca cell tumor, and testosterone is elevated in about 50% to 60% of cases.[47] Horses with functional granulosa theca cell tumors almost invariably have a nonfunctional contralateral ovary and are anovulatory. Therefore a serum sample demonstrating elevation in inhibin or testosterone and low progesterone concentrations is consistent with ovarian neoplasia. Normal ranges in nonpregnant mares for inhibin and testosterone are 0.1 to 0.7 ng/ml and less than45 pg/ml, respectively.[6,47]

Measurement of estrogen concentration in blood, urine, feces, milk, and saliva has been used for monitoring the cycle,[48] determining pregnancy status,[49–51] and monitoring fetal viability.[52] Peripheral blood estrogens may be unconjugated or conjugated (bound to sulfates), and different forms of estrogens are found in the mare, particularly during pregnancy. Conjugated estrogens generally exist in the circulation at much higher concentrations than unconjugated forms, and measuring estrone sulfate or total conjugated estrogens appears to offerthe most use for the indications mentioned previously. A dramatic rise in peripheral blood estrone sulfate concentrations occurs in pregnant mares after day 60 of pregnancy, whereas in fecal assays this rise may take up to 150 days to occur.[53,54] A rapid decline of estrone sulfate concentration in a pregnant mare may be an indication of loss of fetal viability.[52]

Monitoring serum or plasma concentrations of gonadotropin-releasing hormone, gonadotropins (follicle-stimulating hormone and luteinizing hormone), and steroids appears to offer promise in the diagnostic assessment of the hypothalamic-pituitary-ovarian axis. Measuring these products appears to offer considerable future potential, but additional research and development are required before such assays can offer value to field practitioners. Currently, little information is availableon the existence of abnormalities in the hypothalamic-pituitary-ovarian axis, their potential relationship to subfertility, or their detection by measurement of various hormone concentrations. Practical difficulties exist in assessing the production and release of gonadotropin-releasing hormone or follicle-stimulating hormone and luteinizing hormone, because 1037
assessment may require cannulation of the pituitary venous sinuses.[55] In addition, these hormones are released 1038
in a pulsatile manner, with pulse frequency and amplitude influencing their ultimate effect.[56] Finally, varying isoforms of these hormones exist, each with a differing bioactivity that may be difficult to distinguish on enzyme-linked immunosorbent assay or radioimmunoassay.[57] Currently, measurement of these and other central nervous system hormones appears to be restricted to researchers.

16.2.10

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16.3 16.3—Mare Reproductive Pathology

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16.3.1 Vulva

The integrity of the vulvar lips and their anatomic relation to the perineal area and anus are an essential component of the fertility of a mare because they provide the first barrier to contamination between the external environment and the uterus. The endocrine patterns associated with each stage of the estrous cycle and pregnancy can influence the disposition of the vulva, affecting vulvar length and tone. In general, the vulva should have at least two thirds of its length below the pelvic brim, the slope of the vulva in relation to the vertical axis should not be greater than 10 degrees, and the vulvar lips should have an even and firm apposition. Absence (natural or acquired) of a normal perineal conformation can facilitate the entry of air (pneumovagina), feces, and potential pathogens into the reproductive tract, which jeopardizes the fertility of the mare.

16.3.1.1 BODY WEIGHT

Severe loss of body condition, as experienced by some pregnant mares not adequately supplemented during the winter, results in a sunken anus and an increased slopeof the vulva. An apparently normal perineal conformation noted in mares in good body condition may become less than adequate if loss of body weight is extreme. Contamination of the vagina with feces during mid to late gestation may lead to ascending bacterial placentitis, one of the leading causes of abortion and neonatal septicemia in the United States.[1]

16.3.1.2 PARITY AND AGE

Aging of the mare associated with repeated foaling can cause stretching and loss of tone of the perineal muscles that provide the ability of the vulva to form a barrier to external contamination and entry of air into the vagina. Injury to the mare during foaling also aggravates this condition, resulting in loss of vulvar tone and apposition of the labia. Mares undergoing episiotomy also may have permanent damage to vulvar structures.

16.3.1.3 CASLICK VULVOPLASTY

Surgical closure of the dorsal part of the vulvar labiais intended to correct poor perineal conformation. By decreasing the length of the vulvar cleft, one decreases entry of air and potential pathogens into the vagina 1039
ina mare otherwise susceptible to pneumovagina, fecal contamination, and associated complications. This 1040
procedure, however, is overused in mares in which such intervention is not warranted. Paradoxically, a mare with successive Caslick surgeries can experience considerable loss of vulvar tissue, thus generating an abnormal perineal conformation.

Another indirect complication associated with the Caslick vulvoplasty is the increased incidence of vulvar lacerations and dystocias in mares in which the vulva was not opened before foaling. Regardless of the originating cause, Caslick operation is used to correct first-degree perineal laceration affecting only the perineal skin and vulvar mucosa. Second-degree (laceration of deeper tissues of the perineal body) and third-

degree (a defect resulting in communication of the ventral rectumwith the dorsal vagina) lacerations require more elaborate reconstructive surgery to correct.[2] As a rule, one should delay any corrective surgery of the perineal body and vulva until inflammation and edema of the involved tissues has resolved. Mares with third-degree rectovestibular laceration invariably develop endometritis, but endometrial biopsy of mares affected with third-degree laceration have shown a rapid endometrial response to the surgical repair of the laceration; mares then can be artificially inseminated by 2 weeks after surgery.[3]

16.3.1.4

EQUINE COITAL EXANTHEMA

Equine coital exanthema is a venereal disease caused by equine herpesvirus type 3. Mares infected with equine coital exanthema develop pustules and ulcers in the vulvar mucosa and perineal area. Once the lesions start to heal (usually by 14 days after the onset of clinical signs), characteristic depigmented areas in the vulvar labia and perineal skin are visible.[4] Similarly to other herpesvirus-induced diseases, lifelong infection is the rule. Recrudescence after stimuli such as stress, systemic disease, or trauma to the genital area can occur. No specific treatment other than disinfection of pustules and ulcers to prevent secondary bacterial infections during the acute phase is recommended. Natural service should be avoided while active lesions are present to prevent transmission of the disease. One can artificially inseminate affected mares during the symptomatic stage of the disease or wait for 6 weeks after complete healing of equine coital exanthema–associated lesions before natural service.

16.3.1.5

NEOPLASIA

Melanoma is the most common disease affecting the vulva, affecting 80% to 100% of adult gray horses, and less frequently, aging horses of other colors. Common sites for melanoma include the anus, perineum, and vulva. No effective treatment is available for treating melanomas; however, oral treatment with cimetidine (a histamine$_2$ antagonist) has been reported with variable success to result in partial or complete regression of melanocytic nodules.[5] Occurrence of squamous cell carcinoma is less common than melanomas.

16.3.1.6

CLITORIS

The clitoris is enclosed in the clitoral fossa in the ventral part of the vulva but is rhythmically exposed following urination or teasing (winking) as a result of contractions of the vulvar constrictor muscle. The clitoris has several sinuses in which are found a natural smegma. Whereas the lateral sinuses are shallow, the median sinuses are deep enough to allow the growth of bacteria. The anatomy of the clitoris in the mare has become particularly important because the clitoris is an important reservoir for the bacterium *Taylorella equigenitalis* in mares affected with contagious equine metritis. The disease is highly contagious, and the organism can be harboredin the clitoral fossa and sinuses (especially the median sinuses) for prolonged periods. To test a mare for contagious equine metritis, one should swab the median clitoral sinuses and then seed an Amies charcoal medium to be transported (kept preferably at 4° C) to a diagnostic laboratory.

Even in contagious equine metritis–free areas, one must keep in mind that the clitoral sinuses may function as nidi for uterine infection, especially infection iatrogenically induced during diagnostic procedures of the reproductive tract or artificial insemination. Careful asepsis of the perineal and vulvar area, including the clitoral fossa, with a mild disinfectant before any invasive procedures are performed minimizes the risk of introducing potential pathogens into the uterus.

Congenital anomalies of the external genitalia occur in intersex animals that may have underdeveloped vulvar labia associated with an abnormally enlarged clitoris. Treating prepubertal mares with anabolic steroids may lead to enlargement of the clitoris, resulting in a partially and permanently exteriorized clitoris.

16.3.2 Vagina

The vestibule is the area that separates the vulva and clitoris from the vagina proper. At the cranial border of the vestibule, where it meets the vagina, lies the vaginovestibular fold. This folded mucous membrane actsas the second and most important[6] physical barrier between the uterus and the external environment. In young horses, the hymen is usually a weak membranous extension of the vaginovestibular fold. Occasionally, a persistent 1040
hymen may be present in a maiden mare. Usually, a manual examination of the vagina is enough to rupture the 1041
persistent hymen. Some mares with a persistent hymen may accumulate fluid in the vagina proper and uterus (mucometra). Once the persistent hymen is disrupted, evacuation of the fluid is uneventful.

16.3.2.1 PNEUMOVAGINA

Listening for an inrush of air into the vagina when one gently parts the vulvar labia can test the adequacy ofthe vaginovestibular fold as a physical barrier to external contaminants. A positive test (noticeable sound of air rushing in to the vagina) indicates that the vestibular fold is not properly restricting the vagina proper from the outside environment.

Improper functioning of the first barrier (vulva) and second barrier (vaginovestibular fold) may lead to the constant or frequent entry of air into the vagina. The condition can be exacerbated during estrus, when the perineal body is more relaxed than in other stagesof the estrous cycle. Accumulation of small amounts ofa frothy fluid in the cranial vagina may indicate pneumovagina, as may the presence of air in the uterus (pneumouterus) that is visible as hyperechoic particles between the endometrial folds during ultrasonographic examination.

In mares with severe alteration of the perineal conformation, vulvar closure using Caslick vulvoplasty may not correct the problem, and surgical reconstruction of the perineal body (perineoplasty) is recommended.

16.3.2.2 UROVAGINA

This condition, also known as vesicovaginal reflux or urine pooling, refers to the presence of urine in the cranial vagina and possibly in the uterus. As with pneumovagina, mares with marginal perineal conformation may be predisposed to accumulate urine in the vagina during estrus when reproductive organs and the perineal body are relaxed. In older mares with splanchnoptosis, the reflux of urine into the genital tract may be permanent. Urovagina can cause vaginitis, cervicitis, and endometritis, which ultimately result in infertility. Perineoplasty and urethral extension are common surgical procedures to correct this condition.

16.3.2.3 VARICOSE VEINS

During estrus and especially during pregnancy, varicose veins may develop in older mares. Varicose veins can be present in any part of the vagina; however, they often are found in the vaginovestibular area. Bleeding may occur after natural service or spontaneously during mid to late gestation. Occasionally, a persistent

hemorrhage results in considerable blood loss; however, the condition usually subsides with the end of pregnancy. Cautery or ligation of varicose veins is warranted if hemorrhage is persistent or frequent.

16.3.2.4

NEOPLASIA

Vaginal neoplasms are not common in mares. Leiomyomas and squamous cell carcinoma have been reported.

16.3.2.5

VAGINITIS

Minor tears, lacerations, and hematomas are common findings in the vagina of mares after foaling. Medical treatment generally is not needed, and most mares do not show complications in the postpartum period. If more serious trauma occurs, the mare may develop vaginitis. Mares with extensive vaginitis or with a large hematoma may show signs of pain and refuse to stand quietlyto nurse the foal. Vaginal abscesses can develop when a vaginal laceration becomes infected. Clinical signs caused by swelling from a hematoma or abscess include stranguria and straining to defecate. Treatment with antibiotics and antiinflammatory drugs is warranted when one finds extensive vaginitis or abscesses in the vagina. An abscess impinging on the urethra or interfering with defecation may require drainage. Occasionally, vaginal trauma may result in adhesions that can interfere with uterine drainage.

16.3.2.6

RECTOVAGINAL FISTULAE

During parturition, the foot of the foal may be directed toward the dorsal vagina/ventral rectum and if unattended may cause a third-degree perineal laceration. However, if the dystocia is corrected in time, the damage may be limited to a first- or second-degree rectovaginal tear or rectovaginal fistula. Surgical correction of the anatomic defect is necessary to restore fertility.

16.3.2.7

BREEDING TRAUMA

Vaginal lacerations may occur during natural service when the stallion size is disproportional to the mare. Based on the location of the tear in relation to the peritoneal reflection, a vaginal tear may communicate with the peritoneal cavity, likely resulting in peritonitis, or may be retroperitoneal. Treatment includes broad-spectrum antibiotics, antiinflammatory drugs, and tetanus prophylaxis. Peritoneal lavage may be beneficial if the tear communicates with the peritoneal cavity. A breeding roll positioned under the tail of the mare and dorsal to the penis of the stallion prevents the stallion from introducing the penis its full length into the vagina and consequently helps to prevent mating-induced trauma.

16.3.3

Cervix

The cervix is the last of the three physical barriers protecting the uterus from the external environment. Cyclic hormonal changes dictate the tonicity of the cervix. During estrus, the cervix is relaxed and open. High 1041
concentrations of progesterone during diestrus or pregnancy cause the cervix to be tubular, firm, and tightly 1042
closed. These changes are readily palpable per rectum. Moreover, a vaginal examination during estrus reveals the cervix positioned low in the cranial vagina and relaxed, easily dilated, and allowing access to the uterus. During diestrus or pregnancy, a vaginal examination reveals a tightly closed cervical os, pale and positioned high up off the floor of the vagina.

16.3.3.1 CERVICITIS

Inflammation of the cervix often accompanies vaginitis or endometritis. Cervicitis occurs most commonly in the postpartum period, especially after a dystocia. Severe cervicitis associated with metritis also may occur in mares infected with organisms such as *T. equigenitalis* that cause copious purulent discharge.[7] Infusion of certain chemicals such as chlorhexidine or strong iodine solutions into the uterus to treat endometritis may irritate not only the endometrium but also the cervix and vaginal mucosa. If one intends to use such solutions, vaginal speculum examinations between treatments to assess the condition of the cervix are warranted.

16.3.3.2 TRAUMA

Although cervical lacerations may occur during natural service, these lesions are usually small and resolve without major consequences. Occasionally, maiden maresare found in estrus with a tightly closed cervix, which may suffer laceration during natural service, especially if the stallion is disproportionately larger than the mare. However, these tears are usually small and heal without further treatment. Most serious lacerations occur during parturition. They may occur during normal parturition or can be iatrogenic during intervention to correct a dystocia by mutation or fetotomy. Although one should examine the cervix digitally after a difficult foaling or dystocia, especially if a fetotomy procedure is involved, one can evaluate the extent and severity of a cervical laceration best once the cervical lesion is healed. One should evaluate the competency of the cervix during diestrusor when the mare is under the influence of exogenous progestogens, thus verifying its ability to close tightly. One can more easily diagnose transluminal adhesions and anatomic defects by digital examination of the cervix rather than by vaginoscopy. Because surgical correction is difficult and not always rewarding, one should takea biopsy sample of the uterus to assess the potential of the mare to maintain a pregnancy before one attempts surgical correction of a cervical laceration.

16.3.3.3 POLYPS OR CYSTS

Occasionally, pedunculated cystlike structures are apparent on visual or manual examination of the cervix. These structures are attached to the cervical os or emanate from the cervical lumen and protrude into the vaginaor uterine body. Although of unknown cause, they do appear to be associated with infertility, and removal by laser or ligation is recommended.

16.3.4 Uterus

16.3.4.1 VENTRAL SACCULATIONS

Aside from changes associated with pregnancy, other pathologic conditions cause focal enlargements in the ventral portion of the uterus. These uterine changes usually are associated with increased age and parity and invariably are found at the base of either or both uterine horns, where pregnancies usually are established. Inexperienced palpators may mistake these enlargements for a pregnancy, especially if they do not use ultrasonography to back up their palpation findings. Mechanisms contributing to formation of ventral uterine enlargements have been identified, including but not limited to endometrial atrophy, focal myometrial atonia, and lymphatic lacunae.[8] Furthermore, in older and multiparous mares the uterus may tilt ventrally in relation to the pelvic brim (uterine splanchnoptosis). Mares with ventral sacculations and uterine splanchnoptosis have a higher incidence of delayed uterine clearance than normal mares.[9]

16.3.4.2

ENDOMETROSIS

Endometrosis has been referred in the past as chronic infiltrative endometritis and currently refers to the presence of fibrosis in the stromal and periglandular compartments. The degree of endometrosis is associated closely with the ability of a mare to establish and maintain a healthful pregnancy until term. Parity and age contribute to degenerative changes occurring in the endometria of mares.[10]

Fibrotic changes may occur around the endometrial glands and in association with the basement membrane in the stratum compactum. The amount and pattern of distribution of the fibrotic tissue has been classified descriptively as slight, 1 to 3 layers of periglandular fibrosis; moderate, 4 to 10 layers; and severe, more than 10 layers of periglandular fibrosis.[11] Cystic glandular dilation is another manifestation of endometrosis. Periglandular fibrosis, glandular epithelial hypertrophy, or inadequate lymphatic drainage may lead to dilationof the endometrial glands.

Other degenerative alterations in the endometrium that lead to endometrosis include lymphatic lacunaeand 1042
angiosis.[10,12] Lymphatic lacunae are histopathologic indications of lymphangiectasia. Angiosis (a vascular 1043
pathologic condition) is associated with aging and parity, especially in uteri with ventral sacculations and associated venous congestion, which are pathogenic factors for angiosis.[12] No treatments exist for these anatomic and vascular degenerative changes in the uterus. Mares with lymphatic lacunae and disseminated uterine angiosis are at risk for infertility caused by delayed uterine clearance and persistent mating-induced endometritis.[10,12]

Pregnancy loss attributed to endometrial fibrosis more commonly manifests during the embryonic period. Abortion during the early fetal period may occur, however, if uterine fibrosis interferes with implantation of the placenta. Microcotyledonary attachments begin to develop by 80 to 120 days of gestation.[13] Ultrastructural evaluation of the placenta in mares with chronic degenerative endometritis (endometrosis) has shown a delay in microcotyledon development and a reduction in the number of microcotyledons and villi per surface area. Endometrial atrophy may not result in abortion butcan influence fetal growth. Fetal weights in mares with degenerative endometritis were lower than in normal mares.[14] Evidence of endometrial atrophy may be evident on inspection of the placenta after delivery.[15]

16.3.4.3

ENDOMETRITIS

Whereas endometrosis reflects chronic structural changes associated with age and parity, endometritis encompasses endometrial changes associated with acute or chronic inflammation. These changes are modulated by the action of a local immune system and influenced by the hormonal milieu. A transient endometritis normally occurs in all mares that were mated naturally or artificially inseminated. Mares mount an inflammatory reaction in response to the presence of semen in the uterus, but this apparently normal inflammatory response subsides (histologically) within 2 to 3 days. Detection of intrauterine fluid by ultrasonography per rectum 24 hours after mating suggests delayed clearance. Persistent mating-induced endometritis is a clinical entity that has been recognized as a major cause of infertility in mares.

16.3.4.4 ENDOMETRIAL BIOPSY

An endometrial biopsy often is considered a routine part of a complete breeding soundness examination. Because an endometrial biopsy can aid in predicting the chances of a mare carrying a foal to term, one should consider the information provided by a biopsy before purchase or undertaking reproductive surgery such as repair of a cervical tear. Biopsies in some cases provide information that is useful in the diagnosis of infertility and may provide a basis for treatment. One must realize, however, that an endometrial biopsy alone is not the only, nor usually the most important, piece of information and must be taken in context with other information obtained from the history and reproductive examination.

Generally, one takes a biopsy specimen from a siteat the base of one of the uterine horns. When procuringa biopsy, one should take care not to obtain tissue from a site near the internal cervical os. Glands are less dense near the cervix, making a biopsy obtained from that area less representative of the uterus and more difficult to interpret. Moreover, accidentally taking a biopsy sample from the cervix can result in adhesions.

A single biopsy long has been considered to be representative of the entire uterus; however, studies have shown that variation by as much as an entire category may exist between sites.[16] Therefore one first should perform a thorough examination by palpation and ultrasonography to determine whether any areas of the uterus appear to be abnormal. If one detects an abnormal area, one should obtain biopsy samples from the abnormal and the normal areas. Repeated or multiple biopsies do not significantly affect fertility. A mare may become pregnant when bred just a few days after taking of a biopsy specimen.[17]

One may take biopsy samples at any time during the year or during any stage of the estrous cycle, becausethe cervix is dilated easily. Some clinicians prefer to obtain a biopsy sample during diestrus when the endometrial glands are under the influence of progesterone, whereas others recommend taking the sample during estrus because of the ease in passing the instrument through the cervix. One must relay all pertinent history, including the estrous stage during which the biopsy sample was obtained, to the pathologist reading the sample. Periglandular fibrosis may appear worse in biopsy samples taken during anestrus because of the sparseness of glands. In addition, biopsy samples taken during anestrus or transition may have evidence of increased inflammation because the cervix has been in a relaxed state for a prolonged period because of the absence of progesterone.

Endometrial biopsies are classified into four categories (I, IIA, IIB, III).[18] A mare with a category I biopsy has an essentially normal endometrium. The likelihood of the mare becoming pregnant and carrying a foal to term, estimated at 80% to 90%, depends more on broodmare management than on the inherent fertility of the mare. Mares with a category III biopsy have severe pathologic changes in the endometrium and an estimated 10% chance of carrying a foal to term even with good breeding management. Most mares are classified as a category IIA or IIB with an estimated 50% to 80% and 10% to 50% chance, respectively, of carrying a foal to term, reflecting a combination of management practices and the inherent fertility of the mare.

The pathologist usually provides a complete histopathologic description, but for the practitioner to review the biopsy slide is beneficial because this can be helpful in developing a therapeutic plan for the mare. The primary concern to a clinician is the severity and distribution of inflammation and the presence of 1043
degenerative changes such as periglandular fibrosis, angiosis, and lymphatic lacunae (enlarged and dilated lymphatics). Degenerative changes carry a worse prognosis than inflammatory changes because they are considered to be permanent and progressive. No effective treatment for these conditions has been identified. The cause of such degenerative conditions is not known but is presumed by many to be caused by repeated 1044

insults to the uterus. These conditions more commonly occur in older mares.[10] Dilated lymphatics often indicate a uterine clearance problem. However, one can diagnose delayed clearance more reliably by ultrasonographic examination in the postmating period.

Although biopsy can reveal the presence of an inflammatory condition, one must use other methods (suchas examination of perineal conformation) to reveal why the condition is present, and a culture is needed to identify the particular pathogen. A repeat biopsy after appropriate therapy may reveal the degree of success of treatment and aid in determining a prognosis for future fertility.[19]

16.3.4.5 ENDOMETRIAL CYSTS

Endometrial cysts often are cited as a cause of infertility; however, a cause-and-effect relationship has not been established clearly. The proportion of mares with endometrial cysts increases with age. Mares more than 11 years of age are more than 4 times as likely to have endometrial cysts as are younger mares, and most mares more than 17 years of age have endometrial cysts. Reports that associate endometrial cysts with a lower pregnancy rate or increased embryonic loss fail to account for the effect of advancing age. When one controls for confounding effects such as parity and age, the assumption of cysts causing infertilityis not supportable. When confounding factors were accounted for in the analysis of nearly 300 mares, endometrial cysts did not have a statistically significant effect on establishing or maintaining pregnancy, although the time of initial pregnancy diagnosis was not controlled strictly.[20] Another report by a different group of researchers who did control the time of pregnancy diagnosis similarly found no difference in pregnancy loss between mares with cysts versus those without, although mares with endometrial cysts tended to have a lower day 40 pregnancy rate. The effect of cysts on fertility appeared to be quantitative because an effect was not evident until a mare had numerous cysts or the cysts were large. However, even then the effect of endometrial cysts on fertility was much less than that with delayed uterine clearance or intrauterine fluid accumulation. A quantitative effect of endometrial cysts could be caused by interference with embryonic mobility. A well-known fact is that the equine embryo undergoes a period of mobility after entering the uterus, finally becoming fixed in place at approximately 16 or 17 days of gestation. If mobility is restricted during this period and the embryo is not permitted to contact a sufficient portion of the endometrium, maternal recognition of pregnancy may not occur, resulting in luteolysis and embryonic loss.

Rather than viewing endometrial cysts as a cause of infertility, one should consider them as an indication of underlying pathologic changes in the uterus. Endometrial cysts are of lymphatic origin, and their occurrence may be associated with a disruption of lymphatic function.

Endometrial cysts are diagnosed best with ultrasonography. One can identify cysts as hypoechoic, immovable structures with a clear border, as opposed to intraluminal fluid, which is movable and has a less distinct shape or border. Endometrial cysts are usually multiple and most commonly are found at the base of the uterine horns. Cysts may change in size and number between estrus and pregnancy.

Endometrial cysts can complicate early pregnancy diagnosis. Often an endometrial cyst can be similar in size and appearance to an early conceptus. Cysts that appear spherical often can be shown to have a more irregular shape if the ultrasound probe can be reoriented in relation to the cyst. To make early pregnancy diagnosis easier and more reliable, one should record the size and location of endometrial cysts using a diagram or by storing ultrasonographic images during a prebreeding examination. Even so, one may need to repeat the pregnancy examination or delay confirmation in some mares with endometrial cysts. In most cases of endometrial cysts, no treatment is necessary other than recording their size and location for future reference during pregnancy examination. However, if the cysts are sufficientin size or number that they pose

a potential threat to embryonic migration, treatment can be aimed at facilitating establishment of pregnancy by providing exogenous progestogen.

Progestogen, usually in the form of altrenogest (0.044 mg/kg p.o. daily), can maintain pregnancy even when the signal for maternal recognition of pregnancyis lacking. Numerous studies have shown the ability of altrenogest to maintain pregnancy after luteolysis orin ovariectomized mares. One must emphasize that if progestogen therapy is deemed necessary, the correct dose and frequency of administration is required or the effort is wasted. For example, once weekly injections of progesterone in oil or once a month administration of medroxyprogesterone are insufficient to maintain pregnancy and therefore would not be beneficial in mares with large or numerous endometrial cysts.

Alternatively, one may remove endometrial cysts surgically. Laser surgery is an ideal method if the equipment is available. Ligation and transection of the stalk of pedunculated cysts is an alternative. Merely 1044
puncturing and draining the cyst or incising its wall does not provide long-term remission. 1045

16.3.4.6 TRANSLUMINAL ADHESIONS

Severe infectious or chemically induced (following intrauterine infusion of irritating chemicals) endometritis may induce the formation of transluminal adhesions in the uterus. These lesions are asymptomatic and found during hysteroscopy. Uterine adhesions may cause the retention of endometrial secretions, resulting in mucometra or pyometra. Early embryonic motility, a phenomenon paramount for maternal recognition in horses, may be impaired by intraluminal adhesions. Treatment modalities include manual disruption of intraluminal adhesions or ablationvia laser surgery.

16.3.4.7 PYOMETRA

Pyometra is an accumulation of purulent exudate in the uterus. Unlike cows, mares with pyometra do not necessarily have a persistent corpus luteum and many may cycle normally. A cervical anatomic defect that prevents the clearance of fluid from the uterus may predispose mares to pyometra. However, this condition also can affect mares without an apparent anatomic defect in the reproductive tract. One can diagnose pyometra readily by transrectal ultrasonography when intraluminal fluid with moderate echogenicity is visible in the uterus. Because most mares with pyometra are brought to the veterinarian's attention at an advanced stage, degenerative changes such as endometrial atrophy may preclude mares from returning to normal fertility after treatment. One should examine a biopsy sample of the uterus before treatment to determine the prognosis for potential fertility. Although one can attempt medical evacuation of the uterus, hysterectomy is an option for mares refractory to treatment or with advanced degeneration of the endometrium (severe endometrosis).

16.3.4.8 NEOPLASIA

Uterine neoplasms are uncommon in mares. Leiomyomas, often referred to as fibroids, are benign mesenchymal neoplasms derived from smooth muscle and often are associated with the presence of fibrous tissue. Leiomyoma is the most common neoplasm affectingthe uteri of mares and if small does not necessarily cause reproductive failure. Leiomyosarcoma, lymphosarcoma, and adenocarcinoma are rare malignant neoplasms affecting mares.

One usually discovers neoplasms affecting the equine uterus when palpating per rectum and performing transrectal ultrasonography in broodmares during the breeding season. If one suspects the presence of a

uterine neoplasm, one should perform uterine endoscopy and take a biopsy sample of the tissue for final diagnosis. Surgical excision of neoplasms is indicated when extensive hemorrhage and endometritis are present or when the presence of the neoplasia would be incompatible with establishing a pregnancy. Prognosis for future fertility is reduced, but pregnancy has been reported in mares with partial hysterectomy.[21]

16.3.4.9 UTERINE LACERATIONS

Uterine lacerations can occur during unattended or assisted parturition. Palpation per rectum and per vaginum and abdomino-centesis aid diagnosis.[22] Adhesions involving the serosal surface of the uterusmay occur following cesarean section or uterine tears. No effective treatment, other than attempted surgical excision, exists once the adhesions form. Therefore one should take steps to minimize the likelihood of adhesion formation, including daily palpation of the uterus after cesarean section.

16.3.4.10 UTERINE ARTERY RUPTURE

Age and parity are contributing factors for mares atrisk for uterine artery rupture.[23] Uterine vessels undergo extensive dynamic remodeling during pregnancy and the postpartum period, and this is thought to contribute to progressive degenerative vascular changes. Intravenous administration of formalin or naloxone (a μ-opioid receptor antagonist) have been reported anecdotally to control hemorrhage after uterine artery rupture in postpartum mares. Recently, administration of safe doses of formalin to healthy horses has been shown not to alter any of the hemostatic variables analyzed.[24] If a mare survives an acute episode of rupture of the uterine artery, antimicrobial therapy is indicated to prevent the hematoma from becoming an abscess. Monitoring the mucous membranes and pulse for 30 minutes after foaling is indicated in the event the parturition was induced or assisted by a veterinarian, and especially when an intervention to correct a dystocia is performed.[25] In mares that survive a ruptured uterine artery, fertility is not altered, but mares may suffer fatal hemorrhage during the subsequent parturition.

16.3.5 Uterine Tubes

The function of uterine tubes is essential for normal fertility. The passage of equine embryos throughout the uterine tube is longer than for other domestic species, which underlines the importance of the tubal environment for normal fertility. Mares are also unique in that unfertilized oocytes are retained in the uterine tubes and are not transported to the uterus. The mechanism(s) accounting for this phenomenon (selective transport of fertilized versus unfertilized oocytes) has not yet been elucidated fully but appears to involve secretion of 1045

1046 prostaglandin E_2. A healthy uterine tube responds to embryonic signals, resulting in proper timing of tubal transport. Diagnosis of pathologic conditions in the uterine tubes is difficult and often occurs only in postmortem examinations.

Postmortem examination of reproductive tractsfound that salpingitis was common in mares; 37% had infundibulitis, 21% had ampullitis, and 9% had isthmitis. In that study, 50% of mares were more than 15 years old and 85% were more than 11 years old. The infundibulum generally was found adhered to the uterus, mesovarium, or ovary. The incidence of adhesions on the right side was significantly higher than on the left side.[26]

Postmortem analysis of uterine tubes has suggested that tubal patency is not a major problem in mares. Oviductal obstructions are less common in the mare than in the cow, although masses of collagen have been found in the oviducts of young maiden mares and pregnant mares and were observed more often in mares more than 7 years old.[27] An examination of 700 postmortem specimens, primarily from mares more than 11 years old, found that almost all oviducts were patent, although more than 40% had adhesions involving the infundibula.[26] Only one uterine tube was found to be occluded among 1248 pairs of uterine tubes that were flushed post mortem.[26,28] Based on these findings, diagnostic procedures to determine the patency of uterine tube in mares with unexplained infertility are not warranted.

16.3.6 Ovary

The presence of an enlarged ovary in a mare may be normal or may be an indication of a pathologic ovarian condition. Consideration of the various possibilities and careful diagnostic procedures are necessary to avoid surgical removal of normal ovaries. A thorough history, including changes in behavior, estrous cycle characteristics, sexual behavior, and the last observed estrus is important to consider. Ultrasonography, palpation, and hormonal assays are helpful in arriving at an accurate diagnosis. In some mares, sequential examinations are beneficial in determining changes in the size of ovaries or various structures on an ovary.

Discovery of an enlarged ovary may be an incidental finding during a normal reproductive examination or may be stimulated by specific clinical signs. Behavioral changes or signs of colic in a mare warrant examination of the reproductive tract, with special attention to the ovaries. Mares with a history of infertility often are suspected of having abnormalities of the ovaries and deserve a thorough examination before surgical removal of an enlarged ovary. One must take into consideration various factors such as season and pregnancy status when interpretinga finding of ovarian enlargement. Large ovaries may be normal during the transitional periods in the spring and fall and are expected during certain stages of gestation.

16.3.6.1 NEOPLASIA

Granulosa-theca cell tumors are the most common tumors of the reproductive tract in mares. They are benign sex-cord tumors that can occur in any age mare and have been reported in foals, aged mares, and pregnant mares. Although the granulosa and the theca interna cell layers may be involved, the granulosa cell layer most commonly is affected.

Behavioral changes are common in mares with granulosa cell tumors. Behavior may be stallionlike, persistent estrus, or persistent anestrus, depending on the steroid production of the tumor. In other cases of granulosa cell tumors, behavior may be unchanged yet the mare may be showing signs of abdominal discomfort, lameness, or anemia or other signs seemingly unrelated to the reproductive system. Stallionlike behavior is the most commonly reported behavioral change observed, possibly because the change from previous behavior is obvious to the owner and causes an increased challenge in handling the mare. In one report of 63 mares diagnosed with granulosa cell tumors, 20 exhibited anestrus, 14 were in persistent estrus, and 29 showed stallionlike behavior. Stallionlike behavior usually is associated with elevated serum testosterone. However, persistent estrus has not been correlated with elevated estrogen.

On palpation per rectum, the affected ovary is enlarged, whereas the contralateral ovary is typically small and inactive. Atrophy of the contralateral ovary canbe misleading during winter anestrus when ovaries are typically small and inactive. Atrophy of the contralateral ovary is not absolute. Although unusual, granulosa

cell tumors have been reported in pregnant mares and in cyclic mares with a functional contralateral ovary and even in both ovaries.[29] The enlarged ovary may be smooth or knobby, hard or soft, and may feel like multiple follicles are present. Typically, one cannot palpate the ovulation fossa on the enlarged ovary, although with any greatly enlarged ovary, the fossa may be difficult or impossible to palpate.

Ultrasonographic evaluation often shows the classic multiloculated appearance. However, sometimes the tumor may appear solid or with larger cystlike hypoechoic areas.[30] Although ultrasonography is a useful adjunct, it may not yield a definitive diagnosis in many cases. The ultrasonographic image of granulosa cell tumors can be similar to that of other ovarian abnormalities, especially ovarian hematomas. A variety of reported appearances make diagnosis based solely on ultrasonography impossible in many instances.

Mares with granulosa cell tumors may have elevated concentrations of estrogen or testosterone, but 1046
progesterone is almost always less than 1 ng/ml. Measurement of estradiol is of limited value. Although 1047
testosterone often is elevated in mares with granulosa cell tumors exhibiting stallionlike behavior, testosterone is within normal limits in 10% to 50% of cases. Testosterone in normal cycling mares is approximately 45 pg/ml and often is greater than 100 pg/ml in mares with stallionlike behavior. McCue reported that only 54% of mares with granulosa cell tumors had elevated testosterone, yet 87% had elevated inhibin, leading to the conclusion that inhibin is a better indicator of the disease.[31] Inhibin suppresses follicle-stimulating hormone, which leads to a decline in follicular growth, thus explaining the profound negative feedback effect on the contralateral ovary.

Overall, the prognosis for life and reproductive function in a mare with a granulosa cell tumor is good. Depending on the time of year when the ovary is removed, the individual mare, and the length of time the tumor has been present, resumption of ovarian activity usually occurs83 to 392 days after surgery, with a mean of 209 days. If the intended use of the mare is solely as a broodmare, a reproductive examination, including a uterine biopsy, is recommended before surgery.

Teratomas, although uncommon, are the second most common ovarian tumors. They contain at least two ifnot all three germinal layers. Most teratomas found in mares are benign. They usually contain hair and alsomay contain bone, teeth, and neural tissue. Teratomas are usually an incidental finding because most are small and do not often cause significant ovarian enlargement. However, on occasion, large teratomas develop that result in ovarian enlargement. Teratomas do not affect the estrous cycle and therefore lack obvious outward clinical signs.

Serous cystadenomas are neoplasms of epithelial origin usually found in older mares. These tumors do not metastasize. Although they have been found in mares with high plasma testosterone,[32] behavioral changes are not characteristic. The contralateral ovary is not affected, continues to have normal activity, and does not atrophy; and affected mares continue to cycle. On the affected ovary, the ovulation fossa is not obliterated and is palpable.

Dysgerminomas are highly malignant tumorsalso of germ cell origin. They metastasize rapidly tothe abdominal and thoracic cavities and are considered the counterpart of the testicular seminoma. Because of their nature, dysgerminomas can affect other organ systems, and cases have been reported of associated hypertrophic pulmonary osteoarthropathy.[33] Clinical signs on presentation therefore often are unrelated tothe reproductive system. These tumors carry a poor prognosis.

16.3.6.2

OVARIAN ABSCESS

Ovarian abscesses often are attributed to procedures involving puncture of the ovary, such as biopsy or follicle aspiration. As assisted reproductive techniques became more successful and therefore more popular, the incidence of ovarian abscesses was thought likely to increase. However, this has not proved to be the case. Moreover, not all ovarian abscesses can be attributed to iatrogenic causes. Ovarian abscesses have been reported in mares that have had no such procedures performed on them. In these cases, they are likely caused by the hematogenous spread of bacteria or may be associated with strongyle migration.

Affected mares may be febrile and anorectic with an elevated white blood cell count. On ultrasonographic examination, the enlarged ovary typically has a thick-walled, fluid-filled structure. The fluid is usually hyperechoic. Medical management with long-term antibiotic therapy has been successful in treating these cases. Surgical removal of the affected ovary is an alternative treatment, but one must take care that the abscess does not rupture in the abdominal cavity.

16.3.6.3

OVARIAN TORSION

Ovarian torsion, a condition not uncommon in women, has been reported in a mare with a large granulosa-theca cell neoplasm and showing signs of abdominal discomfort.[34] One may suspect ovarian torsion in mares with known ovarian enlargement, if sudden signs of abdominal pain develop.

16.3.6.4

NONNEOPLASTIC OVARIAN ENLARGEMENT

An ovarian hematoma forms as hemorrhage occurs into the previous follicular lumen after ovulation. On occasion, this hemorrhage can be excessive, possibly because ofan anticoagulant in follicular fluid. Hematomas can be large, up to 20 cm in diameter or more. They are able to produce progesterone and do not affect the estrous cycle. Although the ovulation fossa is still present, palpating it can be difficult if the hematoma is large. The ultrasonographic appearance can vary, causing confusion with a granulosa cell tumor. One may observe large, fluid-filled cavities, or the hematoma may have a more solid appearance, sometimes with fibrin strands. A trait that is at times useful in differentiating a hematoma from a granulosa cell tumor is the responsiveness to prostaglandin. Hematomas that are at least 5 or 6 days old often respond to prostaglandin by decreasing in size because of the luteolytic effect. Granulosa cell tumors do not respond to prostaglandin treatment with any change in size, shape, or ultrasonographic appearance. Because the follicle wall still undergoes luteinization despite the presence of the hematoma, cyclicity remains unaltered, 1047
thus normal fertility is not compromised. However, the enlargement of the ovary because of the presence of a 1048
hematoma may persist for several estrous cycles even though the life span of the luteal tissue is normal. Because this structure isa postovulatory phenomenon, the oocyte has been released and the mare may become pregnant if she was mated.

Anovulatory follicles most commonly occur in mares near the end of the breeding season as they go through the autumn transition. These follicles grow to an unusually large size (70 to 100 mm) yet fail to ovulate. Instead, they fill with blood and develop a gelatinous consistency. A thick (compared with a normal follicle) wall commonly forms. The follicles become firmer and then regress over time, usually disappearing within a month. With ultrasonography one observes free-floating echogenic spots in the antrum of the follicle that increase in number as the follicle grows. When the follicle stops growing, the contents become organized with an echogenic appearance and fibrin strands. Formation of luteal tissue around the periphery of the

anovulatory follicle is usually minimal in a true autumnal follicle that occurs at the end of the season. The cause of anovulatory follicles is unknown, although they are hypothesized to be caused by changes in the hormonal status of the mare that occur with autumn transition. This hypothesis does not explain the occasional occurrence of anovulatory follicles during the breeding season, however. Luteinized anovulatory follicles, although unusual during the ovulatory season, most often occur in older mares and may be associated with senility. Their response to prostaglandin varies.

During certain periods of gestation, ovarian enlargement is normal and should be expected. Ovarian enlargement is associated with increased follicular activity and subsequent ovulation (secondary corpora lutea) or anovulatory luteinization (accessory corpora lutea). An increase in follicular growth begins before day 20of gestation. New corpora lutea form around day 40 of gestation. Corpora hemorrhagica and hemorrhagic follicles commonly occur from day 40 to 60. Maresbred early in the season have greater follicular activity during the first 4 months of gestation than mares bred after July.

During the spring as mares undergo transition from winter anestrus to the normal ovulatory season, they undergo periods of prolonged anovulatory follicular development. These periods are characterized by the development of numerous follicles, at times large, on either ovary or usually both ovaries. The follicles may persistfor varying lengths of time and then regress, and new follicles develop. This period lasts for a variable time depending on the mare, photoperiod, and other undetermined factors. The ovaries can be large during this time and mistakenly may be called cystic. No treatment is necessary, although a combination of progesterone and estrogen often is used to suppress follicular activity in an attempt to hasten the onset of ovulation and normal cyclicity. The transition period ends with the first ovulation of the season, after which follicular activity and ovarian size return to normal.

Small and inactive ovaries normally are found in mares in deep anestrus, prepubertal mares, and pregnant mares in the last third of gestation when, curiously, the fetal gonads are larger than the ovaries of the dam. Mares subject to severe malnutrition, mares of advanced age, mares treated with anabolic steroids, and mares with chromosomal alterations leading to gonadal dysgenesis may have abnormally small and inactive ovaries.

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16.4

16.4—Endometritis and Uterine Therapy

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Endometritis is a major problem facing veterinarians treating studs and attempting to maximize per cycle conception and foaling rates. Recent advances have improved understanding of the pathogenesis of endometritis and have resulted in more effective methods to minimize the affect on fertility. Endometritis has been categorized into persistent mating-induced endometritis, chronic infectious endometritis, chronic degenerative endometritis, and sexually transmitted diseases.[1–3]

Failure of mechanical clearance of fluid, debris, and inflammatory by-products from the uterine lumen is recognized as the major predisposing factor associated with development of infectious endometritis.[4–6] Diagnosis of infectious endometritis is based on one or more of the following: ultrasound detection of echogenic uterine luminal fluid and acute inflammatory change on endometrial cytologic examination or biopsy along with a positive endometrial culture. One may suspect the condition in mares with cervical, vaginal, or vulval discharge during estrus.

Diagnosis of chronic degenerative endometritis is based mainly on observation of fibrotic changes in endometrial biopsy samples and is most commonly a condition of older mares. Mares may have a history of failing to conceive over successive cycles or seasons, recurrent or chronic endometritis, and pregnancy loss before 60 days.

Sexually transmitted diseases include contagious equine metritis *(Taylorella equigenitalis)*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Susceptible mares bred to a contagious, bacteria-shedding stallion with contagious equine metritis usually show shortened interovulatory intervals with a copious mucopurulent genital tract discharge associated with vaginitis, cervicitis, and endometritis. Diagnosis depends on recovery of the causative organism from culture samples taken fromthe uterus, cervix, clitoral fossa, and clitoral sinuses. The organism is difficult to culture and requires careful sampling, specific transport media, chilling during shipment, and appropriate incubation environment in a laboratory skilled at microbiologic procedures. The veterinarian should speak to the regional laboratory before culturing a mare for suspected contagious equine metritis. *P. aeruginosa* and *K. pneumoniae* are present in most equine breeding populations as occasional genital tract

pathogens withthe possibility of sexual transmission. Diagnosis and treatment in the mare is as for other causes of infectious endometritis, though owners should withhold mares from natural breeding until the infection is eliminated. 1049

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One should base the choice of uterine therapies for endometritis on the same fundamental principles as for pharmacotherapy in other body systems. The anticipated therapeutic benefit should outweigh the potential risks associated with the disease and the treatment. Historical use of a particular treatment does not justify continued use unless clear evidence documenting efficacy and safety accompanies the treatment. Many of the therapies described in this section are based on empirical evidence alone.

Drug preparations must be nonirritating if they areto be used in the genital tract of the mare. Almost any substance, including physiologic saline, induces an inflammatory response when introduced into the uterus of the mare.[7] Understanding this is particularly important when one treats the mare around the time of breeding, because an inflammatory response that is severeand that persists for longer than 5 days after ovulationis likely to interfere with embryo survival once the embryo enters the uterine lumen at about day 6 after ovulation.

16.4.1 Antimicrobial Therapy

The use of antimicrobial agents to treat and prevent endometritis is based largely on empirical evidence. Pharmacokinetic studies on which to base recommendations regarding choice of drug, route, dose, and frequency of administration are few. Table 16.4-1 shows recommended doses for various preparations commonly infused into the uterus of the mare.

16.4.2 Intrauterine Versus SystemicAntibiotic Therapy

Antibiotics, which have been recommended as possible systemic treatments for equine endometritis, include amikacin sulfate, ampicillin trihydrate, gentamicin sulfate, procaine penicillin, and trimethoprim-sulfadiazine. Dose rates, routes, and frequency of administration usually follow published guidelines for administration of these drugs for other conditions (see Chapter 4 for a detailed discussion).[8]

Several hypothesized advantages have been associated with systemic antibiotic therapy as opposed to intrauterine infusion. Parenteral administration eliminates the risk of bacterial contamination of the reproductive tract associated with repeated intrauterine infusions. Uterine tissue concentrations of ampicillin and enrofloxacin are higher than serum concentrations after intravenous or oral administration of the drugs.[9,10] Some drugs may be associated with an adverse local tissue reaction when administered into the uterine lumen and yet not have any adverse effect on uterine tissue when administered systemically. For example, intrauterine infusion of enrofloxacin has been linked to uterine pathologic conditions including adhesion formation. Enrofloxacin has been associated with cartilage and skeletal damage in young, growing horses and with joint effusion and mild cartilage lesions in adult horses following systemic use.[10] Nontheless, mares receiving enrofloxacin orally (5 mg/kg every12 hours p.o. for 21 days) had endometrial tissue concentrations higher than those observed in serum, and all tissue levels of drug exceeded minimum inhibitory concentrations (MICs) for common equine pathogens. Further work is required before this product can be recommended as a treatment for endometritis.

Little documentation exists of efficacy of systemic therapy in treating endometritis. Authors have suggested that systemic therapy be considered in cases in which deeper layers of the uterus are infected or inflamed, asin

the case of postpartum metritis.[3,7] The author routinely uses systemic antibiotic therapy only in cases in which the mare shows systemic signs of illness associated with the reproductive tract.

Most uterine infections in the mare are limited to the lumen and the superficial endometrium. Local uterine therapy via uterine infusion is favored for treating such conditions because it is considered likely to result in high antibiotic concentrations within the lumen and superficial layers of the endometrium. In addition, uterine infusion of antibiotic is often more economical than systemic therapy.

16.4.2.1 CHOICE OF ANTIBIOTIC

Factors to consider in choosing an antibiotic to use in the uterus include types of organisms isolated, antibiotic sensitivity patterns of isolated organisms, and potential efficacy of the drug in the uterus. Antibiotic selection should be as specific as possible and should be based on microbiologic isolation of the causative organism(s) by uterine culture, followed by sensitivity testing.[11] The use of broad-spectrum antibiotics in the absence of culture and sensitivity results is discouraged because indiscriminate use of antibiotics is considered likely to increase the risk of adverse sequelae such as resistance and superinfection with different bacterial strains or with fungi or yeast.[7] Use of antibiotic combinations also may be inappropriate because of the risk that incompatibilities will reduce efficacy. An example is the suggested inactivation thought to occur when β-lactam antibiotics such as penicillin are combined with aminoglycosides such as gentamicin.[12]

However, a strong argument also exists that the first choice of antibiotic for intrauterine use should be a broad-spectrum combination of drugs aimed at successfully treating all components of the mixed aerobic and anaerobic infections that commonly occur.[13]

In cases in which culture and sensitivity results arenot available, one should base the choice of drug on the 1050
expected bacterial organisms involved and the predicted antibiotic susceptibility patterns.[12] 1051

TABLE 16.4-1 Recommended Doses of Intrauterine Drugs

DRUG	DOSE	COMMENT
Amikacin sulfate	1–2 g	Gram-negative microorganisms
Ampicillin	1–3 g	
Carbenicillin	2–6 g	*Pseudomonas*
Ceftiofur	1 g	Broad spectrum
Gentamicin sulfate	0.5–2 g	Excellent gram-negative
Kanamycin sulfate	1–2 g	*Escherichia coli;* toxic to sperm
Neomycin	3–4 g	
Nitrofurazones	50–60 ml	Questionable efficacy
Penicillin (Na or K salt)	5,000,000 IU	Streptococci
Penicillin G procaine	3,000,000–6,000,000 IU	Streptococci
Polymixin B	40,000–1,000,000 IU	
Ticarcillin	1–6 g	Streptococci, *E.coli, Pseudomonas;* poor against *Klebsiella*
Trimethoprim-sulfadiazine	120 mg	
COMBINATIONS		
Neomycin	1 g	
Polymixin B	40,000 IU	
Furaltadone	600 mg	
Penicillin (Na or K salt)	3,000,000–5,000,000 IU	
Neomycin	2 g	
Penicillin G procaine	3,000,000 IU	
Gentamicin sulfate	0.5–2 g	
Penicillin G procaine	3,000,000–6,000,000 IU	
ANTIFUNGAL THERAPIES		
Nystatin	250,000–1,000,000 IU	Mix with sterile water. Precipitates in saline.
Amphotericin B	200–250 mg	
Clotrimazole	300–600 mg	Every 2 to 3 days for 12 days
Clotrimazole cream	500 mg	Daily infusion for 1 week
Vinegar	2% solution	20 ml wine vinegar in 1 L saline
Povidone-iodine	1%-2% solution	
IRRITANTS		

Chlorhexidine	1%-2% of stock solution	
Dimethyl sulfoxide	5% solution	Infuse 50 to 100 ml.
EDTA-tris	1.2 g EDTA plus 6.05 g tris per 1 L water	Titrate to pH 8.0 with glacial acetic acidic. Infuse, wait 3 hours, and then infuse antibiotic.
Hydrogen peroxide	1:4 dilution with saline	
Kerosene	50 ml	Avoid reflux through cervix.
Povidone-iodine	0.05% solution	
Saline	Variable volume	Consider warming to 45 to 50 C.
Streptococcal cell–free filtrate		
Modified from Perkins NR: Equine reproductive pharmacology, *Vet Clin North Am Equine Pract* 15(3):687–704, 1999.		

One may isolate virtually any bacterial species from acute endometritis. Several studies have reported that 70% to 80% of acute, aerobic endometritis cases were associated with *Streptococcus zoopepidemicus*, *Escherichia coli*, *P. aeruginosa*, and *K. pneumoniae*.[12,14,15] Large-scale data from routine endometrial cultures in the United Kingdom appear to show a slightly different pattern of bacterial involvement. Of 3414 isolates associated with cytologic evidence of endometritis, 38% were recovered in pure culture and 62% in mixed culture.[16] The role of anaerobic bacteria in endometritis remains unclear. One study has reported that in 18 of 71 (25%) mares in which endometrial cytologic tests and cultures were positive, an anaerobe was the sole isolate cultured. The most common anaerobe recovered was *Bacteroides fragilis*.[17] Others have suggested that anaerobes are unlikely to play a significant role in endometritis.[18]

No single antimicrobial therapy is effective against all of the commonly isolated uterine pathogens. β-Lactam antibiotics (penicillins and cephalosporins) are effective against streptococci. Synthetic penicillins such as 1051
1052 ampicillin and ticarcillin are also effective against many strains of *E. coli* and *P. aeruginosa*. Aminoglycosides are used primarily to treat gram-negative organisms. Bacterial sensitivity patterns often vary, particularly for gram-negative organisms, and accurately predicting sensitivity patterns is not possible in many cases.[12]

The difficulties described in identifying the causative agent in cases of endometritis and in predicting antibiotic sensitivities and the time constraints of a short breeding season mean that in most cases, broad-spectrum antibiotics are used as a first-choice treatment. In many cases, use of a combination of drugs achieves a broad spectrum of activity.

Commonly used broad-spectrum antibiotics include ticarcillin, ampicillin, and drug combinations such as penicillin/aminoglycoside and penicillin/furaltadone/neomycin/polymixin B (Utrin Wash, Univet Ltd, Wedgewood Road, Bicester, UK). Recently, ceftiofur sodium (1 g by intrauterine administration per day for 3 successive days) was used in clinical trials as a broad-spectrum, intrauterine treatment for equine endometritis and was considered to be an effective and safe alternative to the combination of penicillin, neomycin, polymixin B, and furaltadone.[13]

16.4.2.2 ANTIBIOTIC FORMULATION, TREATMENT FREQUENCY, AND VOLUME

Little evidence is available on which to base recommendations regarding dose rate, formulation, volume of drug infused, diluent, and frequency of treatment. Ideally, one should base dose regimens on MIC data along with pharmacokinetic studies of drug disposition following intrauterine administration. In the absence of any information, dose rates apparently are based on the recommended parenteral dose rate for the drug in question. In many cases the dose rate recommended for intrauterine use is lower than the systemic dose. In some cases this is based on clinical evidence, whereas in others it appears to be more a response to convenience or cost. Use of a 2-g dose of amikacin sulfate per intrauterine infusion appears to produce optimal results in mares experimentally inoculated with *K. pneumoniae*.[19,20] Intrauterine treatments are performed commonly every day or every second day for 3 to 7 days. Once daily treatment with 3 g ampicillin has been shown to maintain uterine levels above MIC levels for common equine uterine pathogens for the entire 24-hour period between treatments.[21] Possibly, maintaining uterine antibiotic levels above MIC forthe duration of the time interval between successive treatments is not necessary because exposure of bacteria to large doses of antibiotic for brief periods of time has been reported to increase the susceptibility of bacteria to leucocyte killing.[22] One usually gives treatment during estrus, largely because uterine defense mechanisms are more effective during estrus than diestrus. Treatments may proceed for up to 2 to 4 days after ovulation provided the infused preparation results in little endometrial irritation.

The volume of fluid infused into the uterus of the mare with each treatment ranges from 30 to 250 ml. Several authors have suggested that sufficient volume of fluid should be used to obtain complete uterine coverage when infused.[11,14,23] The capacity of the typical, nonpregnant uterus of a mare is about 35 ml, whereas the uterus of an older mare may hold 60 to 150 ml.[11,14] Another study suggested that infusion of fluid volumes ranging from 30 to 250 ml does not result in uniform distribution of fluid within the uterine lumen. Most of the infused fluid pools in the uterine body and at the body-horn junctions.[24] Most cervical reflux was observed 10 to 15 minutes after infusion, which is consistent with endogenous oxytocin or prostaglandin $F_2\alpha$–stimulated uterine contractions. Larger volumes were more likely to result in reflux through the cervix. Apparently, some of the benefit from infusing antibiotic into the uterus in volumes large enough to encourage cervical reflux actually is from the lavage effect rather than a direct antibacterial effect.

One must take care to avoid insoluble or irritant preparations that might cause chronic endometritis.[25] Drugs for uterine infusion commonly are diluted in saline, balanced electrolyte solutions, or water for injection. Some authors suggest that acid drugs such as gentamicin and amikacin be buffered by mixing equal volumes of antibiotic and 7.5% sodium bicarbonate before infusion into the uterus.[8] Others reported that uterine infusion of gentamicin diluted in saline was no more likely to result in endometrial inflammation than saline alone.[26] Choice of diluent may influence pharmacokinetics of some drugs. Infusion of gentamicin into the uterus of cattle was followed by systemic absorption of more than 60% of the dose when the gentamicin was diluted in water versus less than 20% ofthe dose when diluted with saline.[27]

Veterinarians traditionally have avoided infusing procaine penicillin into the uterus of a mare, perhaps because of an early study based on hysteroscopy that reported that the endometrium was visibly covered with a white, chalky material thought to be antibiotic, following infusion of ampicillin. The material was visible at 3, 7, and 14 days after infusion.[28] Nonetheless, procaine penicillin is used widely as an intrauterine infusion and commonly is mixed with aminoglycosides, particularly gentamicin and neomycin. The use of procaine penicillin in the equine uterus requires additional investigation.

16.4.3 Antifungal Preparations

Dilute povidone iodine or 2% vinegar (20 ml white vinegar in 1000 ml saline) has been proposed as intrauterine 1052
therapy for fungal endometritis. A variety of antifungal medications also have been used as intrauterine 1053
treatments. Too little or no data exist on which to make recommendations regarding sensitivity patterns, doserates, or frequency of treatment. Fungal endometritis hasbeen reported as being difficult to treat, though this perception in part may be caused by use of ineffective or inappropriate treatments.[29]

16.4.4 Antiseptics and Irritants

Antiseptic or irritant therapy commonly has been recommended in cases in which a diagnosis of endometritis has been made but no specific causative agent has been isolated or for aged, subfertile mares with distinct degenerative changes on endometrial biopsy. Irritants have been used to reduce the size of the uterus and increase uterine tone. Antibiotics are not useful in this manner unless they are irritating. In addition, irritants may decrease fluid viscosity within the uterus to aid in its expulsion. Irritant therapy also has been credited with stimulating a severe, acute inflammatory response within the endometrium that may help resolve the chronic, monocytic inflammation commonly associated with degenerative endometritis or endometrosis. Mechanical curettage has been reported to have similar effects, although the technique is difficult because of the bicornuate nature of the equine uterus. The typical endometrial response to infusion of an irritant is a nonspecific inflammatory reaction with hyperemia and neutrophil influx, which then is followed by a slower infiltration of mononuclear cells that peaks 4 to 7 days after treatment. The duration of inflammation and the extent of tissue damage have been reported to vary considerably between individual animals given the same treatment. Irritant therapy also appears to produce a nonspecific activation of endometrial glands.[30] Excessive irritation of the endometrium associated with too harsh or concentrateda solution may result in worsening of the degenerative condition and even in adhesion formation. Chlorhexidine solution has a propensity to cause endometrial adhesions and severe reactions and should not be used within the uterus.[23] Recently, anecdotal evidence suggests that enrofloxacin administered as a uterine infusion in mares may be highly irritating, resulting in a severe inflammatory reaction with serosanguineous uterine fluid containing fibrin tags.

16.4.5 Povidone-Iodine

Dilute povidone-iodine solution is a commonly recommended intrauterine treatment because of its irritant, bactericidal, and fungicidal properties. Bactericidal activity of povidone-iodine is maintained in vitro down to concentrations of 0.01% to 0.005%, whereas more than 0.2% povidone-iodine has been claimed to inhibit neutrophil migration.[31] Stock povidone-iodine solution used in practices to disinfect surgical sites is described as a solution containing 10% povidone-iodine, equivalent to 1% available iodine.

Discussions regarding safety of dilute povidone-iodine solutions in the mare are a good example of the need for critical information on the effects of all potential irritant therapies on the uterus. Earlier studies found severe inflammatory responses in the endometrium following infusions of 2% or more povidone-iodine.[32,33] A similar study reported severe, prolonged irritation of the endometrium following infusion of 1% povidone-iodine solution with mares still showing evidence of inflammation at the conclusion of the study, 30 days following infusion.[34] Others have reported resolution of inflammation on uterine biopsy within 6 days of uterine infusion of 0.05% povidone-iodine solution.[7] In addition, infusion of 1 L of 0.05% povidone-iodine into the uterus 4

hours after breeding was found to have no adverse effect on pregnancy rates.[35] Recently, Bracher reported that infusion of 0.05% povidone-iodine solution into the uterus (alone or combined with dimethyl sulfoxide or collagenase) induced adhesions, ulceration of the endometrium, and severe stromal fibrosis and gland degeneration in three of five treated mares.[30] Individual mares have been postulated to be hypersensitive to povidone-iodine solution. Asbury and Lyle[8] have suggested that any signs of discomfort or irritation on clinical observation or observation via vaginal speculum following povidone-iodine infusion be followed by a saline flush of the genital tract and discontinuation of povidone-iodine therapy. The author suggests that povidone-iodine solutions be avoided or used with caution until further information is available on their safety as a uterine therapy.

16.4.6 Hydrogen Peroxide

Hydrogen peroxide acts as an irritant and has been recommended as an alternative approach for treating intrauterine infections caused by *P. aeruginosa*. Hydrogen peroxide is an oxidizing agent that readily breaks down to release oxygen and water, accompanied by effervescence. Hydrogen peroxide should be used in mares only during estrus.[36]

16.4.7 Tris-aminomethane–EDTA

Intrauterine infusion of tris-aminomethane–EDTA has been reported to result in a degree of inflammation similar to that observed following saline infusion.[37] Tris-aminomethane–EDTA is reported to have a potentiating effect on the antimicrobial activity of a variety of antibiotic and antibacterial solutions.[38] Tris-aminomethane–EDTA may have direct antibacterial effects in some cases and in others may cause a reduction 1053
in the MIC for some organisms in association with particular antibiotics, including penicillin, oxytetracycline, 1054
and gentamicin.

16.4.8 Chlorhexidine Gluconate

Chlorhexidine has been described as being a strong irritant with the potential to cause intrauterine adhesions and therefore has been disregarded for potential uterine therapy.[23] Pony mares have been reported to tolerate uterine infusions of 0.25% chlorhexidine gluconate (Hibitane, Schering-Plough Animal Health, Upper Hutt, New Zealand) with little evidence of irritation onvaginal inspection, whereas dilutions of 0.5%, 1%, and 2% caused severe irritation in a proportion of mares treated.[39] Others have reported that a solution containing 100 µg/ml chlorhexidine gluconate exceeds the MIC for *S. zooepidemicus*, *P. aeruginosa, K. penumoniae, Actinobacillus equuli*, and *Candida albicans*.[40] Infusionof 200 ml of this solution on 3 successive days during estrus had no adverse effect on pregnancy rate when mares were given prostaglandin $F_{2}\alpha$ 8 to 9 days after ovulation and bred at the successive estrus (D. Freeman, personal communication, 1998). More research into the safety and efficacy of chlorhexidine as a uterine therapy is needed.

16.4.9 Dimethyl Sulfoxide

Dimethyl sulfoxide (DMSO) causes an inflammatory response following intrauterine infusion that is similar to other irritants.[30] DMSO infused into the uterus improves endometrial biopsy classification scores by one or more points based largely on reduction of periglandular fibrosis,[36] though others have reported that DMSO did

not have any fibrolytic effect.[30,41] One mare in a treatment group receiving concentrated DMSO subsequently was found to have developed transluminal adhesions.[30] A clinical trial involving intrauterine infusion of DMSO into subfertile mares failed to detect any improvement in fertility following infusion.[36]

16.4.10 Kerosene

Kerosene has been used as an irritant therapy in the equine uterus for many years.[42] Recently, an experimental comparison was performed to evaluate the uterine response in subfertile mares to infusions of kerosene, dilute povidone-iodine, DMSO, and collagenase. The uterine response to kerosene, collagenase, and DMSO was similar, resembling the response after mechanical curettage. Gross and histologic evidence of inflammation had resolved within 14 to 21 days following infusion. Mares treated with kerosene differed from other treatment groups in having improved gland activity on uterine biopsy and higher pregnancy rates following breeding. Nine of 11 (82%) mares conceived, and 5 of 11 (45%) subsequently foaled. Bracher[30] concluded that the increase in secretory activity of the glands observed after intrauterine infusion of kerosene was a major factor in achieving the higher conception and foaling rates.

The standard approach for infusing kerosene involves infusing 50 ml of commercial-grade kerosene into the uterine lumen during diestrus. Diestrus is preferred because the closed cervix is more likely to prevent reflux of kerosene into the vagina with resulting inflammation.[36] Alternatively, one should follow infusions during estrus with digital occlusion of the cervix for a short time to prevent vaginal reflux.

16.4.11 Saline

Saline is useful as a means of flushing or mechanically removing fluid and debris from the uterus and for stimulating the uterus to contract and expel contents. Saline also irritates the endometrium and causes migration of polymorphonuclear leukocytes into the uterine lumen. Warmed saline has been reported to result in rapid and transient enhancement of uterine tone in mares with atonic or atrophic uteri, and saline flushes on day 6 after ovulation have been reported to result in luteolysis via endometrial prostaglandin F release.[8,23]

16.4.12 Plasma

Plasma may be beneficial in mares with chronic and chronic-active endometritis by providing immunoglobulins and complement, both important components in opsonization of bacteria within the uterus.[43] Recent studies suggest that suppression of uterine neutrophil function in susceptible mares is likely to result from the accumulation of fluid, debris, and bacteria that occurs following an underlying failure of uterine mechanical clearance.[44] Treatment and prevention therefore are better aimed at aiding clearance than by infusing plasma. This hypothesis is supported by studies failing to demonstrate any beneficial effect of plasma in susceptible mares.[2,45]

Pascoe[46] recently described a large field study involving the routine postbreeding use of plasma with antibiotics. The pregnancy rate improved significantly when plasma was infused into lactating mares but not in maiden or barren mares. Plasma possibly may be beneficial in foaling mares or in mares that do not have a mechanical clearance problem. Further work is needed to determine factors that may influence the effect of plasma in the uterus of the mare.

Autologous plasma is believed to be preferable to heterologous plasma because of the risk of hypersensitivity, 1054
anaphylaxis, or transfusion illness following heterologous plasma infusion. However, many mares have 1055
received heterologous plasma without problem. Preparation of plasma requires aseptic withdrawal of venous bloodinto heparin-based anticoagulant (typically 5 to 10 units of heparin per milliliter of blood).[3] Complementrapidly degrades at room temperature, and therefore oneshould separate plasma quickly. Gravity sedimentationis acceptable but should be performed under refrigeration. Once separated, one should infuse plasma immediately or stored it frozen in 100-ml aliquots ready forthawing and infusion. One must thaw plasma in warm water or in a microwave on a low setting. Thawingat too high a temperature results in denaturation of protein and loss of immunoglobulin activity. In thefield, preparation of plasma invariably is performed under less than ideal conditions. The author always combines field-derived plasma with a broad-spectrum antibiotic as a uterine infusion to minimize the risk of inadvertently introducing infectious organisms when infusing plasma.

16.4.13 Uterotonics

The importance of mechanical clearance of the uterusin resistance to bacterial infections has been documented clearly, particularly in several recent studies performedby Troedsson and colleagues and LeBlanc and colleagues.[2,6,44,47,48]

Inability physically to clear contaminating material from the uterus within a short period of time now is believed to be the major factor resulting in mares being susceptible to chronic and recurrent uterine bacterial infection.[3] Treatments that assist the uterus physicallyto clear bacteria, fluid, and associated inflammatory by-products and debris from the uterine lumen are critical to the effective treatment and prevention of infectious endometritis. Uterine lavage is effective at removing material from the uterine lumen. Pharmacologic alternatives that induce uterine contractions include oxytocin and prostaglandin $F_2\alpha$ and analogs.

Prostaglandin $F_2\alpha$ or cloprostenol, given by intramuscular injection, causes an increase in intrauterine pressure within 10 minutes, and contractions last for about 5 hours. Oxytocin produces a response within 60 seconds of intravenous administration, but contractions last for only 40 to 60 minutes. Recently, investigators concluded that oxytocin was more effective than prostaglandinin clearing the uterus of colloid. The same study also concluded that cloprostenol was more effective than prostaglandin F or fenprostalene, although all mares treated with prostaglandin analogs did have an increased clearance of radiocolloids.[47] Cloprostenol also has been recommended in favor of dinoprost in mares that have lymphatic stasis. Lymphatics drain particulate matter from the uterine lumen and reabsorb the intramural fluid that accumulates within the uterine wall during estrus. Lymphatics drain dorsally into the vessels in the broad ligament and then into the iliac and aortic lymph nodes. Lymphatic vessels do not contain smooth muscle and therefore must rely on uterine contractions to push fluid dorsally within lymph vessels.[48] One should not administer prostaglandins more than 48 hours after ovulation to minimize the risk of inducing luteal regression.

Concurrent medications may interfere with uterine clearance in susceptible mares. Phenylbutazone and acepromzaine administration have been reported to interfere with clearance of uterine radiocolloid in susceptible mares. The effect is reversed with the administration of oxytocin.[48] Xylazine has been suggested to be a preferable sedative to acepromazine for susceptible mares inthe breeding shed because xylazine causes an increase in uterine pressure. In the event that mares receive acepromazine or phenylbutazone around the time of breeding, one should consider administering oxytocin concurrently to assist in uterine clearance.

Intrauterine administration of uterotonics has received a small amount of study and warrants further work. Intrauterine oxytocin administration has been shown to increase intrauterine pressure but not as much as intramuscular or intravenous administration.[49] Preliminary studies involving the intrauterine administration of10-mg doses of dinoprost suggest it is effective at inducing uterine contractility, but detailed comparative work comparing the effect of uterine versus systemic administration of uterotonics on uterine clearance remains to be completed.[2,50]

The use of oxytocin and prostaglandin (or analogs) as aids for uterine clearance remains empirical. Dosesof between 10 and 25 IU oxytocin have been used toaid mechanical evacuation of the uterus.[51] Information is lacking on the most effective dose, route of administration, and frequency of administration. Little information is available on safety of these products when used around the time of breeding, although both products commonly are used during estrus before and after breeding. In addition, little information is available on practical methods of identifying mares with delayed uterine clearance suitable for application in the field. The presence of uterine fluid detectable by ultrasonography appears to be an indicator of reduced clearance, particularly if fluid persists for more than 6 to 12 hours after mating.[52]

16.4.14 Uterine Lavage

Uterine lavage is a traditional and highly effective method of removing fluid and associated debris from the 1055
lumen of the uterus. Uterine lavage commonly is performed before and after breeding and often is combined 1056
with infusion of antibiotic, antiseptic, or other materialssuch as plasma.[23] In addition, lavage commonly is applied in the early postpartum mare to treat retained placenta or metritis. Emptying the uterus of fluid before antibiotic infusion is likely to improve the efficacy of uterine antibiotic therapy by removing foreign material and inflammatory products from the lumen. Such emptying is likely to be more important for aminoglycoside and polymixin antibiotics that bind to purulent material.[7]

Saline induces an inflammatory response within the uterus. Infusion of acidic saline resulted in an immediate release of endogenous prostaglandin F, whereas infusion of pH-neutral saline did not.[53] Heating the lavage fluid to 45° to 50° C has been reported to result in a rapid and transient increase in uterine contractility.[23]

One normally performs uterine lavage with a Foley catheter or embryo transfer catheter with a large balloon cuff (75 ml). One infuses fluid until the uterus is distended (500 to 1000 ml), removes it by gravity flow, and collects it in a vessel to allow measurement of lavage and recovered volumes and the appearance of the recovered fluid. The opacity of the recovered fluid indicates the degree of inflammation present within the uterus and guides the need for additional flushing. One should repeat lavage until the recovered fluid is clear. Apparently, no controlled studies document the efficacy of lavage as a treatment modality for endometritis.

16.4.15 General Treatment Strategies for Management of Uterine Disease

Successful treatment of acute or chronic infectious endometritis is based on elimination of the causative organism, reduction of any accompanying inflammation, correction of anatomic defects, and prevention of recurrence. One must correct predisposing anatomic causes of endometritis such as poor vulval conformation, cervical or rectovestibular lacerations, and urine pooling surgically before applying therapies aimed at resolvingan endometritis. The most common routine procedure performed on broodmares is Caslick surgery.

One should perform more extensive surgical procedures during the non–breeding season to allow the mare time to recover before breeding.

The approach to uterine therapy depends on the diagnosis and presenting clinical signs. One manages infectious endometritis by eliminating fluid and debris from the uterine lumen by lavage and administration of a uterotonic, followed by uterine infusion of a topical treatment (antibiotic, antifungal, antiseptic, etc.). Choice of uterine therapy, formulation, dose, volume infused, diluent, and treatment frequency are discussed elsewhere in this chapter. Lavage of the uterus on subsequent treatment visits is only necessary if the uterine lumen continues to accumulate fluid or if one is performing the lavage to stimulate uterine contractility.

Many treatments have been suggested for chronic degenerative endometritis (endometrosis), but consistently favorable results have not been reported. All of the techniques and treatments described for acute and chronic endometritis have been used. In addition, mechanical and chemical curettage or irritant therapy is commonly used in mares with this condition. Regardless ofthe treatment, the prognosis is considered to be poor,[3] with the exception of those mares that improve from a pretreatment grade III biopsy to a posttreatment grade II biopsy classification.[54]

Topical treatment of the uterus must be accompanied by strict attention to hygiene. One must pay attention to management principles to achieve successful resolution of endometritis accompanied by a reduced risk of recurrence and an optimal opportunity for pregnancy.[7]

16.4.16 Postmating Endometritis

An area in which important advances have been made in the past several years is in understanding and management of postmating endometritis. A transient and physiologic endometritis occurs after breeding because of the intrauterine deposition of semen. In normal, fertile (resistant) mares this transient inflammation resolves within 36 hours after mating. Contamination and inflammation of the uterus following breeding has to be resolved before the embryo enters the uterine lumen at about 6 days after ovulation in the pregnant mare. Impaired physical clearance of bacteria, fluid, and inflammatory products is considered to be the primary factor responsible for susceptibility to persistent, infectious endometritis.[3] One should examine mares in early estrus using ultrasonography to evaluate the presence and echotexture of uterine luminal fluid. Presence of large volumes of echogenic fluid indicates acute endometritis, and one may prefer to treat such mares aggressively through estrus and shorten the cycle to aim for breeding on the successive estrus. One can remove large volumes of anechoic fluid in early estrus using uterine lavage with saline, followed by injection of oxytocin to prevent fluid accumulation from recurring. One may manage smallto moderate volumes of fluid using oxytocin alone. One should examine mares 20 minutes after administrationof oxytocin to ensure uterine fluid has been expelled;in some cases, manual dilation of the cervix is neededto facilitate effective expulsion of fluid. One may use intrauterine infusion of antibiotic in early estrus in an attempt to eliminate endometritis or reduce the 1056
risk of endometritis before breeding. Any product used for uterine lavage or instillation in the prebreeding or 1057
postbreeding estrous period should have minimal irritating effect on the endometrium. Physiologic saline, Dulbecco's phosphate buffered saline, and lactated Ringer's solution are believed to be associated with less inflammatory response than water. One should choose antibiotics with care and not use antiseptics or irritants at all in this period. One may continue to perform ultrasound examinations through estrus right up to immediately before breeding, with the goal being to eliminate fluid from the lumen of the uterus before breeding.

Clinicians generally agree that mares should be bred once only to minimize contamination of the uterus and genital tract. One should consider the use of ovulation induction agents (human chorionic gonadotropin or

synthetic gonadotropin-releasing hormone) routine for susceptible mares because these agents greatly improve the likelihood of a single breeding per ovulatory cycle. Normal practice is to aim for a single breeding in the time period from 24 hours before ovulation to 12 hours after ovulation. Depending on stallion fertility, mares may be bred as early as 48 to 72 hours before ovulation without reduction in fertility. Recently, Pycock has proposed that susceptible mares be bred only once, at least 24 hours before ovulation and perhaps earlier, assuming suitable stallion fertility.[55] Breeding at this time ensures that any contamination associated with breeding occurs when circulating estrogen concentrations are maximal, progesterone concentrations are minimal, uterine defense mechanisms and physical drainage should be optimal, and more time is available after breeding to implement uterine therapy before progesterone rises and the cervix begins to close after ovulation. If breeding is by artificial insemination, one should add a semen extender containing antibiotic to the ejaculate and allow the extended semen to stand undisturbed for a minimum of 15 minutes to allow elimination of possible pathogens from the ejaculate. One also may infuse antibiotic-containing semen extender into the uterus of the mare immediately before natural breeding in an attempt to obtain a similar effect. One should examine susceptible mares by ultrasoundas early as 4 hours after breeding and preferably before12 hours, again to check for accumulation of fluid.[35] Examination ensures that one can eliminate uterine fluid using lavage or uterotonics or a combination of these treatments before the development of an inflammatory response or proliferation of potentially pathogenic organisms. One infuses into the uterus a broad-spectrum antibiotic about 20 minutes after administration of uterotonic. Pycock suggests using a small-volume infusion (30 ml) for the postbreeding treatment because susceptible mares are expected to have clearance problems.[55] Administration of oxytocin can continue, and one can examine the mare the following day to ensure that uterine fluid is not accumulating. If fluid is still present, one can repeat the treatment approach (lavage, uterotonic, and infusion). One should not continue intrauterine treatment past day 3 after ovulation to minimize the risk of endometrial inflammation persisting past day 5 or 6 when an embryo may be expected to be entering the uterus. One should not administer uterotonics past about day 2 after ovulation because the cervix generally is starting to close, thus inhibiting uterine drainage. One should use prostaglandins with care after ovulation because of the added risk of possible induction of luteal regression. The use of a single postbreeding uterine infusion ofa broad-spectrum antibiotic combined with uterotonic administration has been reported to result in higher pregnancy rates in field trials than an antibiotic or uterotonic alone.[56]

The combination of prebreeding and early postbreeding estral management, with the use of oxytocin, has improved the ability to prepare an optimal uterine environment greatly for breeding and for establishmentof early pregnancy. The use of systemic antibiotics and nonsteroidal antiinflammatory drugs in susceptible mares after breeding has been suggested anecdotally as offering promise in reducing the risk of establishment of endometritis and endometrial prostaglandin release.[57,58] Such therapies remain unproven.

16.4.17

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16.5

16.5—Hormonal Manipulationof the Mare

Elizabeth Metcalf

Veterinarians attempt to mimic or change the physiology or behavior of the mare through the administration of exogenous hormones. This chapter examines the effects of hormonal preparations administered to mares in an attempt to enhance their reproductive efficiency.

16.5.1 Vernal Transition

As day length increases, nonpregnant mares gradually emerge from a state of ovarian quiescence to regular cyclicity. Through the hypothalamic-pineal-pituitary-ovarian axis, mares begin to show clinical signs of a prolonged estrous period followed at last by the first ovulation of the season. Section 16.1 presents more precise physiologic events of this period.

Many hormonal regimens aimed at shortening the interval to first ovulation have been examined. Increasing the exposure of a mare to light from 6 A.M. to 10 P.M. has proved a potent and effective means of shortening the time to first ovulation of the season. In an attempt to hasten the interval further, researchers have examined other hormonal preparations including melatonin,[1] progestins[2] (plus estrogens),[3] and dopamine antagonists such as domperidone[4,5] and perphenazine.[6] Intravaginal inserts containing progesterone also have been successful in induction of cyclicity in anestrous mares.[7] Although many of these results are promising, progestin therapy is used most widely. One should note that providing good nutrition, especially in aged mares, may hasten the onset of the first ovulation,[8] possibly by involving hormones that are associated with digestive metabolism.

16.5.2 Synchronizing the Estrous Cycle

In the attempt to optimize breeding efficiency, the ability to predict estrus and ovulation becomes an important management tool. Unfortunately, mares vary more in response to hormonal treatment than ruminant species do. Thus the bovine concept of fixed-timed insemination has not been applicable in equine reproduction. The objective of most attempts to manipulate the timingof the estrous cycle of the mare is synchronization ofa group of mares (e.g., in an embryo transfer program) or more commonly strategic management of insemination with shipped or frozen semen.

To manipulate the estrous cycle of the mare with some precision, one must understand the basic endocrine events of the normal 21- to 22-day cycle of the mare.[9] The day of ovulation is considered to be day 0 and is preceded by a period of estrogen domination (5 to 7 days). The high estrogen levels are responsible for the signsof behavioral estrus and cause the palpably decreased tone of the uterus and cervix. Under the influence ofthis estrogen-priming, a cascade of preovulatory events occurs in the hypothalamic-pituitary axis, and the prolonged increase in luteinizing hormone (LH) blood levels ultimately results in ovulation. The diestrous phase is dominated by progesterone secretion from the corpus luteum. This diestrous phase lasts for 14 to 16 days. In the absence of maternal recognition of a pregnancy, endometrial prostaglandin $F_2\alpha$ ($PGF_2\alpha$) surges—possibly stimulated by or at least coordinated with oxytocin—cause luteolysis and regression of the corpus luteum. Concomitant with the decline in progesterone, estrogen levels begin to rise as a new cohort of follicles develops.

Most hormone regimens aim at manipulating the luteal phase of the cycle. Perhaps the simplest method of synchronizing the cycle of a mare is by using synthetic preparations of $PGF_2\alpha$, which effectively and reliably may shorten the cycle of the mare if used after the corpus luteum is well-formed—after day 5 to 6 of the cycle. The mare returns to estrus within 3 to 5 days following administration of $PGF_2\alpha$ and subsequent regression of 1059

the corpus luteum. Depending on the structures present on her ovary at the time of administration, the mare ovulates approximately 7 days later.[10] Although administration of $PGF_2\alpha$ before day 5 is usually ineffective in returning a mare to estrus, treatment of mares with a $PGF_2\alpha$ analog on day 0 (ovulation) or day 1 still does affect the developing corpus luteum. A transient but significant suppression in progesterone levels occurs, with less echogenicity and size of the corpus luteum and decreased subsequent pregnancy rates.[11,12]

A number of $PGF_2\alpha$ preparations have been used successfully in mares; however, they are associated with many undesirable side effects including profuse sweating, anxiety, diarrhea, and signs of colic. The severity of the side effects depends on the preparation of $PGF_2\alpha$ andthe susceptibility of the individual mare. Cloprostenol (Estrumate, Bayer Corp., Shawnee, Kansas) administered at lower doses (250 μg) usually is associated with fewer side effects than the less expensive analog, dinoprost tromethamine (Lutalyse, Upjohn Co., Kalamazoo, Michigan) when administered intramuscularly. The absence of side effects is *not* related to the effectiveness of the drug in inducing luteolysis.

Treatment with progestins represents one of the oldest and most reliable means of synchronizing estrus in the mare, especially when coupled with appropriate light exposure and $PGF_2\alpha$ administration. Progestins simulate the diestrous period of the estrous cycle. Repositol progesterone (not commercially available), progesterone in oil (Sigma Chemical Co., St. Louis), or oral progestin compounds (altrenogest [Regumate, Intervet Inc. Millsboro, Delaware]) are effective in suppressing estrous behavior but not necessarily ovulation. Treatment is only effective in synchronizing mares that are transitioning into regular cyclicity or are cycling regularly; treatment is not effective in mares that remain in seasonal anestrus. Therefore examining the reproductive tract of the mare by transrectal palpation and ultrasound before initiating treatment is prudent. Ovaries in anestrous mares feel smooth, firm, and small on palpation. Any follicles are small, usually less than 10 mm in size on the ultrasound examination. Conversely, mares that are ready to begin regular cycles or are cycling regularly have larger, irregular-shaped ovaries of variable firmness with multiple follicles greater than 10 mm in diameter. One may image a corpus luteum with ultrasound.

Even though the drug is more expensive, the oral progestin preparation altrenogest perhaps remains the most popular progestin compound to use in mares. Altrenogest is easy to administer, is nonpainful, and has few side effects. Fillies born to mares treated with altrenogest may reach puberty earlier than fillies from nontreated mares and rarely are born with transiently enlarged clitorides.

One should use all progestin preparations with some caution in mares that are susceptible to delayed uterine clearance or uterine infections, for several reasons. Progestins increase the tone of the cervix, and their use may inhibit the physical clearance of fluid. Progestins also have been associated with lower immunoglobulins levels (especially IgG) in the uterus.[13] The combination of these side effects ultimately can lead to delayed uterine clearance, endometritis, pyometra, etc.; therefore, during treatment, one should monitor mares susceptible to these conditions.

When synchronizing mares, one recommendationis that progestins be used for a minimum of 10 daysin cycling mares and 14 days in transitional mares. On the last day of progestin treatment one may give a $PGF_2\alpha$ injection to lyse any remaining corpora lutea. Time to ovulation depends on the structures that are present at the end of progestin administration. The mare should return to estrus in 3 to 5 days and on average, ovulation occurs by 7 days after treatment.

16.5.3 Synchronizing Ovulation

A number of agents that shorten the interval to ovulation in the mare have been used to synchronize the cycles further. The most effective agents possess LH activity with varying degrees of follicle-stimulating hormone (FSH) activity. Human chorionic gonadotropin (hCG [HCG, Intervet]), with its potent LH-like activity, is at the time the least expensive and perhaps the most popular agent used for the induction of ovulation. Human chorionic gonadotropin has been reported to be effective at doses ranging from 1000 to 5000 IU given intramuscularly, intravenously, or subcutaneously.[14]

Use of hCG in the mare has some limitations. First, with its repeated use, antibody development has been documented in several studies[15]; however, the clinical impression of many practitioners is not in agreement with these studies. Secondly, mare owners often complain about the apparent pain associated with administration of some hCG products. Finally, the reliability of hCG in its ability to hasten the interval to ovulation, especiallyin the older or compromised mare, has been questioned. Regardless of its potential disadvantages, hCG remainsa popular, inexpensive, and effective means of inducing ovulation in most mares.

Synthetic gonadotropin-releasing hormone analogs also have proved to be effective in inducing ovulation (deslorelin acetate [Ovuplant, Fort Dodge, Iowa]; and buserelin [Buserelin, Intervet; not commercially available in the United States]).[14,16,17] Although the use ofthese latter agents may delay the return to estrus by 1 to 2 days 1060
if the mare fails to conceive, the general impression of practitioners is that because these gonadotropin- 1061
releasing hormone analogs are more reliable in inducing ovulation, especially in mares that are more prone to ovulation failure (older mares, mares in vernal transition, mares concomitantly treated with prostaglandin inhibiting agents such as many antiinflammatory drugs), the advantages far outweigh the disadvantages of choosing this drug. Furthermore, removal of the implant a few days following insertion can ameliorate this delay.[18]

Ovulation induction agents are more reliable and effective if given at the appropriate time during estrus. If endometrial folds are apparent on the ultrasound examination, and a dominant softening follicle is present, usually greater than 30 mm in diameter, then hCG, deslorelin acetate, and buserelin are expected to induce ovulation an average of 36, 41 to 48,[14] and 24 to48[17] hours, respectively, following administration. Samper reported that 98% of mares with maximal endometrial edema given hCG or deslorelin consistently ovulated within 48 hours of administration.[19] Most remarkably, most mares to which Ovuplant has been administeredat the proper time, ovulate 38 ± 2 hours later,[20] truly allowing appointment breeding.

16.5.4 Synchronizing Estrus and Ovulation

One can use progestins along with estradiol-17β (P+, Franck's Veterinary Pharmacy, Des Moines, Iowa) to obtain greater synchrony between mares. Although not commercially available, biodegradable microspheres impregnated with progesterone (1.25 g) and estradiol (100 mg) also have been used to synchronize the estrous cycle of the mare[21–23]; they appear to suppress follicular development and ovulation. However, these drugs have been replaced to some extent by intravaginal devices (CIDR-B, InterAg, Hamilton, New Zealand) that contain progesterone; one administers estradiol (10 mg) at the time of insertion. Administration of a $PGF_2\alpha$ analog is advised at the time of insert removal (12 to 14 days). Finally, one uses a deslorelin implant when the dominant follicle reaches a size of 40 mm. This regimen nicely synchronizes ovulation and estrus[24]; however, neither these intravaginal devices nor the encapsulated microspheres currently are licensed for horses.

16.5.5 Delaying Estrus and Ovulation

Although most hormonal regimens act to hasten the interval to estrus, numerous managerial reasons to delay estrus exist as well. Altrenogest originally was designed to prevent show mares from exhibiting estrous behavior, and it has proved to be effective in this regard, suppressing signs of estrus in 95% of mares that were given a daily dose of 0.044 mg/kg for 3 days.[25] Although reports represent only anecdotal testimony, many horses owners have found that altrenogest not only is effective at estrus suppression but also appears to exert a calming effecton horses.

In an attempt to find a more economic and less time-consuming means of suppressing estrous behavior in their mares, progestin/estradiol implants (Synovex, Syntex Animal Health, Des Moines, Iowa) licensed for food animals have been investigated in mares. Although mare owners often report an improvement in the behavior of the mare, scientific reports have been less supportive of their use. McCue, Lemons, Squires, et al.[26] found that mares treated with these implants, even at 20 times the usual dose, did not show suppression of estrous behavior and cylicity.

Altrenogest recently has been tested for its effectsin delaying ovulation after the mare is in estrus. Theoretically, if the LH surge has not occurred yet, oral progestin treatment should inhibit LH and delay ovulation. Altrenogest at label dose or 2 times the label dose is effective in delaying ovulation compared with control mares as long as an ovulation-induction agent has not been administered.[27] Intraluminal fluid retention, probably caused by increased cervical tone, has been witnessedin some mares. Although this application is promising, one should intrepret these results with caution, because treated mares had lower pregnancy rates (though not statistically significant).

Delaying the interval to first ovulation of recently foaled mares results in higher pregnancy rates for the first heat cycle. Although the natural foal heat of a mare may be highly fertile, many early pregnancies are lost, likely because of an adverse uterine environment. To optimize the maintenance of those early pregnancies and yet still increase the reproductive efficiency of the mare by breeding her back as soon as possible, delay of the first postpartum interval to ovulation using a combinationof progesterone and estradiol-17β (E2-17B, Sigma Chemical) in oil is beneficial. In an attempt to increase the fertility of foal-heat breeding, Sexton and Bristol[28] reported that this protocol successfully delays the postpartum interval to first ovulation, thereby increasing the period of time for uterine involution and pregnancy rate.

16.5.6 Superovulation

In efforts to increase the number of offspring produced in the lifetime of a mare or in any given season, researchers have investigated numerous means of superovulating mares. Overall, attempts to cause mares to superovulate reliably and effectively have been disappointing. The increased numbers of ovulations/embryos have been minimal or pregnancy rates have been low. The administration of porcine FSH preparations is 1061
ineffective in mares, except at cost-prohibitive doses.[29] Stimulation of endogenous FSH secretion by indirect 1062
and direct immunization against inhibin has not resulted in a superovulatory responsein mares.[30] The administration of equine pituitary extract preparations in research trials only increases the number of embryos recovered from 0.5 per cycle to 2.2 per cycle.[31,32] Purified equine FSH has failed to increase the numbers of ovulations or recovered embryos compared with equine pituitary extract.[33]

16.5.7 Teaser Mares

Breeding facilities require at least one mare exhibiting behavioral estrus to be able to collect semen from most stallions. Some stallions appear to prefer a particular mare or a certain color of mare and therefore a varietyof mares in heat is sometimes desirable for ease of collection. Thus use of exogenous hormone regulation to encourage a mare to display signs of estrous behavior often becomes necessary.

One strategy for ensuring the availability of a teaser mare is to have a rotating supply of mares thatare constantly short-cycled with $PGF_2\alpha$ analogs. If one injects the mare with $PGF_2\alpha$ 5 days after the cessation of behavioral estrus, in theory she will return to heat in a few days because the mature corpus luteum on her ovary will be lysed by the injection. Ideally, a minimum of two teaser mares suffices for this program. An additional benefit is that the fertility of the mare is unaffected by the prostaglandins.

Administration of estrogen compounds also causes some mares to exhibit estrous behavior. Estradiol cypionate (ECP, Upjohn), a long-acting estrogen, and estradiol-17β, a shorter-acting estrogen have been investigated; the results of administration of either compound vary, especially over time and in particular with estradiol cypionate. The estrogen compounds are far more effective in ovariectomized mares than in intact mares, because even the smallest amount of progestin secretion from the ovary inhibit signs of behavioral estrus. Interestingly, ovariectomized mares often demonstrate low-grade signs of heat even in the absence of exogenous hormonal administration. Theoretically, these signs are caused by the absence of ovarian progesterone and the continued secretion of estrogens from other sources, particularly the adrenal glands. Moreover, even the smallest amounts of circulating estrogens are postulated to cause some mares to show constant signs of estrus in the presence of a stallion. The responseof ovariectomized mares to exogenous estradiol-17β or estradiol cypionate has not been reliable, especially with administration of these hormones in increasing amounts and frequency. The hormones appear to reach a threshold at which the clinical response actually is contrary to the signs expected, and treated mares actually may show increased aggressiveness to stallions. Low doses (1 mg intramuscularly) of estradiol cypionate, given every 2 to3 weeks, appear to be most effective in preserving the ovariectimized teaser mares for many years of service. Old, anestrous mares often function as ideal teaser mares for some stallions. In the absence of functional ovarian secretion, the low levels of circulating estrogens may cause a mare to exhibit signs of estrous behavior continually. Unfortunately, a number of stallions appear uninterested in these old mares despite their overt display of heat.

16.5.8 Maintenance of Pregnancy

Although the mechanism is unknown, progesterone supplementation in the form of injectable or oral preparations often is used to maintain pregnancy at all stages of gestation. The well-accepted fact is that early pregnancy maintenance in the mare requires the function first of the primary corpus luteum (approximately 45 days) and second of the accessory corpora lutea (45 to 90 days or more). Still, some mares appear to require progesterone supplementation during this period; otherwise, they lose the pregnancy. Hormonal assays can be confusing because progestin levels in these mares may appear well within the normal range for mares that do not appear to require supplementation.

Some mares also appear to require progesterone supplementation for maintenance of pregnancy in the later stages of gestation as well. Altrenogest may be effective in preserving the pregnancy in a compromised mare. In an experiment that examined the maintenance of pregnancy in mares suffering from experimentally induced endotoxemia, Daels, Besognet, Hansen, et al.[34] demonstrated that mares treated with altrenogest administered

at 2 times the recommended dosage suffered significantly less pregnancy loss than the untreated mares. Pregnancy loss rates appear higher in the Miniature horse mare (25%)[35] than those reported in larger breeds (<10%).[36] Moreover, unlike reports of studies on larger breeds, most of the pregnancy loss in the Miniature horse mare occurs during late term.[35] Thus many Miniature horse mares receive supplemental progesterone for the duration of their pregnancy. Speculation is that placental insufficiency may contribute to the increased loss of pregnancy in this breed, because the size of the foal often is larger with respect to its dam than in the larger breeds of horses.

16.5.9 Endometritis

Mating-induced endometritis is regarded as a normal transient physiologic event in the mare in response to sperm deposited in the uterus.[37] However, some mares are susceptible to a delay in uterine clearance, especially older, multiparous mares in which the uterus lies well beneath the pelvic brim.[38] Warm saline lavage followed 1062

by small doses of oxytocin (Oxytocin Injection, Vedco, St. Joseph, Missouri; intramuscular or intravenous)[39,40] 1063
are especially beneficial in these susceptible mares. The response to oxytocin appears to be greatest with administration just before ovulation, and this preovulatory insemination results in higher pregnancy rates.[41–45] Rigby, Hill, Miller, et al.[46] have reported that administrationof exogenous oxytocin to mares immediately following insemination does not improve pregnancy rates and that certainly this approach is not required for all breedings. Those mares with delay in uterine clearance in which intraluminal fluid remains or recurs despite multiple small doses of oxytocin may respond to $PGF_2\alpha$ therapy. Prostaglandin exerts a more sustained effect on uterine myometrial activity than oxytocin and thus may promote uterine evacuation and enhanced lymphatic drainage in difficult cases.[47]

16.5.10 Cervical Dilation

One of the most frustrating conditions encountered when breeding mares is failure of cervical dilation during estrus. On transrectal palpation, the cervix may feel toned, instead of “melted,” as it should during estrus just before ovulation. Because previous damage to the cervix and the subsequent formation of adhesions can presenta similar and confusing picture of failure of cervical relaxation, a thorough visual and manual cervical examination is prudent. In maiden mares, inserting a finger through the cervix to act as a guide for the insemination pipette is often difficult. Although this condition does not prevent artificial insemination, it can prevent not only penetration of the cervix by the penis of the stallion but also the normal evacuation of fluid in the hours following breeding, thereby setting the stage for delay in uterine clearance and persistent endometritis. Although not objectively studied, this condition does appear to affect older maiden mares more commonly. However, even normal maiden mares often do not have the same degree of cervical relaxation during estrus that is typical of pluriparous mares. A synthetic PGE_1 analog, misoprostol (Cytotek, Searle Corp., Chicago, Illinois), has been used successfully in women for cervical dilation. This compound has not yet been evaluated objectively in horses and currently is not approved for veterinary use. However, many clinicians have seen some encouraging results, simply by covering the external cervix with PGE_1 cream several hours before cervical dilation is desired. If only PGE_1 tablets are available, practitioners have crushed 1 to 2 tablets (200 μg) and mixed them with a small amountof sterile lubricant before application. Finally, digital insertion of a tablet into the external cervical os may be effective as well. Occasionally, veterinarians have used this preparation combined with other parturition induction agents if attempting to terminate a pregnancy prematurely. Again, only anecdotal reports of efficacy currently exist.

16.5.11 Oviductal and Uterine Contraction

The early embryo secretes PGE_2, which is believed to play a role in the contractility of smooth muscle of the oviduct and thus to promote propulsion of the embryo through the oviduct.[48] Prostaglanding E_2 also is secreted during the high mobility phase of the embryo and is suspected to stimulate uterine contraction and enhance uterine tone.[49] Laparoscopic application of PGE_2 along the serosal surface of the oviduct can modify embryo transport. Interestingly, not only is embryo transport hastened, but also unfertilized oocytes that are rarely recovered in the mare are transported to the uterus.[50]

The addition of PGE_2 to semen just before artificial insemination has increased pregnancy rates in mares bred with a fertile semen but not with a subfertile semen.[51] These results are disappointing because the addition of PGE_2 to semen has been associated with higher fertility rates in other species.[52,53] Furthermore, the role of PGE_2 at the uterotubal junction of the mare has been speculated to affect spermatozoal selection. Selection is believed to be based on morphology, but motility of the spermatozoa may play a larger role in this selection process than once suspected. Selection is an area of research that deserves continuing attention.

16.5.12 Future Use of Hormones

Many of the hormone preparations discussed were not designed for use in the mare and they may not be available commercially. Still, great strides have been made in manipulating the endocrine environment to enhance pregnancy rates, at least partially because of the growing artificial insemination industry. Manipulation of the hormonal milieu of the broodmare allows regulationof the estrous period, promotes prediction of ovulation, enhances the reliability of ovulation, and prepares the uterine environment for the maintenance of pregnancy. If per cycle pregnancy rates can be enhanced, then this regulation represents an economically sound investment to owners and veterinarians. Owners have more offspring in a shorter amount of time and lower veterinary costs, and veterinarians have time to use optimal management in breeding more mares.

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16.6

16.6—Breeding Management to Optimize Pregnancy Rates

Elizabeth Metcalf

Where foals once were born only as a result of natural field breeding, the art and science of modern horse breeding has continued to expand its innovative techniques to keep pace with advances in reproduction. Now foals are born as a result of more advanced procedures that include artificial insemination, oocyte transfer, embryo transfer, embryo splitting, intracytoplasmic sperm injection, gender selection, and in the future, cloning from a single cell. Furthermore, through improvements in cryogenic techniques, gametes and embryos may be preserved indefinitely. Veterinarians and scientists function primarily as facilitators of these processes that serve to preserve and perpetuate valuable genetic lines. The following sections examine the procedures currently used in equine private practice to facilitate the inseminationof horses.

Although increasing the number of pregnancies produced per mare in a given year is possible through the techniques of embryo and oocyte transfer, pregnancy rates per cycle have not improved significantly from field breeding to artificial insemination. In the past decade, many changes have been implimented in breeding facilities and include (but are not limited to) the housing ofmares and stallions, the methods used for teasing mares and

stallions, the design of the breeding shed, stallion and mare handling techniques, promoting appropriate behavior in the breeding shed, the processing of stallion semen, and the management of the mare population.

In an attempt to mimic the physical and physiologic events that occur in nature, breeders must understand not only equine behavior but also the processes of intromission, ejaculation, and eventually fertilization of the oocyte.

Although natural and artificial insemination of the mare entail deposition of semen within the uterus of the mare, natural service invokes several physiologic processes that are absent in artificial insemination.[1] When the penis of the stallion enters the vagina of the mare, several rhythmic pelvis thrusts occur before ejaculation directly into the uterine body. Not only does this action cause rapid vasodilation of blood vessels that supply the corpus spongiosum penis, resulting in the characteristic "belling" of the glans penis, but also it stimulates strong uterine contractions that in turn propel the deposited sperm toward the oviductal papillae of the mare. The engorged glans penis also may function to prevent the immediate backflow of semen during ejaculation. On completion of ejaculation, detumescense of the penis follows and the mare moves forward out from under the stallion. This sequence of events may occur many times on a given day under natural conditions.

Fertilization of the oocyte in the mare takes place in the ampulla of the oviduct. Before this, the spermatozoa must undergo capacitation and the acrosome reaction, a complex series of events that results in remodeling of the sperm plasma membrane and release of specific enzymes that enable the sperm to penetrate the oocyte. Although one 1065
can recover capacitated sperm from the uterus of the mare, uncapacitated sperm[2,3] are selected[4] to reside in the 1066
isthmus of the oviduct until the oocyte is present. Spermatozoa actually bind to oviductal epithelial cells, a process that appears to be enhanced in estrous mares but not in diestrous mares.[5] Abnormalities of this process may contribute to the subfertility of the mare or the stallion.

Mares with poor fertility have fewer sperm residingin the storage sites of the oviduct.[6] Furthermore, stallions with poor fertility may have spermatozoa with inherent damage that prevents them from being selected to reside in the oviduct. The effect of this damage more likely is caused by poor binding capacity rather than poor motility or reduced sperm transport.[3] Spermatozoa have the ability to survive in oviductal cell culture for at least 6 days.[7] This supports the report of a mare that became pregnant after a single mating 7 days before ovulation[8] and the studyby Woods, Bergfelt, and Ginther[9] in which mares were bred successfully more than 6 days before ovulation. Conversely, an oocyte is viable in the oviduct for only a short time, perhaps as little as 12 hours,[10] as indicated by the low pregnancy rates of mares bred more than 12 hours after ovulation.[9,11,12] Capacitated spermatozoa therefore must be available for successful fertilization.

Before any type of insemination, assessment of fertility of the mare and stallion is advisable. Many tests are available that evaluate fertility of stallion semen, but the most informative measure is the per cycle conception or foaling rate. Although no single in vitro laboratory test has proved to correlate precisely with stallion fertility, stallions with semen that demonstrates a high percentage of morphologically normal, progressively motile spermatozoa with little reduction in motility over the first24 hours of storage often prove to have better fertility than those with semen that lacks these attributes. Other means of predicting stallion fertility with in vitro tests include flow cytometric analysis of sperm membranes, organelles, and DNA; computer-assisted motility analysis; membrane "stress" tests; glass wool–Sephadex bead filtration; binding and penetration assays; and antisperm antibody assays (see discussion elsewhere in this chapter). Unfortunately, because of the expense, most stallion semen is not evaluated routinely by such sophisticated testing. Thus the reproductive history of the stallion becomes most important in predicting future fertility.

One also should evaluate the fertility of the mare. Too often the mare is selected as a broodmare candidate simply because of her availability. One should performa breeding soundness examination based on her age, history, and parity. The breeding soundness examination may range from a rectal and ultrasonographic examination of the reproductive tract to a cytologic testing, culture, and biopsy of the endometrium to a videoendoscopic examination of the endometrium. Inclusion of an ultrasound examination at every rectal examination not only enhances the continual education of the veterinarian but also aids in detection of many unpalpable changes. Most importantly, ultrasound examination ultimately enhances breeding efficiency and pregnancy rates by using the most sophisticated tools available.

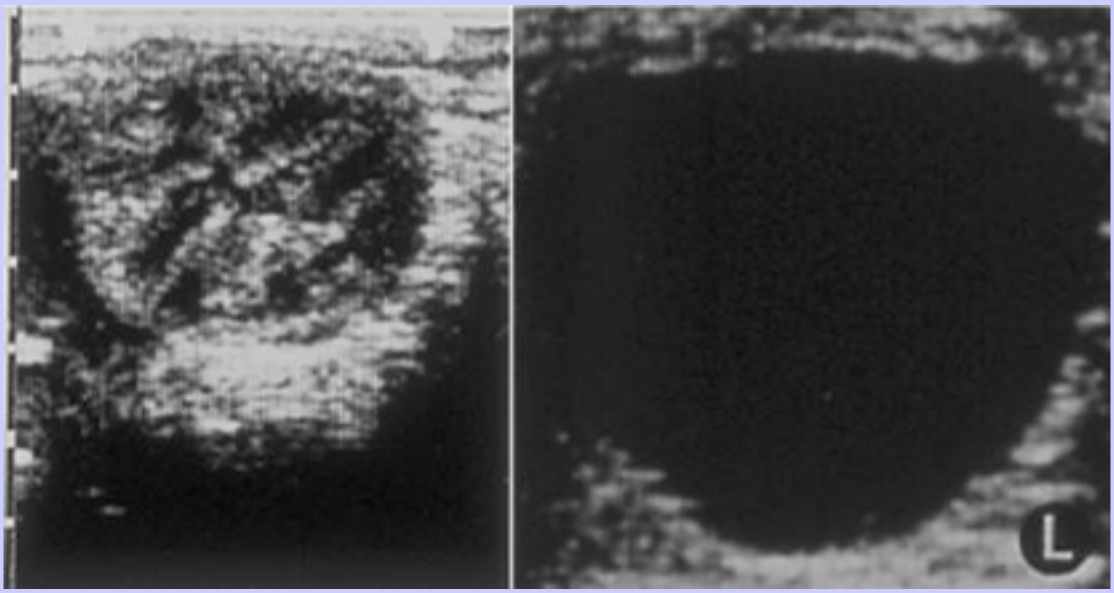
16.6.1 Preparation of the Mare for Breeding

The ultimate goal of insemination is to provide semen in a time frame that coordinates the availability of capacitated spermatozoa with the arrival of the transported oocyte within the oviduct of the mare. Therefore the timing of insemination with ovulation is critical.

One should examine mares in heat daily. During the estrous phase of the heat cycle, one performs an ultrasound examination of the reproductive tract to support and enhance the results of the rectal examination. The appearance of a dominant, growing follicle and the classic spoke-wheel pattern of endometrial edema, which one may quantify subjectively,[13] indicate pending ovulation (Figure 16.6-1). The ultrasound examination also may demonstrate the presence of echogenic particles within the follicle and increasing echogenicity of its wall, both of which indicate impending ovulation within the next 24 hours.[14] Finally, detection of a drop in follicular pressure by measuring transrectal tonometry is even possible and indicates pending ovulation.[15] Although the duration of heat in the mare may vary, most mares ovulate near the end of this estrous phase.[16] This follicular edema is less apparent on ultrasound examination just before ovulation. Because the timing of insemination with ovulation is so critical, the presence or absence of this edema can be a powerful tool to optimize pregnancy rates through the control of ovulation timing.

A number of agents are available that effectivelyshorten the interval to ovulation.[17–20] These agents includehuman chorionic gonadotropin and gonadotropin-releasing hormone analogs (see the discussion of hormonal manipulation in Section 16.5). These induction agents are reliable and effective if given at the appropriate time during estrus. If endometrial folds are apparent on the ultrasound examination and a dominant softening follicle is present, usually greater than 30 mm in diameter, one can expect human chorionic gonadotropin (HCG Intervet, Millsboro, Delaware), deslorelin acetate (Ovuplant, 2.2 mg; Fort Dodge, Iowa), and buserelin (Buserelin, Intervet, Millsboro, Delaware [not commercially available in the United States]) to hasten ovulation on average of 36,[17] 41 to 48,[17] and 24 to 48[13,18,21] hours, respectively, following administration. However, some disadvantages are associated with the use of deslorelin acetate. McCue, Farquhar, and Squires[22] have reported that in mares treated with deslorelin acetate, the interestrous interval may 1066
be prolonged on the average by 2 days because of a diestrous inhibition of pituitary follicle-stimulating 1067
hormone secretion. Removal of the implant at 2 days after administration appears to prevent the delay in return to estrus.

Figure 16.6-1 The appearance of a dominant, growing, softening follicle *(right)* and the classic spoke-wheel pattern of endometrial edema *(left)* indicate pending ovulation. (From Ginther OJ: *Reproductive biology of the mare*, ed 2, Cross Plains, Wis, 1992, Equiservices.)



16.6.2

Pasture Breeding

Successful pasture breeding requires an understandingof equine social structure. Every time a new memberis introduced to the herd, disruption of the social strata and a reorganization of the ranking occur. Theoretically, optimal field breeding management aims for a closed herd to minimize stress and enhance breeding efficiency.

Under field conditions, mares in heat approach the stallion for teasing. If interested, a stallion may mount a mare numerous times with or without an erection, with or without copulation, and most interestingly, with or without ejaculation. This behavior rarely is sanctioned in breeding facilities, often leading to undesirable behavior in breeding stock.

16.6.3

Breeding in Hand

Many breeding facilities continue to breed their stallions in hand, especially if the stallions belong to registries that do not allow registration of foals produced by artificial insemination. One perceived advantage to this management practice is that it may require fewer personnelor less time to breed the mare and therefore is more cost-efficient. However, this is not usually the case, for in-hand breeding poses far more risk to the mare, stallion, and handlers and therefore requires more personnelto ensure the safety of all involved. In-hand breeding also may require far more preparation of the mare than artificial insemination, especially in applying

restraining devices or removal of the foal for breeding. Thereforein-hand breeding does not necessarily reduce the time needed to breed a mare, especially if the cover does not go as planned.

If breeding in hand, good communication between personnel involved is paramount to ensuring the safety of the breeding. First, one prepares the mare in heat for natural cover by wrapping her tail to prevent laceration of the stallion's penis by the hair during breeding and washes her perineum with a mild soap, rinses it well, and dries it with a clean paper towel. One may apply a leather harness to her crest to prevent the stallion from biting her during breeding. One may apply hobbles to her rear legs to deter the mare from kicking the stallion.

Many choices of hobbles are commercially available. Desirable hobble attributes include comfort, ease of application, ease of removal (ensuring a quick release if needed), and simplicity so that the stallion cannot become entangled in the ropes or straps. Perhaps the simplest, safest, and most effective hobble design consists of a two pieces of soft cotton rope that fit many different mares (Figure 16.6-2). The first cotton loop slips loosely around the neck of the mare to rest against the point of both shoulders. The second portion is a longer cotton rope attached to a padded leather anklet designed to fit snugly around the rear pastern of the mare. One straps the anklet around the left rear pastern, brings the left rear leg well under the body of the mare, pulls the rope gently between the front legs of the mare, and ties a quick release knot just below the point of the shoulder on the left side. One may use additional restraint devices. One may apply a twitch just minutes before the 1067
stallion arrives or may sedate the mare. These additional restraints may inhibit the desired response, however, 1068
and the mare may fail to show signs of heat in response to the stallion.

Figure 16.6-2 A simple yet effective hobble.

All personnel stand on the left side of the horses, thereby allowing the horses to move unencumbered to the right if necessary. The mare handler steadies the mare as the stallion mounts and breeds her. The stallion handler is perhaps at the most risk during in-hand breeding (or semen collection) and therefore may direct the cover. Neither size nor strength is necessary in a stallion handler; rather a calm, firm, knowledgeable horse

person fares the best in the breeding shed. The quality ofthe ejaculate often reflects the expertise of the stallion handler.

After ejaculation a variable latency period occurs until the stallion begins to dismount. In field breeding the mare initiates the dismount by moving away from the stallion. Patience and ingenuity are needed duringin-hand breeding because theoretically the restrained mare cannot move. Because of a possibility that a mare may kick during the dismount, the heads of both horses are turned to left simultaneously, causing their hind ends to move to the right and thereby creating an adequate space between them. If one requires a dismount semen sample, expediency and experience are necessary to obtain it between the end of ejaculation and the dismount.

16.6.4 Artificial Insemination

Most equine breed registries, with the notable exception of the Jockey Club, not only have sanctioned but also have encouraged the use of artificial insemination. The advantages to breeding horses by artificial insemination are numerous and range from safety for the horses and their handlers to enhancing the value of the stallion by increasing the number of his offspring produced per year (Box 16.6-1). Use of frozen semen for inseminationhas additional advantages (Box 16.6-2). Althoughcryopreserved semen offers an attractive alternative for many reasons, it also can require more intensive mare management and in some cases results in lower pregnancy rates.

16.6.4.1 BOX 16.6-1 ADVANTAGES OF ARTIFICIAL INSEMINATION USING COOLED SEMEN

Allows many mares to be bred with a single ejaculate.

Prevents overuse of the stallion.

Enables mare owners to breed to any stallion regardless of distance, especially with cryopreserved semen.

Enhances and encourages genetic variability and hybrid vigor.

Controls management of the difficult breeder.

Reduces the chances of spread of infectious disease, venereal and systemic.

Reduces possibility of injury to mare and stallion.

Eliminates transportation and board expenses of the mare.

Allows evaluation of semen quality at each insemination.

Allows advance shipment of cryopreserved semen and increased availability.

May result in less variability in semen quality because of central processing station.

In an attempt to mimic the events of natural mating and yet ensure minimal contamination of the reproductive tract of the mare during artificial insemination, the following techniques have been described.[23] The mare should be restrained properly, preferably in stocks to protect the inseminator. Other devices, such as a twitch, have been used effectively for restraint but are usually unnecessary. If chemical restraint is warranted, the use 1068

of α-agonists such as xylazine (Rompun, Bayer Corp., Shawnee Mission, Kansas) may be preferable to other agents because of their effect on contractility of the uterus while it is under the influence of estrogen, particularly in mares exhibiting a delay in uterine clearance following insemination.[24] One bandages the tail of the mare and ties it away from contact with the vulva and perineum and washes the vulva well with liquid soap and rinses it thoroughly with water. One repeats the washing a minimum of three times until the area is visually free of any debris. One then should dry the vulva and perineal area with a clean paper towel. 1069

16.6.4.2

BOX 16.6-2 ADDITIONAL ADVANTAGES TO USING CRYOPRESERVED SEMEN FOR INSEMINATION

Ability to breed any stallion in the world, regardless of distance.

Ability to ship the semen well in advance of breeding so that it is available when needed.

Preservation of stallion genetics for many years.

Insurance against unavailability, injury, or death of the stallion.

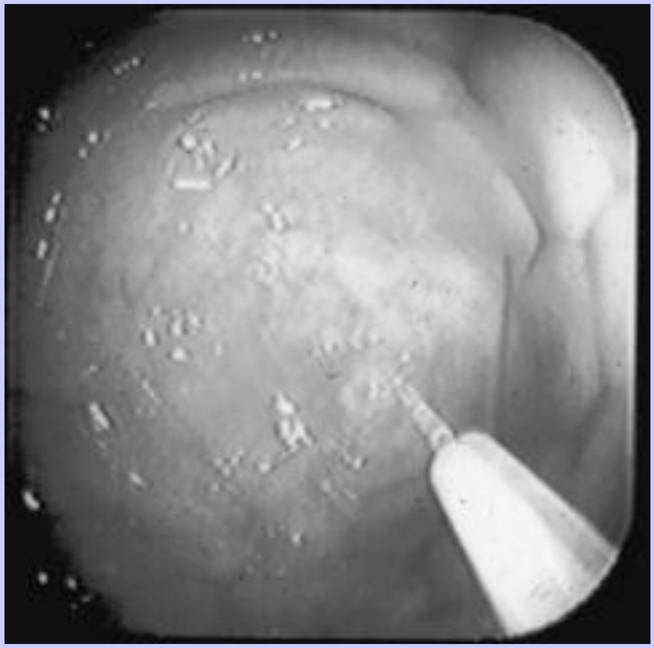
Less variability of semen quality.

If one is breeding with cooled semen, the semen does not have to be warmed after removal from the storage container. If one is breeding with frozen semen, one must thaw the semen before insemination. One must maintain stored semen at −196° C until ready for use, at which time one must follow the thawing and insemination instructions that accompany the frozen semen. Thawing protocols at high temperatures (50° to 75° C) require closer adherence to exact immersion times than do low temperatures (37° C). Depending on the cryopreservation techniques and sperm concentration per straw, a breeding dose may consist of a single straw(0.5 to 5.0 ml) or multiple straws.

The inseminator dons a sterile sleeve and grasps a sterile insemination pipette between thumb and palm to protect the tip in a sterile environment. If one is breeding with 0.5-ml straws of frozen-thawed semen, one mayuse a sterile insemination gun instead of the pipette. One applies nonspermicidal sterile lubricant sparingly to the sleeve covering the forefinger. Prepared semen should be contained in a nonspermicidal syringe and protected in the nonsterile hand from adverse environmental conditions such as ultraviolet light, cold, heat, and air.

The inseminator inserts the sleeved hand, continuing to protect the tip of the insemination pipette, through the lips of the vulva and into the vaginal vault and inserts one to two fingers through the cervical os. The finger(s) then act as a guide for advancement of the insemination pipette through the cervix approximately 1 cm into the uterus of the mare. If the advancement of the pipettehas not encountered resistance once in the uterus, one can advanced it carefully and gently into the desired (usually ipsilateral to the developing follicle) uterine horn. This advancement is not always possible because of the amount of endometrial edema and folding, and one must take extreme care to avoid damaging the endometrium. Once the pipette is satisfactorily in place, one depresses the plunger of the syringe and deposits the semen inthe uterus. If one encounters resistance on depressing the plunger, the tip of the pipette may be obstructed by the endometrium and may require repositioning. One may introduce a small amount of air to clear the pipette. Massage of the vaginal vault during withdrawal of the inseminator's hand may stimulate uterine contraction and aid in propulsion of spermatozoa to the uterotubal junction.

Figure 16.6-3 Through hysteroscopic insemination, lessthan 0.25 ml of semen is deposited on the oviductal papilla. (Courtesy E.L. Squires, Colorado State University, Fort Collins.)



The area of semen deposition has received great attention recently. Although pregnancy rates appear to be independent of volume of semen,[25] minute volumes and numbers of motile spermatozoa (as low as 1×10^6 motile sperm) deposited at the uterotubal junction (Figure 16.6-3) or within the oviduct have shown promising pregnancy results.[26–28] Furthermore, deep uterine insemination also enhances pregnancy rates.[26] Withthe procedure described by Rigby,[29] one first passes the insemination pipette transcervically into the uterine body; then directs it into the uterine horn ipsilateral to the dominant follicle; and through transrectal manipulation, moves the pipette into the horn by gently threading the uterus around the pipette. However, because ofthe degree of edema of the mare endometrium during estrus, one must exercise caution to avoid damaging the endometrium with this technique.

16.6.5 Optimizing Pregnancy Rates

Insemination of mares with a minimum insemination dose of 500×10^6 progressively motile spermatozoa every other day long has been accepted to maximize pregnancy rates.[30] Some stallions with apparently superior fertility may provide maximum pregnancy rates at much lower dosages. As with bulls, the minimal insemination dose for optimizing pregnancy rate may be male-dependent.[31] However, unlike with bulls, no evidence is currently available that increasing the insemination dose can compensate for morphologic defects in 1069
stallion sperm. Fortunately, the number of sperm in a single ejaculate from a normal stallion far exceeds this 1070
dose, provided that the stallion is not suffering from overuse. Therefore depending on the intensity of management, most breeding operations using in-hand breeding aim to cover mares shortly before ovulation or,

if the stallion is not breeding other mares, every 48 hours while the mare is in standing heat. To prevent overuse of the stallion and to enhance pregnancy rates, one should examine mares with transrectal palpations and ultrasound at least every other day and should breed them when ovulation is expected within 24 to 48 hours. Postovulation breeding, although at times successful, more often is associated with embryos of inferior quality.[10,32–34]

In a breeding program that uses artificial insemination, generally accepted fact is that the fertility of most stallion semen is greatest on the farm, followed by that of shipped cooled semen, with frozen semen being least fertile.[35] Exceptions to this rule invariably occur. Because the fertility of semen appears to be stallion-dependent, good breeding management requires an awareness of the minimal insemination dose necessary to maximize pregnancy rates for each stallion. The aim in a cooled semen insemination protocol is to provide a minimum insemination dose (500×10^6 progressively motile sperm) tobe inseminated every other day before ovulation. Jasko, Moran, Farlin, et al.[36] have reported further that the semen must be extended to 25 to 50×10^6 motile sperm per milliliter to achieve maximal pregnancy rates.

In an artificial insemination program that uses cooled shipped semen, often more than a single dose of semen is sent. Controversy exists regarding the fate of the unused doses: Should they remain in storage at 4° C, or should they be inseminated immediately into the mare? Supporters of the Equitainer (Hamilton-Thorne Research Inc., Danvers, Massachusetts) hypothesis argue that the sperm storage sites in the oviduct of the mare are filled with the first insemination and do not need to be replenished for at least 24 hours[37]; therefore using the mare as the incubator for the second dose is wasteful because the excess spermatozoa will be expelled. Those in favor of the mare as the optimal reservoir believe that although the motility of the second dose of semen fares well in the Equitainer, it does not necessarily reflect the fertility of this dose and therefore the sperm fare better in the mare.[38] The studies that have addressed this argument have used few stallions in the experimental design or have failed to take into account the interval from insemination to ovulation. In the study presented by Love, Thompson, Lowry, et al.,[39] maintaining the fertility of the sperm when cooled at 5° C may yet again be stallion dependent; stallions with poor fertility appear to have more chromatin damage with cooling than stallions with good fertility. The most convincing evidence for sequential breedings arises in a studies by Heiskanen, Huhtinen, Prihonen, et al.[40,41] These researchers found that mares bred with semen stored at 5° C for up to 40 and 80 hours have pregnancy rates of 87% and 65%, respectively, thus demonstrating acceptable pregnancy rates with stored semen. Still, the optimal means for preserving the fertility of extra doses of semen remains a controversial subject, and the answer is not entirely clear, perhaps because again the fertility may vary with individual stallions and mares.

Occasionally, a suboptimal dose of semen arrives: the semen may be extended improperly, the sperm numbers may be low, or the motility or morphology may be poor. If the total volume of semen is not so great that it is expelled through the cervix on insemination, one should make every attempt to inseminate enough semen to comprise the optimal dose for pregnancy. If the volume is too great, one may centrifuge the semen, but this may jeopardize the motility of the semen. Therefore reinseminating the mare 6 to 12 hours after the first insemination with a second dose is perhaps best in attempt to cover her with an optimal dose over 24 hours.

Pregnancy rates with transported cooled semen have been reported to range from less than 20% to greater than 80%.[42] Although somewhat dependent on the fertility of the mare and stallion, the extreme variability of the fertility rate often is due to other management factors. These factors include handling and management of the stallion, handling and management of the semen, extension of the semen, shipment of the semen, number of motile sperm inseminated, management of the mare, preparation of the mare for breeding, insemination technique, handling of the semen during insemination, and postinsemination management of the mare.

Therefore pregnancy rates reflect not only the fertility of the horses but also the expertise and conscientiousness of the personnel involved in this endeavor.

With cooled semen, insemination of mares within24 hours before ovulation is recommended to allow ample time for capacitation of the spermatozoa and transport to the oviduct. If ovulation fails to occur in the expected time frame, reinsemination every 24 to 48 hours with at least 500×10^6 progressively motile spermatozoa should optimize pregnancy rates. However, repeated inoculation of the uterus with rebreeding can cause problemsin mares that are susceptible to endometritis or a delay in uterine clearance.

Pregnancy rates with frozen-thawed semen also demonstrate a wide range of success. Cryopreserved semen from most stallions generally is accepted to have lower fertility than cooled semen, but this is not always the case. Frozen-thawed semen probably does not maintain viability within the mare for as long as fresh or fresh-cooled semen; this effect has been demonstrated in culture.[43] Ellington, Ball, Blue, et al. have suggested that 1070
the cause of this short viability may be due to the capacitation-like changes associated with frozen-thawed 1071
sperm.[7] Furthermore, fewer numbers of spermatozoaare found bound to the oviduct of the mare following insemination with frozen-thawed semen. If indeed a selective mechanism exists for sperm-binding capacity within the oviduct and that sperm must be noncapacitated, then in the process of freezing and thawing, irreversible damage may cause spermatozoa to lose this binding ability.[43] Lower pregnancy rates associated with insemination of frozen-thawed semen also could be caused at least in part by the intensive management often required for success. Although many researchers have demonstrated acceptable per cycle pregnancy rates of 25% to 45%[44–46] when breeding every 24 hours or postovulation, much higher pregnancy rates (>70% per cycle) have been achieved with intensive mare management that includes pre- and postovulation breeding.[44–49] In practice, the cost of frozen semen often precludes frequent inseminations.

The results of a recent retrospective study performedby Loomis[49] demonstrated that no significant difference existed in first-cycle pregnancy rates, seasonal pregnancy rates, or number of cycles per pregnancy between cooled and frozen semen shipped from one facility to a large number of mare owners. In this study, the inseminators were instructed to breed the mares with the frozen-thawed semen pre- and postovulation and close to ovulation. In this instance, management and selection of the stallions was optimal, and the stud farm was involved closely (through instruction and communication) with selection and insemination of the mares. Therefore management and selection of breeding stock may assume even greater importance when breeding with frozen semen.

The number of morphologically normal, progressively motile sperm that yields optimal pregnancy rates with frozen-thawed semen is likely, again, to be stallion and management dependent. In one objective study, Leipold, Graham, Squires, et al.[45] found that the optimal concentration at which to freeze spermatozoa, with respectto pregnancy rate, is equal to 800×10^6 total sperm contained in a 0.5 ml straw. However, the number of stallions was limited in this study, and per cycle pregnancy rates, although acceptable, were only 40%. Most researchers suggest that a minimum of 200×10^6 progressively motile sperm are necessary for an optimal breeding dose, and many inseminate with a higher number. Researchers that report per cycle pregnancy rates in excess of 50% aim to inseminate at least 200×10^6 motile sperm pre- and postovulation, with only a 6- to 8-hour interval between inseminations (with ovulation occurring during this interval).[47–49]

Although predicting ovulation is challenging and management of mares to be inseminated with frozen semen is time consuming, timely treatment with deslorelin acetate can ease management greatly. Not only does this drug

reliable stimulate ovulation, but also the window of ovulation is so small that far fewer examinations of the mare are necessary for success. Furthermore, its use can curtail, if not eliminate, late-night breedings.

16.6.6 Future Techniques

Procedures that were once considered to be advanced reproductive technology have become a routine part of many private practices. These procedures incude embryo transfer, gamete intrafallopian transfer, and cryopreservation of spermatozoa and oocytes, to name a few. These techniques offer the possibility of preserving valuable lines that otherwise may be lost in breeding stock. However, the increased awareness of management techniques and fertility evaluations perhaps has permitted the greatest increase in breeding efficiency found in the horse industry today.

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16.7
16.7—Equine Embryo Transfer

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Unlike the stallion, the mare is limited in the number of offspring that she can produce in her lifetime. Embryo transfer offers a means for enhancing production of offspring from mares of superior genetic lines or that are incapable of carrying a foal to term. More specifically, this procedure offers the following advantages:

1. Embryo transfer allows a mare to remain in competition while still producing valuable offspring.
2. Embryo transfer allows production of offspring from mares suffering from pathologic conditions of the uterus severe enough to prevent carrying a foal to term.
3. Embryo transfer allows production of offspring from mares suffering from systemic illness that prevents them from carrying a foal to term.
4. Embryo transfer allows for production of offspring from mares with musculoskeletal disease in which their health may be at risk for carrying a pregnancy to term or delivering a foal (fractured pelvis or hip or severe arthritis, for example).
5. Embryo transfer prevents risk of postfoaling complications in the valuable mare or older mare at risk.
6. Embryo transfer allows more than a single offspring to be born within a given year.
7. Immature (2-year-old) mares yet unable to carry a foal to term are capable of producing embryos that have a good survival rate in mature recipient mares.
8. In breed registries in which the value of the offspring often depends on a determined birthdate, embryo transfer allows mares that foal late in the season to produce an embryo for that year and yet also to be prepared to be bred early on the subsequent season because they have been left open.
9. The procedure has proved invaluable in many research projects comparing effects on twins that arise from embryo splitting or double ovulation.

As breed registries have permitted registration of the foals produced by embryo transfer, the technique rapidly has become a routine procedure offered in many equine private practices. Although most practices do not maintain a herd of recipient mares and most small-scale mare owners do not provide enough recipients, the procedure of flushing embryos from donor mares and shipping the embryos to recipient mares at a distant location has grown in popularity. Still, despite the numerous advantages to transferring embryos, the procedure is an expensive endeavor that may cost in veterinary and mare care alone in excess of $10,000.[1] Coupled with the stud fee, these offspring, if the procedure is successful, are indeed valuable.

16.7.1
Factors Affecting Success Rates

Three major factors affect success rates in an embryo transfer program: the fertility of the mares (donor and recipient), the fertility of the semen, and the expertise of all technical and veterinary personnel. Best management practices optimize all three factors for success. One should evaluate all horses involved by a

breeding soundness examination, the extent of which depends on the age, history, and parity of the mares and the reproductive history of the stallion.

16.7.1.1 FERTILITY OF THE DONOR MARE

The ideal candidate for a donor mare in an embryo transfer program is a 3- to 10-year-old, healthy, reproductively sound mare. Foss, Wirth, Schlitz, et al.[2] reported high embryo recovery rates from show mares (84.2%) followed by an acceptable rate from multiparous, nonbarren mares (59.7%). However, mares with a history of infertility showed a significantly lower embryo recovery rate (30.0%). Unfortunately, the value of many mares is not realized until they are older, and some offspring have exhibited superior performance. Age of the mare appears to play a major role in fertility[3]; early loss of pregnancy is a common and frustrating problem encountered in older mares. Investigation into the causes of this age-associated infertility has led researchers to examine changes that occur in the reproductive tracts of aging mares: the uterine environment, the uterine tube environment, and ovarian function.

The numbers and quality of oocytes differ greatly between young and aged mares.[4] Oocytes from older mares (i.e., >20 years) have significantly more morphologic anomalies.[5] The clinical impression of many practitioners is that delayed development occurs in embryos obtained from older mares.[4,6] In evaluation of equine embryo quality and viability, not only have young mares (<10 years old) been reported to produce more embryos, but also the embryos that are recovered from them are of significantly superior quality compared with embryos from older mares.[7–9] Embryos are graded based on morphologic characteristics and size (grade 1, excellent; grade 4, poor).[10] Transfer of grade 1 embryos results in significantly higher 50-day pregnancy rates (70% to 80%) than transfer of grade 4 embryos (30% to 40%).[9] Transferred embryos classified as grades 1 and 2 demonstrate significantly higher pregnancy rates than grade 3 embryos.[10] Embryo loss between 12 and 50 days of gestation was significantly greater with grade 2 embryos than with grade 1 embryos.[11]

Because the number of blastocysts recovered fromthe oviductal lumen 2 days following ovulation are not different based on mare age,[6,11] fertilization and early cleavage steps may not be the limiting factors in 1073
production of embryos in older mares. However, pregnancy ratesat 4 days demonstrate a significant 1074
difference based on mare age.[6,12] Although age-associated degenerative and inflammatory changes to the endometrium and its associated blood vessels of the mare are well documented and in some cases may contribute to lower foaling rates in old mares, the defect that causes early embryonic losses appears to occur before the mature blastocyst can be found within the uterus.[13] Early (2-day) pregnancy rates are not different based on age of the mare,[12] but 3-day pregnancy rates are decreased significantly in aged mares.[14] Day 4 embryos collected from the uterine tube of old mares and transferred to the uterus of young normal mares show significantly lower survival rates.[15]

Apparently, older mares require superior nutritional support, particularly during the vernal transition period to hasten the interval to ovulation.[16–19] Follicular development is prolonged in older mares, especially as they approach ovarian senescence.[20,21] The most common cause of early pregnancy loss in older mares appears tobe an intrinsic defect within the oocyte itself or in the physiologic events that occur before the formation of a day 3 embryo. Other factors, such as failure of maternal recognition of pregnancy, immune-

mediated causes, chromosomal aberrations, reaction to stress, nutrition, hormonal insufficiencies, and postovulatory insemination may play a role in early pregnancy loss or delayed embryonic development.[22]

16.7.1.2 FERTILITY OF THE RECIPIENT MARE

A young, healthy reproductively sound mare is the ideal candidate to act as an embryo recipient. Factors that further enhance success rates in the recipients include good nutrition, good general health care, minimal stress, and hormonal synchrony with the donor mare. Ideally, the recipient mare should ovulate during a time period from 1 day (+1) before to as many as 6 days after (–6) the donor mare to optimize pregnancy rates.[23,24] Carnevale, Ramirez, Squires, et al. have reported that embryo loss rates are significantly higher in recipient mares that ovulate 7 days or more (–7) after the donor mare.[11] One may encourage synchrony between the ovulations of the donor and recipient mares through the administration of prostaglandin $F_2\alpha$ alone or with progestin compounds.[25] Ovulation-induction agents such as human chorionic gonadotropin or deslorelin may further enhance desired synchrony.

To eliminate the need for synchrony, many researchers have used ovariectomized recipient mares that have been treated with progestins alone or a combination of progestins and estrogen.[26–28] Although McKinnon, Squires, Carnevale, et al.[28] found that pregnancy rates were similar between ovariectomized, progesterone-supplemented mares and synchronized recipients, results in the field have been unreliable using ovariectomized recipients, perhaps because ovariectomized mares that remain open for a season appear to be less optimal candidates for subsequent recipient seasons. From an economic standpoint, ovariectomized mares also require upward of120 days of progestin supplementation.[29] Researchers also have evaluated the suitability of hormone-treated mares in the beginning of the transitional period as embryo recipients, but pregnancy rates are often not as high as those in recipients that are cycling regularly.[11]

Uterine tone at day 5 following recipient ovulation represents a useful predictive indicator of success in transfer. Recipient mares with good uterine tone 5 days after ovulation had significantly higher pregnancy rates at day 50 than mares with poor to fair uterine tone.[11] McCue, Vanderwall, Keith, et al.[30] have shown that mares that qualify as recipients based on uterine tone have significantly higher progesterone concentrationsat day 5 than mares who failed to qualify. This findingis consistent with another study reported by Sevinga, Schukken, Hesselink, et al.[31] that found that the size of the corpus luteum and progesterone production are higher at day 8 or 9 after ovulation in pregnant versus nonpregnant mares.

Although veterinarians generally accept that the parturient mare determines the size of the foal at birth, research has demonstrated the need to avoid choosinga recipient pony mare to deliver the foal of a donor Warmblood mare.[32] Allen[33] has demonstrated that the size of the recipient mare does affect the adult size of the offspring. Wilsher and Allen[34] split equine embryos and transferred these identical twins into recipient mares of varying heights. Although the mare appeared to determine the size of the foal at birth, the adult height ofthe transferred embryo appeared to depend on the size of the recipient mare. Therefore perhaps one is wiserto consider choosing donor and recipient mares of similar size.

16.7.1.3 FERTILITY OF THE SEMEN

The quality of semen affects the success rate of embryo recovery.[35] In general, one obtains the best results when the mare is inseminated with semen collected on the farm (or is bred under natural cover), followed by

sementhat is cooled and shipped to arrive within 24 hours of collection, followed by frozen semen.[36] High pregnancy rates have been demonstrated when the mares are inseminated pre- and postovulation during a 12-hour period.[37] Results from an embryo transfer program ina private practice over a 2-year period of evaluation support this discrepancy in fertility based on the typeof semen, with fresh, chilled, and frozen semen resulting in embryo recovery rates of 55%, 44%, and 32%, respectively.[38] 1074

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16.7.1.4

EXPERTISE OF THE VETERINARIAN

The veterinarian's role is not only to determine the optimal time for breeding but also to recognize abnormalities as they arise. Abnormalities such as delayed uterine clearance[39] or hemorrhagic follicles can be detrimental to successful embryo recovery. The veterinarian also should be experienced in embryo recovery and transfer techniques to achieve the most successful pregnancy rates. Owners need to have realistic expectations before embarking on this expensive endeavor. They must realize that even with a young, fertile mare bred with fertile semen and under the care of an experienced veterinarian, the best embryo recovery rates are only 70% to 80%. If semen shipped cooled or frozen is used, then the success rates are often lower.[40]

16.7.2

Procedure for Embryo Recovery

Most embryo flushes are attempted on days 7 or 8, with the day that the donor ovulated designated as day 0.Day 0 is the first day that a corpus hemorrhagicum is visible on transrectal ultrasound examination, assuming that mare has been examined at least daily. If one examines a mare more frequently, as is often the case when breeding with frozen semen, one still determines day 0 by the first appearance of the corpus hemorrhagicum. Occasionally, another question of ovulation may arise because of the presence of a hemorrhagic follicle; these anomalies occur more often in transitional and older mares. In these cases, ovulation generally is believed not to have occurred.[41]

The developing embryo enters the uterus after day5 at the morula or early blastocyst stage.[42] Enormous growth of the blastocyst and rapid expansion of the inner cavity, the blastocoele, occur during the next few days. Most embryos destined for transfer are flushed as expanded blastocysts (days 7 or 8) because they appear to optimize transfer pregnancy rates.[43–45]

One lavages the uterus of the donor serially with liters of Dulbecco's phosphate buffered saline or synthetic oviductal fluid warmed to 37° C (Figure 16.7-1). One may add antimicrobial agents and 1% fetal calf serum (FCS; Hyclone Laboratories, Logan, Utah) to the lavage medium. Although many Foley-type catheters have been used for the lavage, a large-bore (8.0-mm) sterile silicone catheter (80 cm; Bivona ET flushing catheter, Western Veterinary Supply, Grapevine, Texas) with an inflatable cuff is often ideal for most donors presented in practice. The uteri of some older or multiparous mares may require more than a single liter of fluid to distend the uterus. Likewise, the smaller uteri of maiden mares may take less than 1 liter for maximum distention. Often one can determine this by the ease of fluid flow into the uterus. One evacuates the uterus by gravity flow and passes the recovered fluid through a sterile recovery cup lined with a 0.75-μm filter (Harvet, Spring Valley, Wisconsin). One must ensure that the cup does not run dry; a certain amount of fluid must remain in this cup at all times to bathe the embryo. Even though most embryos are recovered in the first flush, one must repeat this procedure 2 to 3 more times, always measuring the recovered fluid on each flush so that when the procedure is complete, most of the fluid has been recovered. One performs transrectal massage of the uterus for the last two flushes, ensuring recovery of all fluid introduced to the uterus.

Once in the laboratory, one gently swirls the contents of the cup and pours the contents into search dishesthat have been maintained at 37° C. One rinses the cupwith 20 ml of remaining Dulbecco's phosphate buffered saline solution and decants it into the search dishes. Using a stereoscopic microscope, usually at 15× magnification, one methodically seaches for the embryo. Once found, one evaluates the embryo at higher magnification for age, size, and morphology and then washes it 3 times in lavage medium with 10% FCS in preparation for shipping or transfer. Often one adds antimicrobial agents to this medium as well.

16.7.3 Embryo Transfer into theRecipient Mare

Surgical and nonsurgical techniques have been used successfully to transfer mare embryos. Surgical transfer via a standing flank laparotomy demonstrated the most favorable pregnancy rates for many years. However, this procedure is more expensive, more labor intensive, and time consuming for the veterinarian, and it poses a higher risk to the recipient mare. Over the past few years, nonsurgical transfer has been reported to achieve similar, if not superior, pregnancy rates for many clinicians.[2,40,46] This success rate appears to depend on the experience and expertise of the veterinarian performing the transfer.

One loads the embryo into a sterile 0.25-ml or 0.50-ml straw using a ureteral catheter adapter (Cook Urological, Spencer, Indiana) attached to a tuberculin syringe. The size of the straw depends on the size of the embryo. To ensure the midstraw location of the embryo at all times before transfer, one first loads the straw with lavage medium (10% FCS), then with an airpocket, then with medium (10% FCS) containing the embryo, then with another airpocket, and followed at last by medium(10% FCS). For nonsurgical transfer, the first fluid section must penetrate the wick of the straw to prevent loss of the contents of the straw by gravity. This sequence not only allows verification of the location of the embryo but also reduces the amount of fluid inoculated into the uterus of the mare on transfer. 1075

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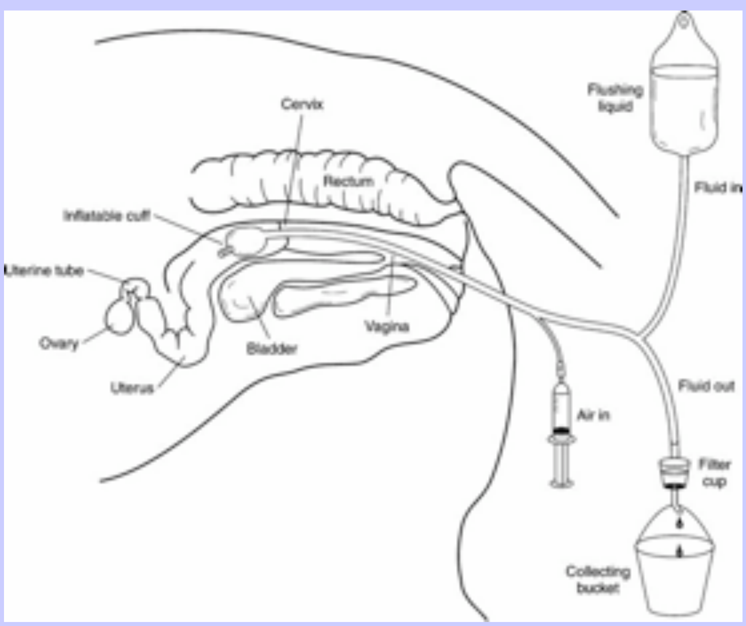
Figure 16.7-1 Embryo flushing: the inflated catheter seals the cervix. (Courtesy T. Ayres, Aurora, Oregon.)



One loads the straw into a sterile insemination gun. Although several commercially available guns have been designed to fit the different sizes of straws, some veterinarians prefer to use a stainless steel reusable gun, and others prefer an intrauterine pipette for transfer of large embryos. Manipulation of the cervix or inoculationof the uterus during transfer long has been considered detrimental to a successful pregnancy. This invasion of the reproductive tract of a diestrus recipient mare also has been suspected potentially to lead to eventual regression of the corpus luteum via prostaglandin and/or oxytocin release. Thus one must take many steps to preserve the corpus luteum and thus the transferred pregnancy.

Although studies have shown that no benefit accrues from treating the recipient mare with altrenogest (Regumate, Intervet Inc., Millsboro, Delaware) or flunixin meglumine (Banamine, Schering-Plough Animal Health, Union, New Jersey), many practitioners continue to administer these drugs to mares before transfer of the embryo. Some also use a single intramuscular dose of procaine penicillin (Aquacillin, Vedco, St. Joseph, Missouri) for its antibiotic action, whereas others administer dexamethasone (Dexaject SP, Burns Veterinary Supply, Rockville Centre, New York) for its antiinflammatory effects, terbutaline (Franck's Veterinary Pharmacy, Des Moines, Iowa) for its tocolytic action on the uterus, and propantheline bromide (Franck's) for transient relaxation of the rectum.[2]

Unfortunately, little scientific data is available to validate the merits of these therapies. Sedation of the recipient mare depends on her temperament and degree of discomfort during the procedure. Based on work by LeBlanc, DeLille, Cadario, et al.,[47] tranquilization using an α-agonist such as dormosedan may cause less stimulation of uterine contraction because the uterus of the recipient mare is under the influence of progesterone from the corpus luteum. When β-adrenoceptors predominate, they inhibit contractions.

One secures the loaded sterile embryo transfer gun, perhaps shielded by a sterile outer guard sleeve, in the palm
with the thumb. One applies a small amount of sterile lubricant and inserts the hand and gun transvaginally. 1076
One digitally palpates the external cervical os and passes just the tip of the gun gently through the guard sleeve 1077
and then the remainder of the cervix. At this point, many prefer to deposit the embryo in the uterine body. Ifone is planning to deposit the embryo in the uterine horn instead, the veterinarian then inserts a well-lubricated arm into the rectum of the recipient, locates and grasps the uterine horn, and gently threads the tipof the gun into the uterine horn. One slowly depresses the plunger, releasing the embryo from the straw asthe gun is withdrawn. Because of equine embryo mobility, into which horn the embryo is deposited does not matter.

One performs surgical transfer of an embryo on a sedated recipient mare aseptically prepared for a routine flank incision. Usually one uses an inverted L or line block over the paralumbar fossa for local anesthesia. The surgeon exteriorizes the uterine horn of the mare and makes a small puncture with a cutting-edge suture needle. The surgeon then enlarges the hole slightly with iris forceps and inserts the sterile straw containing the embryo through the hole into the uterine lumen to deposit the embryo. The surgeon places the uterine horn back into the abdomen and then repairs the incision with a routine three-layer closure.

One can detect a developing embryonic vesicle by transrectal ultrasound examination as early as 5 daysafter transfer. If the embryonic vesicle is not visible at this time, one must plan to recheck the mare every 48 hours at least through day 16. Carnevale, Ramirez, Squires, et al.[11] have shown that the presence or absence of an embryonic vesicle at 5 days is an important predictorof pregnancy loss by 50 days. Recipient mares in which the embryonic vesicle is large enough to be seen on the first pregnancy examination (usually 5 days) have fewer embryonic deaths than mares in which the vesicle is not seen until subsequent examinations. Ginther, Bergfelt, Leith, et al.[48] support this theory; they also found that undersized embryonic vesicles are associated with a higher rate of pregnacy loss.

16.7.4 Shipping Embryos

Carney, Squires, Cook, et al.[49] have shown that no significant differences occur in pregnancy rates between mares that have embryos transferred immediately after collection and mares that receive cooled, transported embryos. The expense of maintaining open recipient mares is considerable, and a number of commercial recipient herds are now available to practitioners. Most of these commercial herds have limited numbers of mares, so having contracts signed and mares reserved early in the breeding season is important.

Preparing the embryo for shipping is uncomplicated. Most embryos in the United States are shipped in Ham's F-10 (Sigma Chemical Co., St. Louis, Missouri) medium buffered to an optimal pH with a mixture of 5% CO_2,5% O_2, and 90% N_2 gas.[50] The final preparation of the medium contains 10% FCS (filtered through a Millipore filter) and antimicrobial agents (usually a combinationof penicillin and streptomycin). Although most larger commercial operations send the buffered medium on ice to the practitioner the day before the embryo flush, this transport medium is simple for the practitioner to make in the clinic, providing that a tank of the appropriate gas mixture is on hand.

Several other media are available for embryo transport that eliminate the need for the gas buffer of Ham's F-10, which is necessary to maintain the correct pH for embryo transport. Moussa, Duchamp, Bruyas, et al.[51] reported that two commercially available embryo holding media (Emcare Holding Solution [ICP, Auckland, New Zealand] and ViGro [AB Technology, Pullman, Washington]) appear to have an effect on embryo viability similar to the gas-buffered Ham's F-10 for 24-hour cooled storage of embryos. However, this study did not examine pregnancy rates after transfer. Fleury, Fleury, and Landim-Alvarenga[52] recently reported that using hydroxyethyl-piperazine ethane-sulfonate (HEPES)-buffered Ham's F-10 (with 0.4% borine serum albumin [BSA]) for temporary storage of embryos gave 15-day pregnancy rates of greater than 75% in recipient mares. This medium also eliminates the need for incubation with the gases. In this experiment, embryos were stored at ambient temperature, between 15° and 24° C, thereby eliminating the need for cooling as well.

One transfers the washed embryo using a sterile0.25- or 0.5-ml straw to a 6-ml snaptop tube containing the transport medium. One tightly closes the top and places the tube in a larger tube (50-ml centrifuge tube) also filled with transport medium. One then places both tubes into an isothermalizer cup (37° C) and places that into an Equitainer (Hamilton-Thorne Research Inc., Danvers, Mass.) for shipment.

Some commercial operations with recipient herds prefer that the embryos be shipped via commercial airlines in attempt to minimize the time that the embryo spends outside of the uterus of the mare. Others believe that commercial transport companies that deliver the embryo within 24 hours are acceptable and that pregnancy rates do not differ between 12-hour and 24-hour shipment.[53] Foss[54] has shown that pregnancy rates do not differ between embryos transported by airlines versus those transported by courier services. The largest factor in choosing the means of transportation is reliability of service. Whatever means of transporting the embryo one ultimately chooses, the imperative is to ensure that the paperwork is filled out correctly and that the shipment is insured. 1077

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16.7.5 REFERENCES

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16.8 16.8—The Pregnant Mare

Grant S. Frazer

16.8.1 Physiology of Gestation

16.8.1.1 MATERNAL RECOGNITION OF PREGNANCY

The zygote begins its journey down the oviduct once the oocyte has been fertilized, reaching the uterine horn by day 6.[1,2] Detailed descriptions of the early development of the equine embryo have been published and are beyond the scope of this text.[3,4] Although the term *embryo* is used routinely to describe the early conceptus, one must understand that what generally is being discussed is the embryonic vesicle and the embryo itself. These two structures ultimately develop into the fetal membranes and fetus, respectively. Embryonic development has progressed sufficiently by day 40 of gestation that the term *fetus* is used thereafter.[4]

The longitudinal arrangement of the endometrial folds may favor mobility of the spherical equine embryo.[4] After entering the tip of the uterine horn on day 6, the embryo is moved down toward the body by day 8.[5,6] The intrauterine mobility phase continues until days 15to 17, and during this period, the embryo may move between horns 10 to 20 times each day.[2,4,6,7] To maintain the pregnancy, the embryo must send a signal to the endometrium to prevent the cyclic release of the uterine luteolysin (prostaglandin $F_{2}\alpha$, or $PGF_{2}\alpha$).[8] An embryo-derived factor is the most likely cause for the suppression of $PGF_{2}\alpha$ release and interruption of the oxytocin-$PGF_{2}\alpha$ interaction in mares during early pregnancy.[9,10] The mobility of the spherical equine conceptus is critical for blockage of luteolysis,[3] and for this reason, large uterine cysts may cause pregnancy loss.[11,12] If the enlargingvesicle cannot pass by a cyst, it may be unable to contact all endometrial surfaces and thus inhibit $PGF_{2}\alpha$ production.[13] Yet another unique feature of equine reproduction is that the luteolysin is delivered to the ovary systemically.[14] Such delivery contasts with the local countercurrent uteroovarian route in domestic ruminants in which intimate contact occurs between the ovarian artery and uteroovarian vein.[15] The embryo typically becomes fixed at the base of one of the uterine horns. In postpartum mares this is usually the most involuted horn.[16–18]

16.8.1.2 PLACENTATION

The trophoblast cells of the early embryo are destinedto form the absorptive placental contact with the endometrium.[4] The mare has a unique nondeciduate, epitheliochorial, diffuse, microcotyledonary placenta.[3,19–22] The entire maternal surface of the fetal membranes becomes covered with delicate microvilli (diffuse) that interdigitate with the proliferating luminal epithelial cells to form intricately branched microcotyledons.[20,21] The interdigitation tends to be deeper at the tip of the nongravid horn, and this helps explain the higher incidence of fetal membrane retention in that horn.[23] In a normal pregnancy the noninvasive allantochorion extends slowly to fill the uterus by days 80 to 85, and its microcotyledonary architecture, which provides hemotrophic and histotrophic nutrition for the growing fetus, is not established fully until days 120 to 140.[2] During the second half of gestation, most of the mitotic activity is confined to the periphery of the microcotyledons, which are still growing.[24]

Degenerative changes (endometrosis) in the maternal endometrium can affect adversely the ability of the 1079
microcotyledonary placenta to facilitate optimal hemotrophic nutrition and exchange of waste products.[25–32] 1080
If the pregnancy is maintained, dysfunctional placental attachment can lead to the birth of small, weak foals (intrauterine growth retardation).[33,34] Likewise, the presence of twin fetal sacs deprives variable areas of each allantochorion of the necessary endometrial contact to promote development of the diffuse microvilli. Failure of development of the microcotyledons results in the characteristic white, avillous portions on the maternal surface of abortedtwin fetal membranes (see Early Pregnancy Loss and Abortion).[35,36] Although the histotrophic form of nutrition is likely to remain important throughout gestation, adequate hemotrophic nutrition is essential to support the rapid fetal growth that occurs during the latterpart of gestation.[2,22,25] The morphology of endometrial blood vessels in uterine biopsy can vary considerably depending on the age and reproductive status of the mare. In a recent study, inflammatory vascular alterations were observed in 20.5% of the endometrial specimens examined.[32] Smaller and larger arterial and venous vessels demonstrated mild to severe degenerative lesions. Unaltered vessels were detected only in maiden mares.

The incidence and severity of angiosis increases with the number of previous pregnancies and with advancing age. Changes in the endometrial vasculature of multiparous mares has been compared with the so-called pregnancy sclerosis of other species, with fraying and disruption of the membrana elastica interna, medial and adventitial elastosis and fibrosis, and calcification processes within the media. Cycles of vascular growth during pregnancy and subsequent involution post partum are thoughtto result in progressive degenerative vascular changes, as occur in multiparous mares. Aging processes, chronic inflammation, and short foaling intervals have been suggested as additional detrimental factors.[28,32] Severe angiosis frequently is combined with phlebectasia and lymphangiectasia, possibly indicating a reduced abilityof the vessels to adapt to the varying demands of uterine circulation, with a decrease of uterine perfusion and lymph drainage. Angiosis in older, multiparous mares therefore might be related intimately to infertility, possibly because of detrimental effects on early embryo nourishment and subsequent placentation.[28,32,37]

Some interesting embryo transfer experiments have demonstrated the effect that uterine capacity—and placental area—can have on the size and birth weight of the foal.[38–40] In one study, pony embryos were transferred into Draft mare recipients, and then the genetic dams were permitted to carry a full sibling to term. The final experimental outcome was three pairs of sex-matched, full-sibling pony foals. The embryo transfer foals were all larger than their siblings at birth. Although the increased milk production of the Draft mares may have explained the subsequent faster growth rates, the three foals gestated in Draft mares notably were still heavier at 4 years of age.[38] A similar embryo transfer experiment used a Thoroughbred-in-pony and pony-in-Thoroughbred comparison, with Thoroughbred-Thoroughbred and pony-pony transfers acting as controls.[40] The pony-in-Thoroughbred foals were heavier than the Thoroughbred-in-pony foals. Comparison of placental measurements (weight, volume, and surface area) confirmed the effect of nutrient supply on birth weight.[39,40] These studies confirmed earlier work in which a Shetland pony mare was inseminated with Shire semen, and a Shire mare was bred with Shetland pony semen. The foal out of the pony mare was 50% of the weight of the half-sibling that was gestated in the Shire mare. The considerable size discrepancy remained even after these half-siblings reached maturity.[41]

Equids possess the unusual ability to interbreedfreely among the phenotypically and karyotypically diverse member species of the genus to produce viable but usually infertile offspring. The mule (female horse crossed with a male donkey) is a historical example of this successful interbreeding. What is even more amazing is that mares and donkeys have been shown to be capable of carrying to term a range of true, xenogeneic extraspecies pregnancies created by embryo transfer. In these instances all of the allantochorion tissue is composed of foreign protein (see the following discussion of endometrial cups). Successful pregnancies include Przewalski's horse (*Equus prezwalskii;* $2n = 66$) and Grant's zebra (*E. burchelli;* $2n = 44$) crossed with the horse (*E. caballus;* $2n = 64$).[42]

16.8.1.3 ENDOCRINOLOGY

By day 35 a specialized, circumferential group of trophoblast cells (chorionic girdle) detach from the embryonic vesicle, and some cells successfully invade the endometrium,[2,3,43–47] where they give rise to discrete aggregates of endocrinologically active tissue (endometrial cups).[2,43,44,48] These endometrial cups become visible by day 38 to 40 as a ring of small, pale plaques that surround the developing conceptus.[3,4] Because the maternal and fetal cells (sire contribution) have differing genotypes, the fetal antigens invoke a cell-mediated immune response.[4,49,50] The influx of lymphocytes creates a pronounced, localized

immunologic reaction, and by day 120 of gestation the endometrial cups are sloughed.[49–52] The necrotic tissue and inspissated secretions form invaginations into the chorioallantois (allantoic pouches).[53] These structures are visible on the allantoic surface of the fetal membranes after the mare has foaled.

The endometrial cups produce equine chorionic gonadotropin, formerly known as pregnant mare serum gonadotropin. Equine chorionic gonadotropin (eCG) is a large-molecular-weight glycoprotein that has
activity like follicle-stimulating hormone and luteinizing hormone.[54] Periodic surges (10 to 12 days apart) of 1080
pituitary follicle-stimulating hormone release drive a series of follicular waves on the equine ovary during 1081
early pregnancy.[2,55] The eCG promotes luteinization (and sometimes ovulation) of the dominant follicle in each wave.[3,56–58] These accessory (secondary) corpora lutea augment the progesterone production from the original structure that developed at the time of conception. This process is critical to ensure pregnancy maintenance before the development of steroid production by the placenta.[2,3,59–62] The fully developed equine placenta produces significant quantities of progestins.[2,3] One can maintain pregnancy in ovariectomized embryo transfer recipient mares, providedthat they receive an exogenous progestin supplement during the early period when progesterone from corpora lutea is critical.[63] The necessity for progesterone therapy after day 100 of gestation is controversial and is discussed later in this section.[2,64,65] One may detect high levels of eCG in the blood of pregnant mares from 40 to 120 days of gestation.[2] This forms the basis of the mare immunologic pregnancy (MIP) pregnancy test. A positive MIPtest can occur when a mare is no longer pregnant, because pregnancy loss following days 35 to 40 doesnot eliminate the endometrial cups.[3] The endometrial cups can produce high levels of eCG in a mare that has suffered fetal loss during the second or third months of gestation.[66] This production results in erratic estrous behavior, unreliable follicular development, and unpredictable ovulation.

Another unique feature of early gestation in the mare is an elevation in plasma conjugated estrogen levels associated with gonadotropin stimulation of the luteal tissue.[3,57,67,68] A second, prolonged rise in estrogen levels begins by day 70, peaking around day 240, and then gradually declining toward basal levels at term.[62,69] Peak estrogen levels range between 300 and 400 ng/ml.[62] These large quantities of estrogens are secreted by the fetoplacental unit after synthesis from precursors produced by the enlarged fetal gonads.[2,61,70–73] Bilateral fetal gonadectomy between days 197 and 253 of gestation causes a precipitous drop in plasma estrogen levels that then remains basal until small foals are delivered spontaneously in 70 to 97 days.[62] After day 220 the enlarged gonads gradually shrink into an insignificant size by birth.[74] Serum estrogens in the pregnant mare include estradiol, estrone, and the equine-specific steroid ring-B unsaturated estrogens equilin and equilenin.[75,76] Estrogen assays have been used by researchers to confirm pregnancy and fetal viability.[77–79]

Four separate components combine to produce the progesterone and biologically active 5α–reduced pregnanes needed to maintain pregnancy in the mare. The primary corpus luteum is prolonged beyond its cycliclife span by the downregulation of endometrial oxytocin receptors to prevent activation of the luteolytic pathway. The waning progesterone production of the corpus luteum is supplemented from day 40 of gestation by the formation of a series of accessory corpora lutea that developin the maternal ovaries as a result of the gonadotrophic actions of pituitary follicle-stimulating hormoneand eCG.[80] The equine fetoplacental unit produces significant quantities of progesterone and C-21 progestagens.[2,59–62,81–83] The placenta (chorioallantois and endometrium) initially metabolizes maternally derived cholesterol into pregnenolone and then into progesterone. A significant amount of progesterone originates from this source,

starting from approximately day 70 of gestation.[59–62,83] In normal pregnant mares the circulating progesterone concentration starts to decline by 4 months of gestation, reaching baseline levels by day 180.[84] Thus plasma progesterone-specific assays may be of limited clinical value after midgestation.[85,86] The circulating progesterone concentration no longer indicates what is happening at the uterine level where the allantochorion secretes progesterone and progestagens directly to the endometrium and underlying myometrium. In the second half of gestation, most of the placental-derived progesterone is metabolized further into 5α–reduced pregnanes (5α-dihydroprogesterone and 20α-dihydroxy-5-pregnan-3-one, together with other 5α-reduced metabolites of progesterone and pregnenolone).[87,88] In the later part of gestation, another steroid, perhaps5α-pregnane-3,20-dione and not progesterone, has been suggested to be the important steroid precursor for the other progestin metabolites found in circulating plasma.[89] A precise biologic explanation for this mechanism has not been elucidated to date.

In the last month of gestation, the enlarging fetal adrenal gland secretes appreciable quantities of pregnenolone, which then is used by the placenta to synthesize progestagens.[2,80,87,90–93] Even at this late stage in equine gestation, adrenal 17α-hydroxylase activity is insufficient to convert the pregnenolone into fetal cortisol, and thus it passes out in the umbilical vessels and is converted to 5α-reduced pregnanes in the placenta.[2,34,87,92–94] Thus unlike the trend in ruminants, a significant increase in the plasma progestagen concentrations in the mare occurs during the last 4 to 6 weeks of gestation, followed by a precipitous drop in levels at the time of parturition.[62,81,87,95,96] A premature rise in progestins has been observed in mares with placentitis, and this phenomenon is thought to be associated with fetal stress and premature maturation of the pituitary adrenocortical axis.[40,91,97] When pony mares were subjected to intrafetal injections of adrenocorticotropic hormone (ACTH) at day 300 of gestation, maternal plasma progestagen concentrations increased significantly.[98]

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Physiology of parturition in the mare does notfollow the well-documented ruminant model.[99–101] The precise mechanism that couples the fetal hypothalamic-pituitary-adrenal axis in the term foal is not known at this time but invariably is triggered when the fetus becomes fully mature.[102] In the mare the fetal cortisol level remains basal until the last couple of days of gestation, when a significant increase culminates in parturition.[34,103] Enhanced adrenocortical activity in the fetus is related to the onset of parturition in many species.[98] Final maturation of the fetus results in increased ACTH release from the pituitary and subsequent stimulation of the fetal adrenal cortex.[87,90,91] Not until the maturing adrenal gland attains 17α-hydroxylase capacity are the high levels of pregnenolone metabolized into fetal cortisol.[93] Disruption of these fetal maturational processes appears to play an integral part in the pathophysiology of fescue toxicosis.[94] The normal fetal maturational change (pregnenolone conversion into fetal cortisol) causes the maternal progestagen levels to plummet, resulting in a rising estrogen-to-progestagen ratio, and in the final hours, estrogen ultimately becomes dominant in the preparturient mare.[62] Studies with intrafetal injections of ACTH have indicated that precocious maturation of the equine fetus and a significant reduction in gestational length is likely to be mediated via adrenal regulation of fetal maturation and production of maternal progestagens.[98] Follow-up work by Ousey JC, Rossdale PD, Palmer L, et al.[104] demonstrated that ACTH administration (high doses) in the mare appears to accelerate fetal maturation and delivery in pony mares. Further work is required to establish the optimal gestational age and dosage for ACTH administration to mares before clinical recommendations can be given for this therapy.

Current knowledge about relaxin activity in the pregnant mare is limited.[105] Relaxin levels may serve to keepthe uterus quiescent until parturition is imminent.[105–108] Relaxin is thought to be involved in softening the pelvic ligaments to facilitate fetal passage. Mares may develop a relaxed croup just before foaling. The myometrium becomes more responsive to oxytocin and prostaglandin, and eventually the high concentrations of oxytocin and prostaglandins may overcome the inhibitory effects of relaxin.[*] The sudden dominance of estrogen is thought to promote cervical production of PGE_2. The contracting myometrium forces the chorioallantoic sac against the softening cervix and stage I of parturition ensues. The expulsive efforts during vaginal passage of the foal are driven by rising oxytocin and prostaglandin levels.[115–117] Oxytocin levels reach peak concentrations during the expulsive (stage II) phase of parturition.[106,118,119] Maternal straining (contraction of the abdominal muscles) is almost always associated with large sustained uterine contractions.

In the last week before foaling the prolactin concentration increases, and this hormone drives mammary development and the initiation of lactation.[120,121] Prolactin release from lactotrophs in the anterior pituitary is regulated by hypothalamic secretion of prolactin-releasing factor. The prolactin-inhibiting factor is thought to be dopamine.[122–125] The supraoptic and paraventricular nuclei of the hypothalamus synthesize oxytocin. The alveoli produce milk, which is expelled into the lactiferous ducts and teat cisterns when oxytocin causes contraction of the myoepithelial cells. Although nursing by the foal is assumed to stimulate the release of oxytocin from the neurohypophysis, recent research has revealed that the process is more complicated in the mare. A significant effect of suckling on oxytocin release by the mare was detected in only two of nine mares, when oxytocin concentrations were evaluated 0 to 3 minutes after suckling. When foals were prevented from sucking for 1 hour by being muzzled (n = 2) or separated from the mare (n = 2), no significant association was apparent between resumption of suckling and oxytocin release by the mare.[118]

* References 90, 96, 100, 106, 107, 109–114.

16.8.1.4

MYOMETRIAL ACTIVITY

The myometrium of the uterus is composed of an inner circular and an outer longitudinal smooth muscle layer. This arrangement of muscle fibers permits regulation of luminal size (circular) and uterine length (longitudinal).[3] The uterus is palpably flaccid during and immediately after estrus and then increases in tone and becomes turgid if the mare is pregnant.[126,127] Myometrial activity is vitally important in the uterine clearance mechanism after breeding.[128,129] Uterine contractions also play an integral role during the embryo mobility phase.[126,130–133] Between at least day 9 and day 16 after ovulation the spherical equine conceptus migrates continuously throughout the uterine lumen, propelled by peristaltic myometrial contractions.[3,134] This unusually long period of intrauterine movement ensures that the conceptus delivers its antiluteolytic signal to the entire endometrium. Conceptus mobility is high between day 10 and day 14 after ovulation but can be reduced immediately and significantly by an intravenous injection of flunixin meglumine. This suggests that prostaglandins are the primary stimulus for the myometrial contractions that drive migration of the conceptus.[134] The embryo itself produces a myometrial stimulant, and the subsequent uterine contractions result in embryo mobility.[5,130] The conceptus produces estrogens by day 12, and these, along with embryonic production of PGE_2, are thought to play a role in stimulating uterine contractions and increasing uterine tone during the mobility phase.[135–140] Progesterone is vital for embryonic development, and it plays a role in the mobility, fixation, orientation, and maintenance of the equine conceptus.[141] 1082

Administration of a progestagen (altrenogest) prevents prostaglandin-induced abortion in the first trimester of pregnancy.[142] Considerable controversy and confusion surround the use of progestagen supplementation in the later trimesters. The cellular mechanisms involvedin myometrial contractility have not been well characterized in the pregnant mare, and extrapolation from studies in nonpregnant mares may not be appropriate.[9,10,143–145] The classic theory in many species is that myometrial excitation and uterine contractility are suppressed by the progesterone block.[146] The assumption has been that progesterone and progestagens are necessary for myometrial quiescence in the pregnant mare, and the binding of 5α-dihydroprogesterone to endometrial progesterone receptors is thought to control uterine prostaglandin production and inhibit myometrial activity.[34,83,90] The oxytocin-neurophysin I gene is transcribed into messenger RNA in the endometrium of mares, and mRNA levels negatively correlate with serum progesterone concentrations.[147] However, progestagens were ineffective at controlling myometrial contractilityin vitro and did not inhibit the effects of oxytocin.[148,149] A pregnancy-induced inhibition of $PGF_2\alpha$ release is not associated with suppression of oxytocin release or oxytocin receptor density, and an embryo-derived factor is thought to be the most likely cause for the suppression of $PGF_2\alpha$ release and interruption of the oxytocin-$PGF_2\alpha$ interaction in mares during early pregnancy.[9,10] An oncofetal protein has been shown to reduce oxytocin-induced myometrial contractions in vitro, and this protein has been suggested to play a role in controlling myometrial quiescence in mares as well.[148] A reduction in circulating progestagen levels by experimental blockade with the 3β-hydroxysteroid dehydrogenase inhibitor (epostane) did not increase myometrial activity (labor) in late gestation pony mares.[82] However, despite a paucity of evidence that a deficiency of fetoplacental progestagen production is a cause of pregnancy loss in the mare, exogenous progestagen therapy is used widely as a form of preventative insurance in the belief that it promotes uterine quiescence and guards against the possibility of pregnancy failure.

Electromyographic activity in the uterus of pregnant mares increases during the last week before foaling, even though the circulating progestagen levels are still high.[96,97,106,150] One proposal is that the high levels of relaxin in the preparturient mare may act by inhibiting myometrial contractions until rising oxytocin and prostaglandin concentrations become overwhelming.[106] The number of oxytocin receptors and myometrial gap junctions may not increase in the mare until just before parturition.[106,148] A rapid membrane depolarization results in the onset of strong, coordinated uterine contractions that characterize the first stage of labor. In the last 6 hours before rupture of the chorioallantois, a significant increase in $PGF_2\alpha$ concentrations occurs before a significant increase in oxytocin concentration.[118] Oxytocin levels reach peak concentrations during the expulsive (stage II) phase of parturition.[106,118,119]

16.8.1.5 TERATOGENESIS

Little is known about embryotoxic and possible teratogenic effects of chemicals, drugs, and other agents that may be administered to pregnant mares. Phenothiazines and thiabendazole and organophosphate anthelmintics have been reported to cause pregnancy loss.[151] Likewise, ingested Sudan grass and sorghum have been reported to be toxic to the fetus.[151,152] In recent years, more definitive documentation has become available concerning the toxic effects of some medications. When griseofulvin was used to treat dermatomycosis in a mare during the second month of pregnancy, the mare carried a male foal to331 days gestation. The foal showed bilateral microphthalmia, severe brachygnathia superior, and palatocheiloschisis. The lesions were incompatible with life and the animal was euthanized. Griseofulvin administration during

pregnancy has been associated with similar lesions in other species. Because the development of the eyes and facial bones in the horse occurs in the second month of pregnancy, the lesions described in this case most likely can be attributed to griseofulvin administration.[153] Three weak, recumbent neonatal foals with skin lesions, including a thin wooly coat, were born to mares being treated for equine protozoal myeloencephalitis. The foals were anemic, leukopenic, azotemic, hyponatremic, and hyperkalemic. The pregnant mares had received sulfadiazine or sulfamethoxazole-trimethoprim, pyrimethamine, folic acid, and vitamin E orally. Serum folate concentrations in the three foals and two mares were lower than those reported in the literature for clinically normal broodmares. At necropsy each foal had lobulated kidneys with thin cortices and pale medullae. The spleen and thymus were small. Histologic examination revealed significant epidermal necrosis without inflammatory cells, thin renal cortices, renal tubular nephrosis, lymphoid aplasia, and bone marrow aplasia and hypoplasia. These observations indicated that oral administrationof 2,4-diaminopyrimidines (pyrimethamine with or without trimethoprim), sulfonamides, and folic acid to mares during pregnancy is related to congenital defects in newborn foals.[154] Further investigations are warranted to ascertain the toxic agent and to determine at what stage of gestation the fetus is most vulnerable.

16.8.2 Pregnancy Diagnosis

Standard transrectal imaging technique permits an experienced practitioner (using a 5-MHz ultrasound 1083
transducer under optimal lighting conditions) to detect up to 98% of embryos as early day 11 after confirmation 1084
of ovulation.[155] The black (anechoic) spherical vesicle is characteristic of early equine pregnancy. Novices should realize that bright white (echoic) spots on the upper and lower surfaces of the black sphere are specular reflections generated by the ultrasound waves.[156] Beginners also must appreciate that the equine embryo literally can be anywhere in the uterine lumen before fixation on day 16. One easily can make diagnostic errors unless one meticulously searches the entire length of both horns and the uterine body down to the cervix during an ultrasonographic examination. Confirmation of mobility obviously is useful in differentiating a vesicle from a cyst. At the time of fixation at the base of a uterine horn the yolk sac has three germ layers (ectoderm, mesoderm, endoderm) near the embryonic pole, and only two layers (ectoderm and endoderm) at the opposite pole. The difference in rigidity between the three-layered ventral wall and the two-layered dorsal wall explains the characteristicguitar-pick shaped image on ultrasound by day 18.[3,4] The thickest portion of the yolk sac wall (embryonic pole) rotates to a ventral position. The change from the previous spherical shape is caused by uterine turgidity and thickening of the dorsal uterine wall.[144] The increased uterine tone is responsible for the failure of the diameter of the embryonic vesicle to enlarge between days 18 and 26, thus creating the classic growth profile that plateaus during this period.[4,155]

By day 21 the amniotic cavity has formed completely and the embryo itself often is detectable by ultrasound. By day 24 one can see the allantoic sac raising the embryo off the ventral floor. The embryo itself is suspended on a thin echoic line that delineates the apposition of allantois and yolk sac.[4,155,157] This separating membrane tends to be horizontal and thus is a useful means for differentiating a singleton from twin embryonic vesicles. In the latter case the adjacent walls of the two vesicles tend to form a vertical separation. One can detect the primitive heart beat between 22 and 25 days, and it is a useful indicator of embryonic viability. By day 40 the embryo (now fetus) has been elevated to the dorsal pole because the allantois almost has displaced the vestigial yolk sac completely. The membranes and blood vessels that separate the allantois and yolk sac give rise to the umbilical cord. The increasing size of the fetus causes the fetus and amniotic sac to descend gradually back to the floor of the chorioallantoic vesicle by day 48. The remnant of the yolk sac is incorporated into the umbilical cord. The site where the umbilical cord attaches to the chorioallantois identifies the horn and site where embryo

fixation originally occurred. The dorsal attachment ensures that the developing fetus does not compress this vital area.[4]

Although ultrasonographic examinations have become standard practice for confirmation of pregnancy in mares, it is still essential that the veterinarian be competent at manual diagnosis. By 18 days after breeding, an experienced clinician may be able to perceive changes in uterine tone and consistency in the mare that, coupled with palpation of a narrow, elongated cervix, are consistent with early pregnancy. However, one should remember that not uncommonly a persistent corpus luteum provides a prolonged progesterone environment in a nonpregnant mare. One must not mistake the curvature at the base of the uterine horn for an embryonic vesicle.[3,127,158] An ultrasound image of the embryonic vesicle is required to confirm pregnancy definitively at this early stage. However, the gradually enlarging embryonic vesicle eventually creates a discernible ventral bulge that has been described as being the size of a hen's egg (day 30), goose's egg (day 35), and orange (days 40to 45).[158,159] Because dorsal distention at this stage of pregnancy is minimal, examination of the ventral aspect of each uterine horn is essential. One can make errors if the fingers are not passed far enough around the cranial aspect of the uterine horn so as to reach well under the uterine body and horns.[158] One can identify a ventral bulge consistent with a 35- to 45-day pregnancy by the distinct margins that are palpable when one moves the fingers along the ventral aspect of the uterus. The conceptus becomes more oval as it expands during the third month of pregnancy. At 90 days the chorionic vesicle has been said to be the size and shape of a football.[158,159] The age of the mare and the number of previous foals affectsthe rate of descent of the gravid uterus over the pelvic brim.[159,160] Positive pregnancy diagnosis becomes progressively more difficult as the large, heavy uterus descends into the abdominal cavity. In a normal pregnancy the uterine wall should be thin and pliable, whereas pyometra causes a thickened uterine wall that contains viscous purulent fluid. The fetus itself is not always palpable before 120 days of gestation, but after that time gentle ballottement usually allows detection of the fetus.[158,159] With palpation of some part of the fetus, one should be able to confirm pregnancy status between 5 months and term.

Blood tests for pregnancy sometimes are indicated, especially in Miniature breeds.[78] Measurement of equine chorionic gonadotropin levels verifies that the mare has endometrial cups but does not guarantee that a viable fetus is still present.[3] With this caveat in mind, eCG is suitable for determining pregnancy status in Miniature 1084
mares between 40 and 100 days after mating.[161] However, mares returning a diagnosis of pregnant should 1085
undergo a blood estrone sulfate test 100 or more days after mating to eliminate the possibility of a false-positive diagnosis. The fetoplacental unit secretes large quantities of estrogen.[69,78] Measuring blood estrone sulfate levels is recommended as the method of choice for determining pregnancy status in Miniature mares 100 or more days after mating.[162] Fecal estrone sulfate measurements provide a noninvasive alternative to blood testing from 150 days after mating. However, the discrimination between pregnant and nonpregnant levels of estrone sulfate is not as great in feces as it is in blood.[161] An early pregnant factor test recently has been reported to be useful for pregnancy confirmation in the mare. Early pregnancy factor is an immunosuppressive protein detected by a rosette inhibition test in the early pregnancy serum. Early pregnancy factor appears in the maternal blood circulation at 24 to 72 hours after mating and is detectable until the second trimester; after that, early pregnancy factor disappears from the maternal serum.[163]

16.8.3 Management of Twins

16.8.3.1 MONITORING FOLLICULAR DEVELOPMENTAND OVULATION

Most twin pregnancies arise from double ovulations.[164–166] A higher incidence of twin ovulations occurs in some breeds (e.g., Thoroughbreds and Warmbloods), andmares that tend to double ovulate can be expected to do this frequently.[3,165,167–171] Double ovulations may occur on the same day but may occur several days apart.[3,172] If a fertile stallion was used to breed the mare on the first ovulation, the viable sperm possibly still are present in the reproductive tract when the second oocyte arrives. One must remember this possibility when scanning mares for pregnancy at 14 to 16 days. At that time, scanning of the ovaries for evidence of luteal tissue from a second ovulation is good practice.

In the past, veterinarians often elected to avoid breeding a mare when two large (>30 mm follicles were palpated or to recheck the second follicle 10 to 12 hours after the first detected ovulation.[173] Because an ovulated oocyte is less likely to be viable after this time, one could perform a delayed breeding in anticipation of the second ovulation. Today the preferred strategy is to breed all eligible mares, irrespective of the number of preovulatory follicles. The widespread adoption of early ultrasonographic pregnancy examinations has permitted the focus to be placed on embryonic vesicle reduction after confirmation of a twin pregnancy.[66]

16.8.3.2 MANUAL REDUCTION

The increasing size of the embryonic vesicle, coupled with the increasing tone of the early pregnant uterus, tends to fix the conceptus at the base of one uterine horn by day 16.[3] A thorough ultrasound scan of the uterus is essential, along with a complete examination of the length of both horns plus the uterine body as far back as the cervix. Such thoroughness is especially important before day 16 because the vesicle moves freely within the lumen of both horns and the uterine body.[172] If twin vesicles are detectable, manual separation of them before day 16 is easier. Successful elimination of one vesicle is more likely at that time because the uterine walls are thin and minimal pressure is required to crush a vesicle. One often can feel a definite pop when the vesicle ruptures, but one always should confirm success by ultrasound. This sensation is attributable to the rupture of the embryonic capsule.[174]

The downside to this approach is that one easily can confuse an early embryonic with an endometrial cyst. The embryo itself does not become readily identifiable until the fourth week of pregnancy. Thus for one to note the size and location of any cysts at the time the mare is being examined for breeding is good practice. If no record of cyst size and location exists, then differentiation between early twin vesicles, versus a singleton anda cyst, with a single examination is virtually impossible. This difficulty is especially true because asynchronous ovulations are likely to result in considerable size discrepancy between the two vesicle.[3] Under these circumstances, measurement of each suspect vesicle and notation of its location may be best. A second scan in 1 to 2 days should note a size increase in any normally growing vesicle (approximately 4 mm per day).[155] Unfortunately, this delay may make separation of unilaterally fixed vesicles more difficult because of their ongoing growth and the increased uterine tone.

Manual reduction of bilaterally fixed vesicles requires less manipulation than with unilateral twins. Manual reduction is an easy procedure, and success rates exceeding 90% are not uncommon if the vesicle is crushed before day 16.[175,176] If the vesicles are fixed unilaterally, then the clinician should attempt to move the more proximal vesicle toward the tip of the uterine horn. At this location the manual reduction procedure is less likely to disrupt the remaining vesicle. One can crush the vesicle by pinching it between the thumb and fingers. Alternately, one can squeeze the vesicle against the pelvis of the mare until it ruptures. If one can separate the twins before crushing, then the success rate may be similar to that for reduction of bilateral twins.[177] If one cannot separate the unilateral twins or more than 20 days of gestation have passed, then the success rate is lower.[66] The extra pressure used to eliminate a twin vesicle after fixation is the reason many clinicians use antiinflammatory medications and progestin therapy. The likelihood of success improves with experience.[176] If one does not detect the unilateral vesicles until after day 20, then manipulations easily can result in the disruption of both vesicles. The best option in these cases may be to wait and see if natural reduction occurs.

16.8.3.3

NATURAL REDUCTION

Almost three quarters (70%) of twin embryonic vesicles become fixed unilaterally, with only 30% of twin 1085
vesicles becoming fixed bilaterally.[178,179] When twin vesicles are dissimilar in size, the incidence of 1086
unilateral fixation appears to be higher. The larger vesicle is thought to serve as an impediment to the continued mobility of the smaller vesicle.[4] Fortunately, natural reduction to a singleton is far more likely with unilaterally fixed vesicles. More than 80% of unilaterally fixed twins are likely to reduce naturally to a singleton, with more than half of these occurring between days 16 and 20.[3,178,179] However, most bilaterally fixed vesicles continue to develop.

16.8.3.4

PREGNANCY TERMINATIONWITH PROSTAGLANDIN

If natural reduction does not occur, one may consider terminating the pregnancy with a prostaglandin injection. The prostaglandin should lyse the corpora lutea that resulted from the double ovulation, and the precipitous decline in progesterone will bring the mare back into estrus. However, treatment failures have been reported.[180] Certainly, one must give this treatment before day 35. After the endometrial cups begin to form, repeated prostaglandin injections may be necessary to terminate the pregnancy. The mare more than likely will not return to normal ovulatory cycles until the cups are sloughed. In the interim they secrete eCG, a hormone that causes the development of accessory corpora lutea and augments the progesterone level in support of the early pregnancy.[3] The result is erratic estrous behavior, unreliable follicular development, and unpredictable ovulation.

16.8.3.5

TRANSVAGINAL ULTRASOUND-GUIDED ALLANTOCENTESIS

Even though the advent of transrectal ultrasonography has improved greatly the ability of veterinarians to make an early diagnosis of twin pregnancies, diagnostic errors still occur.[181,182] Such errors could be due to an early pregnancy diagnosis when the second vesicle was too small to detect, incomplete examination of the entire uterus, poor image quality, or an inability of the clinician to differentiate two embryonic vesicles that are closely apposed to each other.[182] If natural reduction does not occur or the diagnosis of twins is not confirmed until after 30 days, then transvaginal aspiration of one vesicle is an option. The results are best if

one performs the procedure before day 35.[66] Although spontaneous reduction of twin pregnancies can occur even after day 40, the probability of this occurring is low.[36,183] Natural twin reduction is more likely to occur if an obvious size discrepancy is present between the two vesicles at this time.[178,184]

If one is to attempt a transvaginal reduction, one should treat the mare with flunixin meglumine. Many clinicians also administer altrenogest orally. Because sedation causes significant uterine relaxation, most clinicians use a lidocaine enema to reduce straining.[185] The transvaginal aspiration technique uses a 5.0- or 7.5-MHz endovaginal curvilinear transducer. One should cold-disinfect and sterilize the transducer and casing before use. One then places the assembled unit in a sterile transducer cover that has been filled with sterile lubricating gel and advances the transducer aseptically until it is seated lateral to the cervix. The clinician then grasps the pregnancy per rectum and advances a sterile 60-cm, 18-gauge spinal needle with an echogenic tip along the needle guide in the transducer casing. One can use a dotted line on the ultrasound screen to select a path for the needle entryinto the embryonic vesicle. A sharp jab of the needle penetrates the vaginal wall, peritoneal lining, uterus, and ultimately the allantoic or yolk sac. With a 60-ml syringe attached to the needle, one aspirates the embryonic fluid. One should stop aspiration when a danger existsof damaging the adjacent vesicle of unilateral twins. If one is eliminating a bilateral twin, then one can move the needle within the vesicle until all detectable fluid has been aspirated.[66] The success rate is better for bilateral twin reductions.[185] Death of the remaining twin ismost likely to occur within 2 weeks of the procedure. Although reports are scarce, preliminary data suggest that experienced operators may achieve a live singleton birth in about one third of cases.

16.8.3.6 TRANSABDOMINAL ULTRASOUND-GUIDED FETAL CARDIAC PUNCTURE

In advanced twin pregnancies, one can attempt reduction by a transabdominal approach.[66,177,186,187] Fetal intracardiac injection of potassium chloride is effective but requires accurate placement of the potassium chloride into the fetal heart. One obtains the best results when the pregnancy is between 115 and 130 days.[188] At this stage experienced operators can achieve a 50% success rate. Penicillin G procaine can cause fetal death when injected into the fetal thorax or abdomen, but the effect is not instantaneous. The advantage of the latter treatmentis that it does not require precise placement of the injection into the fetal heart.[177] One should start mares on orally administered altrenogest, systemic antibiotics, and flunixin meglumine on the day of the procedure and should continue the antibiotic coverage and antiinflammatory medication for 3 days.[66]

One can use a 3.0-MHz transducer to image the90- to 130-day-old fetus in the caudal abdomen, just cranial to the udder.[182] Once the mare has been sedated, the uterus relaxes and the location of the fetuses shifts cranially. A sedative/analgesic combination that works well for this procedure is acepromazine (10 mg), xylazine (100 mg), and butorphanol (10 mg). One selects the smallest or most easily accessible fetus for reduction. One should prepare the ventral abdomen for surgery and infiltrate local anesthetic at the puncture 1086
site. One can use an 18-gauge, 8-inch spinal needle with stylet for most fetal injections.[182] The distance from 1087
the skin surface to the fetus determines the length of the needle that is required. Specialized needles with echogenic tips are available to provide better visualization via ultrasound.[177] Once one confirms the location of the thorax of the selected twin, one introduces the needle through the prepared skin, abdominal wall, and uterus. If one is to inject penicillin G procaine, then the needle may puncture the fetal thorax or abdomen. One typically injects up to 20 ml into the fetus.[177,182] One should confirm fetal death the following day.

Although the benefits of supplemental progestin therapy are debatable, many clinicians suggest that the mare be medicated for at least 2 weeks if the initial twin reduction has been successful.[182] Regular checking of fetal viability is essential because supplemental progestin therapy may prevent elimination of the dead fetuses if both die.[189] Most abortions occur within 1 to 2 months after the reduction procedure.[66] If the operator is experienced in the technique, one can expect between 40% and 60% of mares to deliver a viable singleton foal.[177,182] The eliminated twin in these cases appears as a mummified remnant contained within an invaginated pouch that protrudes into the allantoic space of the fetal membranes of the viable foal.[187] One theory for the loss of both twins following an intrafetal injection has to do with the presence of vascular anastomoses between the two fetoplacental units.[190] Circulation of the injected solution or other tissue degradation products has been suggested possibly to result in the death of the adjoining twin fetus.[182]

16.8.4 Early Pregnancy Loss and Abortion

16.8.4.1 EMBRYONIC LOSS

Once conception has occurred, any pregnancy failure up to day 40 of gestation is defined as being early embryonic loss.[3] Between 10% and 15% of mares undergo embryonic loss or abortion at some time in gestation, and most of these losses occur during the first 40 days of gestation when the primary corpus luteum is the sole source of progesterone. Yet all the available evidence suggests that untoward luteolysis is not common in this period, and the losses that do occur have other underlying causes.[80] Fertilization failure rates and embryonic losses are higher in aged mares.[191–195] Differentiation between fertilization failure and embryonic loss before day 10 is not easy because this is the earliest stage of development in which ultrasonographic detection is possible under ideal research conditions. Fertilization rates in young, well-managed mares may exceed 90% and appear to be better than80% in aged mares.[194,196] Oocytes from aged mares may be more likely to result in nonviable embryos because of inherent morphologic defects.[194,197] Carnevale transferred oocytes from young and aged mares into young recipients; thus fertilization and early embryonic development occurred in an optimal oviductal environment. The day 12 pregnancy rate in the recipients that received the oocytes from aged mares was significantly less than that achieved with the oocytes of younger mares.[198] Research has shown that embryo recovery rates are considerably lower in aged mares, and that significant losses occur before day 14 of pregnancy.[192,199–201] Ball, Hillman, and Woods have provided evidence to suggest that the uterine environment may not be the only reason for subfertility in some mares. Embryos collected from normal mares resulted in similar pregnancy rates in fertile and subfertile recipients (significant uterine pathologic condition) at day 28.[202] In a reversal of study design, embryos were collected from the oviducts of normal and subfertile mares at day 4 after ovulation and then were transferred into normal recipients. Pregnancy rates were lower in those normal mares that received embryos from the subfertile donors.[199] Thus although the uterine environment may have a delayed effect on embryonic and fetal loss, oocyte quality and oviductal influences apparently play a significant role in the problem of subfertility and early embryonic loss in mares.[203] In practice, embryonic losses that may be detectable between days 14 and 40 can range from 10% to 15% in young, well-managed mares to 25% or 30% in aged mares.[194,204] The presence of endometrial inflammation and uterine fluid accumulation have a detrimental effect on the early embryo survival and can increase the likelihood of early pregnancy loss greatly.[205,206]

16.8.4.2

EARLY FETAL LOSS

Formation of the endometrial cups is a defining moment regarding early pregnancy loss in mares. If the embryo dies before day 35, then the chorionic girdle cells do not invade the endometrium and the endometrial cups do not form. These mares should return to normal estrous cycle activity and may be bred successfully again during the same breeding season. However, if the fetus is lost after day 40, the endometrial cups irreversibly are established.[3] Thus if one performs an assay for eCG (MIP test) after the endometrial cups form, one will obtain a false-positive result for pregnancy until the endometrial cups are sloughed between days 120 and 140 after the original conception occurred.[3] Retention of the endometrial cups after fetal loss results in erratic estrous behavior, unreliable follicular development, and unpredictable ovulation. Thus this unique physiologic mechanism typically prevents mares suffering fetal loss after endometrial cup formation from being bred back during the current breeding season.

The exact mechanism that causes most pregnancy losses in the mare remains to be elucidated.[32,37,207] 1087
Beyond day 40 the secondary corpora lutea receive powerful luteotrophic support from eCG and from days 1088
80 to 100 until term, the supply organ (placenta) and target tissues (endometrium and myometrium) are in direct contact with each other over their entire surface. A paucity of evidence shows that a deficiency of progesterone production is a cause of pregnancy loss in the mare.[80] Certainly, fetal death may follow uteroplacental insufficiency or an overwhelming sepsis.[207] In recent years, a broad consensus has developed that the inflammatory mediator $PGF_{2\alpha}$ may play an integral role in many cases of fetal death.[85] Prostaglandins are well known to be luteolytic.[3] Thus in the first 70 to 80 days when the pregnancy depends on primary and accessory progesterone production of the corpora lutea, the pregnancy is especially susceptible to the luteolytic effects of prostaglandins. However, one should remember that repeated exogenous prostaglandin injections may be required to terminate a pregnancy electively once the endometrial cups have formed, because some of the immature accessory corpora lutea may not be developed sufficiently to respond to the first prostaglandin injection. Another probable abortogenic feature of prostaglandins may be myometrial hypermotility, which may be associated with placental inflammation or high systemic levels of prostaglandin.[208]

Studies by Daels and colleagues have demonstrated that an early pregnancy may be lost following prostaglandin-induced luteal deficiency associated with endotoxemia.[209–211] The detrimental effect of the endotoxin could be prevented only if a cyclooxygenase inhibitor (flunixin meglumine) was administered before clinical signs of endotoxemia were evident.[65,210,212,213] Thus although gram-negative septicemia and endotoxemia associated with many gastrointestinal crises are known to result in elevated levels of inflammatory mediators, any pregnancy-sparing effect of prostaglandin inhibitors is likely to be effective only if antiinflammatory agents such as flunixin meglumine are administered in the acute phase of the disease.[207] Because a healthy fetoplacental unit can produce enough progesterone to sustain the pregnancy after 80 days of gestation, the concept of prophylactic altrenogest past 3 months of gestation is controversial.[65,80,85,142,214]

Although recent in vitro studies suggest that progesterone may not be the primary regulator of myometrial quiescence, in situations in which elevated prostaglandin levels are likely, clinical justification appears adequate at present for providing exogenous progestagen support for high-risk pregnant mares.[148] Based on current knowledge, the administration of a double-dose (0.088 mg/kg s.i.d.) of altrenogest is suggested during the acute phase of a medical or surgical condition when prostaglandin levels are likely to be elevated.

[149] If the condition warrants muzzling the mare (nil per os), then short-term use of progesterone-in-oil (150 to 250 mg intramscularly every 24 hours) is warranted. This approach is based on further work by Daels and colleagues that showed that progestin treatment could prevent abortion at 3 to 5 months of gestation if a higher dose (0.088 mg altrenogest per kilogram) was administered.[65,142] Abortion did not occur in five of eight mares treated with progesterone and eight of eight mares treated with altrenogest, and endogenous $PGF_{2}\alpha$ secretion was inhibited, compared with values in aborting mares.[142] The researchers concluded that circulating progestagen concentrations may play a role in the outcome of pregnancy during prostaglandin-induced abortion.

That separation of the chorioallantois from the endometrium disrupts local endocrine function seems logical.[215] The fetoplacental unit does attempt to compensate for this placental dysfunction by increasing progesterone production.[216] However, endotoxemia may cause deleterious effects to the placental circulation and potentially disrupt vital steroid metabolism within the fetoplacental unit.[217] Thus administration of flunixin meglumine to pregnant mares is indicated early in the course of any condition in which endotoxemia is possible.[212,218,219] If a late pregnant mare develops a surgical colic condition, then the fetus is not only at risk from the maternal endotoxemia that can be associated with gastrointestinal crises but also from any maternal hypoxic episodes that may occur during anesthesia.[217,218,220,221] Acute enteritis or colitis in a pregnant mare also can result in abortion because of the effects of endotoxemia.[220,221] Because maternal hypoxia has been shown to be a risk factor for abortion, one must avoid intraoperative hypoxia if a pregnant mare requires surgery.[220] One can expect 16% to 20% of mares to abort after colic surgery, but superior intraoperative ventilatory techniques may reduce this risk.[220,221] Stage of gestation and duration of anesthesia are less critical factors provided that maternal oxygenation is adequate.[222] Apparently, aberrations in the cardiovascular and metabolic status of the mare and fetus are more detrimental to pregnancy maintenance than the actual medical or surgical condition.[219]

16.8.4.3 PLACENTITIS

Placentitis is a major cause of abortion in mares during the latter part of pregnancy.[181,223] Placentitis tends to be a sporadic, individual mare problem that seldom has any lasting effect on mare fertility.[224] Bacterial (β-hemolytic streptococci, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*) and fungal (*Aspergillus* spp.) organisms may be incriminated.[35,215,225–228] Ascending infections are especially common and result in inflammation and detachment of the placenta in the area surrounding the cervical star. The 1088
cervix is almost invariably softened, with purulent exudates draining into the vagina.[215,226,227] Thus one 1089
should examine this part of the fetal membranes closely whenever a mare aborts. The vestibular sphincter and vulvar lips are important barriers to ascending infections, and many aged mares require a Caslick procedure to reduce the likelihood of ascending infections. Hematogenous infection of the placenta is also possible.[226,227] *Leptospira* spp. induce a diffuse placentitis with large numbers of spirochetes in the placental tissues.[229,230] A unique form of placentitis has been reported as a sporadic cause of abortionand weak foals in central Kentucky over the past decade.[181,223,229,231,232] A review of nocardioform placentitis cases over a 9-year period (1991 to 1999) revealed that of the farms that had cases, 83% had two or fewer over the 9 years and 66% of the farms had only a single case during that time.[232] The causative organism recently was identified as *Crossiella equi*.[233,234] This bacterium is responsible for nocardioform placentitis and is one ofthe few actinomycetes known to cause animal disease. Although numerous gram-positive branching bacilli

are visible on histologic sections, the bacterium is seldom present in fetal tissue.[230,232] In vitro testing has demonstrated that the nocardioform bacterium is susceptible to sulfonamides and trimethoprim.[230] This combination is an ideal choice for systemic medication of a pregnant mare when placentitis is suspected because these antimicrobial agents are known to gain acceptable levels in the fetal fluids.

Although nocardioform placentitis was first diagnosed at the University of Kentucky in 1986, this type of placentitis has not been confirmed elsewhere in North America or in other countries.[230,234] Although the organism has been suggested as gaining access to the uterusat breeding, this unusual form of equine placentitis does not become apparent until the latter part of gestation.[224] The initial lesion is localized in the cranioventral aspect of the uterine body, extending cranially onto the base of the horns and circumferentially around the placenta.[235] Outcomes vary from abortion to birth of a normal foal. Some foals are premature, whereas others are delivered at term but are stillborn or weak and compromisedwith a malnourished appearance.[230,235] Most affected mares appear normal, although many display signs of placentitis (premature mammary development and lactation).[224,230,232,235] Vaginal discharge is not a feature of this condition because the area around the cervical star is not involved. Once the fetus has been expelled, these mares soon clear the infection with no adverse effects on subsequent fertility.[230,232] On examination of the fetal membranes, a line of demarcation is obvious between diseased and normal tissue, with the affected area being covered by a characteristic thick, brownish-tan mucoid material.[230] The underlying chorionic villi are reduced in size, and in the central portion of the lesion the chorionic surface may be denuded completely.[235]

The exact cause of fetal expulsion is not known, but placental thickening and separation from the endometrium are consistent features in many abortions.[181] Placentitis is detrimental to the pregnancy not only because it disrupts nutrient exchange but also because of the release of inflammatory mediators. Thus antiinflammatory medication (flunixin meglumine at 1.1 mg/kgor phenylbutazone at 4 mg/kg every 12 hours) is indicated.[208] Placentitis associated with early abortions tends to be acute, with the fetus succumbing to bacter-emia. Broad-spectrum antibiotics that have been recommended include trimethoprim-sulfadiazine (15 to30 mg/kg p.o. every 12 hours), procaine penicillin (30,000 IU/kg every 24 hours) and gentamicin(6 mg/kg every 24 hours), and ceftiofur (1 to 5 mg/kg every 12 hours).[208] Gentamicin is undetectable in the plasma of newborn foals after mares are treated with the antibiotic (6.6 mg/kg) an hour before parturition. Thus gentamicin apparently does not cross the placenta of mares at term.[236]

Abortions later in gestation tend to be associated with chronic placentitis and severe tissue reaction that results in uteroplacental insufficiency.[229,232] If the localized production of high concentrations of prostaglandins stimulates the formation of gap junctions, then the subsequent hypermotility may impede placental blood flow as the uterus contracts.[85] Impeded blood flow reduces fetal oxygenation and increases fetal stress. Because progesterone is known to inhibit gap junction formation, a clinically valid conclusion is that progestin supplementation may be beneficial when one suspects uteroplacental inflammation. This is the rationale behind the current recommendation to treat suspect placentitis cases witha double dose of altrenogest (0.088 mg/kg every24 hours).[85,208,216] However, one must understand that at this time the practice is controversial, and although the treatment probably does no harm, the expense of long-term progestin supplementation may not be warranted.[80] Recent studies suggest that measurement of relaxin levels may serve as a useful means of monitoring placental function and treatment efficacy in the mare.[237]

The rationale for other treatment regimens is based on extrapolations from the human literature and application of sound reasoning for the potential efficacy of a particular drug. Scientific investigation in this

areais needed desperately. Many equine clinicians havebeen advocating the use of an orally administered β_2-sympathomimetic drug (clenbuterol) to suppress uterine motility.[208,238] In the United States the product is marketed as an oral formulation to treat chronic obstructive pulmonary disease. Research is needed to determine what oral dose of this bronchodilatory compound, if any, is actually effective on the gravid uterus. 1089
Inthose countries where an intravenous formulation is available, research has shown that a 300-μg 1090
intravenous dose of clenbuterol reduces uterine tone for approximately 2 hours.[239–241] The administration of pentoxyfylline (7.5 mg/kg p.o. b.i.d.) is recommended, based on its use for treating tissue ischemia in human beingsand its ability to modulate the inflammatory process.[208,242] Pentoxifylline increases erythrocyte flexibility. Inflammation-driven uterine hypermotility may impede circulation in the placental capillary bed, and pentoxifylline is thought to increase fetal oxygenation by facilitating blood flow.[243,244]

16.8.4.4 LEPTOSPIROSIS

Leptospirosis has been incriminated as a sporadiccause of placentitis, abortion, and premature births in horses.[181,223,231,245–256] Leptospiral infections may cause abortions in the latter part of gestation, generally with no premonitory clinical signs, and occasionally an infected premature or full-term weak, icteric foal.[245,247,250,251,253] The placenta is edematous, with a necrotic chorion covered with a mucoid exudate.[230] The gross placental lesions are associated with thrombosis, vasculitis, and inflammatory cell infiltrates. The spirochetes tend to be numerous and are demonstrated readily in the stroma and villi of the placenta.[253,257] A microscopic agglutination test on fetal fluids (heart and body cavities) or maternal serum is likely to reveal a high titer (1:6400 to 1:819,200 or greater).[230,247] One may detect leptospires in the fetus by the fluorescent antibody test, silver staining, or darkfield microscopy.[230] One should submit the fetal kidney for a fluorescent antibody test because it yields the highest percentage of positive results. The kidney is also the best tissue for culture.[230,247] If one intends to submit urine from an infected mare (fluorescent antibody test or darkfield), one must obtain collection instructions and appropriate transport medium from the diagnostic laboratory.[230] One must obtain the specimen before administration of any antimicrobial therapy.

Although L. interrogans serovar *bratislava* is the serovar most commonly isolated from aborted fetuses in Northern Ireland, in North America most leptospiral abortions have been associated with the *pomona* serogroup, and occasionally the serovars *grippotyphosa* and *hardjo*.[245,247,249] Equine leptospiral infections previously reported as being *L. pomona* are now thought to be identified more correctly as L. interrogans serovar *kennewicki*.[230] In North America, pregnant mares are considered to be incidental hosts that become infected after exposure from maintenance hosts (i.e., wildlife such as skunks and raccoons for serovars *kennewicki* and *grippotyphosa* and cattle for serovar *hardjo*).[230,245,247] Infected mares may shed leptospires in the urine for up to 14 weeks. Thus therapy aims at preventing urinary shedding and possibly prophylactic treatment of pregnant in-contact mares that have high titers.[230] A combination of penicillin (10,000 to 15,000 IU/kg intramuscularly) and streptomycin (10 mg/kg intramuscularly) every12 hours for 1 week has been recommended, but streptomycin no longer is used widely in equine practice.[230,252,258] High doses of penicillin G potassium (20 million units intravenously every 12 hours) may be effective in preventing infection of a fetus if the mare has a high titer.[258] The dosage and duration of treatment appear to be important. Oxytetracycline (5 to 10 mg/kg) also has been suggested, but it was less effective at preventing urinary shedding of leptospires in all cases tested (five of seven infected mares).[230,252,258] No approved vaccine is available to prevent leptospirosis in horses in North America. One should attempt to prevent direct contact between maintenance hosts and pregnant mares and also to avoid exposure to infected urine from

these species (e.g., contaminated water and feed). Vaccination of cattle with a multivalent vaccine if they are present on the same property as pregnant mares may be prudent.[230]

16.8.4.5 MARE REPRODUCTIVE LOSS SYNDROME

In the spring of 2001 the equine breeding industryin central Kentucky was faced with reproductive crisis. Mares were foaling prematurely with premature separation of the chorioallantois (late-term “red bag” abortions), many mares that were 45 to 80 days pregnant suffered acute fetal loss, and an unusual number of weak foals were born.[259,260] The epidemic became known as the mare reproductive loss syndrome (MRLS). Some 318 aborted fetuses were submitted to the diagnostic laboratory between April 28 and May 8, 2001.[259] Fortunately, in the Kentucky epidemic of 2001, no contagious infectious cause was identified, and the condition became self-limiting as the breeding season progressed. Veterinarians and scientists involved in the epidemic considered the possibility of mycotoxins, ergot alkaloids, phytoestrogens, and even cyanide from wild cherry tree foliage. An extensive epidemiologic study was performed subsequently to identify risk factors that predisposed mares to MRLS.[259,260] At the time of writing, environmental toxic agents remain the primary suspect, with increasing attention being focused on the eastern tent caterpillar.[260]

The state diagnostic laboratory was inundated with late-term aborted fetuses, and practitioners identified approximately 2000 early fetal losses.[261] Many of these abortions were detected during what should have been a routine fetal gender determination. An initial questionnaire determined that during the peak period of MRLS in the spring of 2001, some 20% of mares that had been confirmed pregnant at 42 days were
determined subsequently to have suffered fetal loss by 60 days.[259] Approximately 500 late-term abortions 1090
were reported in central Kentucky; the Appalachian region to the north also reported a high number of 1091
similar cases. The author investigated reports of more than 150 late-term abortions in southeast Ohio during the spring of 2001. Reports of weather patterns and an unusually high emergence of eastern tent caterpillars *(Malacosome americanum)* were identical in the central Kentucky and southeast Ohio regions.[262] Results of the initial Kentucky survey confirmed that mares bred in February (45 to 80 days pregnant in late April or early May) were at high risk for fetal loss.[259,260] Mares that were not exposed to eastern tent caterpillars were not at risk, and feeding of hay to mares outside appeared to have been protective. Multiple breeds of horses were affected by MRLS. Smaller numbers of horses of different sexes and ages were diagnosed with pericarditis and unilateral uveitis during the same period.[260]

A study in 2001 involving 288 pregnant broodmares reported an early fetal loss rate of 25%, with a median gestational age at the time of fetal loss being 77 days. In this study early fetal loss was defined as being the loss of a fetus that was viable at 40 days of gestation but was subsequently lost by 5 months of gestation.[261] Although one could assume that maiden and barren mares were most affected because these horses would have been bred soon after the official season began in mid-February, management practices related to housing of mares with foals at foot may have reduced exposure to the toxin in this latter group.[260,261] What is less easily explained perhaps is the fact that fetal losses were significantly greater in mares of 10 years of age and younger.[261] The MRLS in 2001 in central Kentucky cost the local economy millions of dollars, and its effects were manifest in a reduced foal crop in the 2002 season. Most affected mares failed to resume normal cyclic activity until after the official breeding season had closed because endometrial cups had been formed before the MRLS fetal losses in the spring of 2001.

16.8.4.6 VIRAL ABORTIONS

Although equine infectious anemia is not regarded as an abortigenic disease per se, mares may abort because of the systemic effects of this virus.[263] Equine viral arteritis is a venereal disease that can be transmitted in the semen of asymptomatic (shedding) stallions.[264–267] However, the most common mode of transmission is through respiratory infection, and equine viral arteritis can cause abortion if infected animals come into contact with pregnant mares that are in the later stage of gestation (5 to 10 months).[266,268,269] Clinical signs vary but may include fever, conjunctivitis, nasal discharge, and dependent edema associated with the vasculitis.[267,269–271] Often the disease goes undetected because the clinical signs are mild or subclinical and may be clinically indistinguishable from other respiratory infections.[272] Transplacental infection of the fetus follows the development of myometritis, with degeneration of the myocytes and infiltration ofthe mononuclear cells. Epithelial cells of the endometrial gland show sporadic degeneration. The placentae are edematous, and one may observe degenerated fibroblasts in the subvillous layers. Lesions in the fetal tissue include an atrophy of the lymphoid follicles in the spleen and lymph nodes with degenerated lymphocytes. Immunofluorescence can detect equine arteritis virus antigen in the myometrium and the endometrial gland in the dams, in the subvillous layer of the placentae, and in the aborted fetuses. One may recover the virus from the uterus and fetus, but the placenta is likely to yield the greatest amounts of the virus.[273] One may control the disease by an effective vaccination program and screening tests (polymerase chain reaction, microneutralization, and enzyme-linked immunosorbent assay).[264]

The number of abortions caused by equine herpesvirus 1 (EHV1) infections has declined over the past 2 decades, and isolated abortions rather than abortion stormsare now a more common feature of this disease. In the Thoroughbred mare population of central Kentucky the number of EHV1 abortions per 1000 pregnant mares has remained below 5 since 1977, despite the mare population doubling in size.[274] This low abortion rate is due to widespread adoption of stringent vaccination programs combined with improved management practices on broodmare farms. Pregnant mares should be vaccinated with an approved vaccine at 5, 7, and 9 months of gestation. On many farms mares also are vaccinated at 3 months. New arrivals should be isolated for 3 weeks, and groups of pregnant mares should be isolated by stage of gestation. Segregation of pregnant mares from weanlings and other horses is especially important.[275]

Although EHV1 is often a subclinical respiratory infection in the mare, it can infect the fetus during a viremic episode and cause abortion because of rapid detachment of the placenta.[276] In these cases the endothelial cells in the endometrium and allantochorion are often virus-infected, with accompanying vascular lesions. The fetus can be infected via the chorionic vasculature or by inhalation of infected amniotic fluid.[277,278] Blood-borne viremic lymphocytes spread the infection from primary sites of replication in the respiratory tract and draining lymph nodes to the pregnant uterus.[279] The abortion may occur soon after the mare is infected but also may occur after several weeks have elapsed. Thus maternal serologic testing is of little diagnostic value.[280] Although EHV4 (formerly EHV1, subtype 2) occasionally is incriminated, EHV1 is responsible for mostlate gestation (>7 months) herpesvirus abortions.[281] The aborted fetus is fresh, with copious amounts of pleural and peritoneal fluid. The trachea may contain a fibrin clot. Small necrotic foci 1091
may be discernable on the swollen liver. A hyperplastic, necrotizing bronchiolitis may be visible in lung 1092
sections, and large intranuclear eosinophilic inclusion bodies are a characteristic histologic lesion. Although vaccination is practiced widely, owners should be aware that the protection is not absolute. If a pregnant mare is exposed to infected animals that recently have been to a show or that are returning from a training

facility, the possibility is that any protective immunity that the vaccine may have provided will be overwhelmed. Abortions have been associated with reactivation of latent virus that was induced by transport stress.[282] Thus a history of regular vaccination of an aborting mare does not eliminate the possibility of a herpesvirus abortion. Informed management practices are essential to minimize the chances of reproductive loss. One always should submit tissue samples for confirmation and should implement sanitary and isolation protocols. Neutralization tests, indirect immunofluorescence tests, and polymerase chain reaction tests and virus isolation are useful for EHV1 diagnostics. Antigen detection combined with virus isolation and polymerase chain reaction tests from fetal lungs gives reliable results.[283] More detailed reviews have been published.[225,284]

16.8.4.7 NONINFECTIOUS CAUSES OF ABORTION

In North America the incidence of twin abortions has dropped significantly because of early intervention after ultrasonographic diagnosis of a multiple pregnancy.[66,181] Before the widespread adoption of this technology, twin abortions were a major cause of fetal loss.[35] The type of placentation in the mare (diffuse, microcotyledonary) makes it highly unlikely that a twin pregnancy will be carried to term. Obviously, a finite endometrial surface area is available for allantochorion attachment. The more common unicornuate twin vesicles are a problem because one conceptus inevitably is restricted to the proximal aspect of the gravid horn.[16,204] Thus the two conceptuses are literally in a deadly competition for adequate nourishment and subsequent placentation. If the twin pregnancy is maintained until the latter part of gestation, the nutrient demands of the rapidly growing fetuses outstrip the placental attachments. Fetal growth is such that nutrient demands may be met until the latter half of gestation, when the considerable fetal development in the last trimester usually requires more exchange capability than the smaller placenta provides. The fetus becomes stressed as it becomes progressively emaciated, and ultimately dies.[183] Death of one or both fetuses is followed by abortion, with the characteristic avillous areas on the fetal membranes confirming the amountof placental disruption.[36,170,181] Affected mares develop premature mammary enlargement and may “run” milk before aborting.[36,285] Transabdominal ultrasonographic evaluation can be useful to confirm the diagnosis at this late stage. The area of apposition of the two chorioallantoic membranes (twin membrane) can be a useful aid for ultrasonic diagnosis of twins even in late pregnancy.[183] Twin abortions in the last few months of gestation are likely to cause a dystocia. Bicornuate twins are more likely to survive because each membrane can attach to an entire horn and one side of the uterine body, but the resulting foals are likely to be stunted because of intrauterine growth retardation.[4,36] The live birth of twin foals is extremely uncommon, and many of these neonates do not survive.[175] The mares are prone to fetal membrane retention and may be difficult to rebreed. Thus, not surprisingly, the equine breeding industry has tried always to avoid twin pregnancies.

Ultrasonographic studies of fetal mobility have helped explain the characteristic twisting that is a feature of the normal equine umbilical cord.[4,286–289] Unlike the situation in ruminants, the equine amnion floats freely within the allantoic fluid. Fetal rotation within the amniotic cavity, and amniotic sac rotation within the allantoic fluid, results in the characteristic twisting of the equine umbilical cord.[286,290] A recent report reviewed 168 cases of umbilical cord torsion from the University of Kentucky Livestock Disease Diagnostic Center representing 6.0% of equine fetus submissions over a 5-year period.[291] The gestational age of the fetuses ranged from 5 to 10 months, with a mean of 7½ months. The mean umbilical cord length for full-term Thoroughbred foals is reported to be 55 cm.[292] Umbilical cord lengths in the Kentucky torsion cases varied from 62 to 125 cm.[291] The average length of the affected cords (96 cm) exceeded that described in another

abortion study by almost 10 cm.[293] The cords tended to be highly twisted with areas of constriction, edema, hemorrhage, and thrombosis and fluid-filled sacculations. The fetuses were slightly to moderately autolyzed. This is consistent with fetal death before abortion. Urinary bladder dilation was noted in some cases, presumably because of obstruction of urachal outflow. The most consistent histopathologic finding was deposition of calcified material in the blood vessels of the chorioallantois.[291] At this time, no evidence appears to suggest that if a mare aborts because of umbilical torsion, the risk for future umbilical cord complications increases. Another complication that can be associated with an excessively long cord is strangulation. In this instance the cord becomes tightly wrapped around the fetal body or extremity, often causing a deep groove and edema of surrounding tissues.

16.8.5 Gender Determination (Fetal Sexing)

The advent of fetal sexing has permitted early gender determination to influence the value of the pregnant mare. 1092
Factors that may vary depending on the predicted sex of the foal include choice of state for foaling, appraisals 1093
and insurance coverage, sales reserves, bookings for stallion service the next season, and retention or sale of the mare.[294] Gender determination of the equine fetus can be accurate using transrectal or transabdominal ultrasonography.[294–296] One should certify fetal gender only when the identifying structures have been delineated clearly and the accuracy of the determination is guaranteed. Accurate determination of fetal sex may be difficult or impossible in some cases because of excessive mare and fetal movement or the fetus being located too deeply to permit adequate imaging. Although tranquilization (e.g., xylazine and butorphanol tartrate) is used sometimes, it may cause the uterus to relax and drop away from the examiner.[294,297] A 5.0-MHz linear array transducer is adequate for transrectal gender determination, but a 3.5-MHz transducer ensures the depth of penetration that is required to obtain transabdominal images.[155]

Gender determination is based on ultrasonographic assessment of the location of the genital tubercle, an embryologic structure initially located between the rear limbs in both sexes. The genital tubercle differentiates into a clitoris or a penis and has an ultrasonographically distinctive, hyperechoic, bilobulated appearance in both sexes. Curren[297] reported that the optimal time for gender determination is between days 59 and 68, and Holder[294] concurs that a day 60 to 70 window is the ideal period. A second ideal period for gender determination may be between days 110 and 120 because the genitalia are now well developed and the fetus tends to be more accessible again. After this time the increasing depth of the uterus means that one may not be able to make a diagnosis if the fetus is in a anterior (cranial) presentation at the time of the examination.[294] When one uses the transabdominal approach, the optimal window of time in both sexesis between 100 and 220 days of gestation.[295,296] Thereafter, identification of the anatomic structures required to make an accurate gender determination may become increasingly difficult. Detailed instructions for fetal sexing by ultrasonography have been described.[294–297] Transabdominal gender identifications based on the presence of the penis and prepuce in males and mammary glands and teats or fetal gonads in females can be accurate.

16.8.6 Monitoring Fetal Well-Being

One should suspect that mares that develop premature mammary enlargement (with or without lactation) have placentitis. A reduction in plasma relaxin levels may indicate placental compromise, because relaxin is produced by the equine placenta.[105,109–111,114,237,298–300] Low relaxin levels in late pregnancy have been associated with various causes of placental dysfunction, including fescue toxicosis, oligohydramnios, and placentitis.[301] Measurement of an equine fetal protein and estrone sulfate levels in maternal plasma have not

proved useful for early detection of fetal stress associated with medical and surgical colics.[77,78,302–305] Although pathologic conditions ofthe placenta have been correlated with increased plasma progestin concentrations in some studies, others have not detected differences in plasma progestin concentrations in mares with impending abortion and mares with normal pregnancies when monthly blood sampling was performed.[33,87,97,306–308] Pregnant mares that have experienced colic or uterine torsion and that have progestagen levels less than 2 ng/ml are at high risk for fetal loss.[77] Obtaining serial samples from a mare suspected of having placentitis is recommended. This may help to identify a clinically useful trend in progestagen concentrations.[77,208]

One must use strict hygienic procedures when using a vaginal speculum on a mare with a high-risk pregnancy, because one breaches the first two barriers to the pregnant uterus (vulvar lips, vestibular sphincter). Mares at risk for abortion often have a moist, hyperemic, relaxed cervix. Even if a vaginal discharge has not been reported, many of these mares have a purulent cervical dischargeif placentitis is present. However, although cervical softening and vaginal discharge are often present if the infection is localized around the cervical star, in the case of nocardioform placentitis the lesion does not involve the cervical star, and vaginal discharge is conspicuously absent.[230,232,235] Thus although transrectal ultrasound is a useful aid for diagnosing ascending placentitis, the site of the nocardioform lesion makes it of limited diagnostic value in these cases.[238] Transabdominal examination of the ventral uterus may reveal separation of the chorioallantoic membrane from the uterine wall, often with evidence of inflammatory exudate accumulation between the two surfaces. Placentitis and associated placental edema results in a thickened uteroplacental image. The average uteroplacental thickness on a transabdominal ultrasound image should be between 9 and 14 mm.[309,310] If the uteroplacental thickness exceeds 2.0 cm in late gestation, then a pathologic condition of the placenta is likely to be present.[310,311]

Transabdominal ultrasonography of late pregnant mares has become a routine diagnostic aid for evaluating fetal well-being.[285,308,310,311] Although the 5.0-MHz linear array transducer is ideal for transrectal reproductive ultrasonography, its shallow depth of penetration (~10 cm) limits its usefulness for transabdominal examinationsin late pregnant mares. If the mare does not have a pronounced plaque of ventral edema, the 5.0-MHz transducer often is sufficient to image the uteroplacental unit and some of the fetal fluids. A 3.5- or 2.5-MHz curved linear array or sector-scanner transducer is best for transabdominal examinations because these 1093
can penetrate to a depth of 20 and 30 cm, respectively.[156,312] Although one may image the 70- to 90-day fetus 1094
from the ventral abdomen of the mare, just cranial to the mammary gland, the late gestation gravid uterus extends along the ventral abdomen to the xiphoid.[285,309–313] By the ninth month of gestation the fetus should be in anterior (cranial) presentation and dorsopubic or dorsolateral position.[2,287,313] Thus in late gestation the fetal head should be positioned near the pelvis of the mare. An abnormal presentation, or the presence of twins, is possible if one detects a fetal head along the ventral abdomen during late gestation.[312] A more detailed examination is indicated in such cases. The posture of the extremities varies with fetal movement.[313]

One must follow a standardized methodology when scanning the uterus from the ventral abdomen, starting just cranial to the mammary gland of the mare and moving cranially to locate the fetal thorax. The ribs cause multiple acoustic shadows that delineate the thoracic cavity. A complete examination of the fetus and uterus involves scanning cranially to the xiphoid in multiple parasagittal planes and then scanning from left to right sides of the abdomen in multiple transverse planes.[312] Transabdominal ultrasound examination is an important diagnostic tool when one attempts to identify the presence of twin fetuses in late gestation. Identification of the nongravid horn can be useful to help rule out the possibility of twins. Often, obvious size discrepancy serves as

confirmation that twins are present. In other cases, one thoracic cavity does not contain a beating heart, confirming that one of the twins has died already.

In a normal pregnancy most of the fetal fluids are within the allantoic cavity. The amnion is imaged as a thin membrane that surrounds the fetus and actually lies in close contact with the fetus over much of its body. The amniotic membrane divides the imaged fetal fluid into two distinct cavities that one can see most easily around the fetal neck, shoulder, thorax, and foreleg. Typically, one images the largest pocket of amniotic fluid where the forelimb and neck meet the thorax. The maximum vertical depth of amniotic and allantoic fluid, and the qualityof amniotic and allantoic fluid are useful guides to fetal well-being.[312] One should make any measurements of fluid depth as perpendicular to the uteroplacental surface as possible.[311,312] In the normal equine pregnancy the maximum ventral fetal fluid pocket depth for amniotic fluid is 8 cm and for allantoic fluid is 13 cm.[309,312] Extremes in either direction are not normal. Obviously deficient amounts of fetal fluid indicate placental dysfunction, and excessive amounts suggest a hydrops condition.[309,314] Reef suggests that fetal fluid quantities should be considered excessive if the maximal vertical amniotic fluid depth exceeds 14.9 cm or the maximum vertical allantoic fluid depth exceeds 22.1 cm.[312] The quality of the fetal fluid is scored from 0 (clear) to 3 (echogenic fluid with numerous particles).[311,312] For one to note echogenic particles in the fetal fluids is not unusual, especially during periods of fetal activity. These particles represent sloughed cells and proteinaceous debris.[310] An increase in the number of echogenic particles in late gestation may not be abnormal.[311] However, if one is monitoring a high-risk pregnancy regularly and one observes a sudden increase in fluid turbidity (grade 3), the prognosis is not good.[310,311] The clinician should consider the possibility of inflammatory exudates, meconium passage by a compromised fetus, or even hemorrhage. One should remember that hippomanes (allantoic calculi) are a normal feature of the equine pregnancy. One may observe these structures on the ventral aspect of the allantoic cavity.[312]

An equine biophysical profile has been proposed as a guide to assessing fetal well-being and predicting perinatal morbidity and mortality.[310,311,315] Although a low score definitely indicates a negative outcome, higher scoresdo not guarantee the birth of a viable neonate.[312] Fetal breathing, heart rate and rhythm, fetal tone, and general activity are useful guides for evaluating fetal health and well-being. Thus one should avoid chemical sedation of the mare because commonly used drugs are likely to induce fetal bradycardia and suppress normal fetal activity. Fetal breathing is characterized by movement of the diaphragm between the thorax and abdomen, along with rib cage expansion, without any other movement by the fetus. One should monitor fetal breathing patterns forat least 30 seconds.[312,316]

When one monitors fetal heart rate and rhythm, scanning for only 10 or 15 seconds and then multiplying by a correction factor to obtain the number of beats per minute is not appropriate. Beat-to-beat variations and observation of periodic accelerations are important. Heart rate accelerations normally occur in association with fetal activity. Reef recommends that one make multiple measurements of fetal heart rate and assessments of fetal heart rhythm over a 30-minute period while evaluating the fetus, fetal fluids, and placenta.[312] Ideally, one should obtain three measurements with the fetus at rest and another three after periods of activity. Accurately monitoring the heart rate during periods of fetal activity is difficult, unless M-mode echocardiography equipmentis available.[312,313] Fetal heart rates vary with the stage of gestation and the amount of fetal activity at the time of the examination.[317–319] The fetal heart beat is normally regular and decreases from greater than 120 beats/min in midgestation to between 60 and 90 beats/min in late gestation.[309–311,313,317–321] Cardiac accelerations (20 to 40 beats/min above baseline) are normal if they are associated with fetal movement. [309,310,312] However, persistent tachycardia in the absence of fetal activity indicates fetal stress. A resting heart 1094

rate exceeding 104 beats/min indicates stress in a late-gestation fetus. A heart rate of less than 57 beats/min in a fetus that is less than 330 days of gestation and a rate of less than 50 beats/min in a fetus older than 329 days gestation should be considered abnormal.[311,312] A fetus suffering from hypoxemia has a slow heart rate, with minimal limb activity or fetal breathing, indicating central nervous system depression.[310–313] However, if the condition is chronic and ischemic conditions are developing, the fetus will become tachycardic despite a lack of fetal activity, which is a prelude to fetal demise. In terminal cases, extreme bradycardia ensues just before fetal death.[311,316] Although failure to observe fetal activity may be because of the stage of the normal rest/activity cycle, confirmation of a regularly beating heart at least confirms that the fetus is alive, which is a major advantage over transrectal fetal ballottement in which failure to detect movement can raise unnecessary concerns about fetal health.

If Doppler ultrasound equipment is available, one places the Doppler transducer directly over the site where one detected the best image by the ultrasound scan. One can record tracings of fetal heart rate and rhythm over time, usually intervals of 5 to 10 minutes.[322] Such tracings make analysis easier and serve as a permanent record of the fetal status at the time of the recording. If some question arises about the presence of twins after a transabdominal ultrasound examination, fetal electrocardiogram tracings may show two distinct fetal patterns.[320,321] Features of the electrocardiogram tracing that one should note include fetal heart rate and rhythm, accelerations and decelerations, complex polarity changes, and beat-to-beat variation. In the last weeks of pregnancy, fetal foals usually have a baseline heart rate in the range of 60 to75 beats/min. Transient low heart rates of less than60 beats/min are not uncommon. These troughs only warrant concern if they are not interspersed with accelerations. Likewise, transiently elevated rates around120 beats/min (occasionally >200 beats/min) are not abnormal, provided that they return to baseline. If the fetal heart rate is found to be less than 60 beats/min or greater than 120 beats/min during an observation period, then more frequent monitoring is justified to determine if the fetus is distressed. Beat-to-beat variations are normal, and a finding of no variability is an ominous sign. Maternal medications such as detomidine or butorphanol reduce fetal heart rate variability transiently.[316]

The fetus has tone if one observes it flex and extend the limbs, torso, or neck. Tone is poor or absent if the fetus appears flaccid. Fetal movements include partial to full rotation around the long axis of the fetus and less marked activity such as extension and flexion of the extremities. Fetal activity is rated on a scale from 0 to 3, with 3 being an active fetus. A score of 0 indicates that one noted no fetal movement during the examination period.[311,312] Long periods without noticeable fetal activity are cause for concern and should be evaluated along with information about the fetal heart rate and rhythm. The fetus may be distressed, suffering from advanced hypoxia, and have central nervous system depression.[310,311]

Fetal aortic diameter is correlated with the weight of the pregnant mare and with the final neonatal foal weight.[309–311] Thus one can use the weight of the pregnant mare to estimate what the fetal aortic diameter should be, using the regression equation ($Y = 0.00912 \times$ weight of pregnant mare in pounds $+ 12.46$) where Y is the predicted fetal aortic diameter (millimeters).[311,312] One then should measure the actual diameter of the fetal aortain the thoracic cavity as close to the fetal heart as possible.[309,311] An aortic diameter smaller than predicted may indicate a dysmature or growth-retarded fetus (intrauterine growth retardation) or twins. One measures the maximal thoracic diameter from the spine to the sternum over the caudal part of the thorax, and in a late gestation fetus the diameter should be 18.4 ± 1.2 cm. Thoracic diameter has been correlated with fetal aortic diameter and neonatal foal weight in high-risk pregnancies.[311,312] Foal girth measurements and hip height also have been correlated with fetal aortic diameter measurements.[310,312] Fetal biparietal measurements

and orbital diameters also have been used to estimate fetal size.[312] Decreased blood flow to the placental unit inhibits fetal growth, andone should suspect some form of chronic placental insufficiency on detection of small fetal size.

Transrectal ultrasonography provides an excellent assessment of the current status of the caudal allantochorion, and as such it is an invaluable aid for examining a late pregnant mare with signs of placentitis. An image of the ventral placental tissues in the area adjacent to the cervical star provides the ability to diagnose the early stages of ascending placentitis accurately[208] and may provide the best chance for successful medical intervention. Experienced clinicians are able to observe abnormal tissue thickness and even evidence of placental separation with an associated pocket of inflammatory exudate. Normal values for the combined thickness of the uterus and placenta have been established.[323] The combined tissue measurement is useful because it is difficult to determine exactly where the chorioallantoic membrane meets the endometrium. The area cranial and ventral to the cervix provides the most consistent measurement in normal mares, and this is the recommended site for any measurements. One should freeze the ultrasound image after locating the landmark vessel in the ventral uterine wall. One takes caliper measurements from the inner surface of the ventral uterine vessel to the edge of the allantoic fluid.[208] An established fact is that an increased combined thickness of the uterus and placenta any 1095
time from midgestation through until term indicates placental disruption and pending abortion.[306] If the 1096
combined thickness of the uterus and placenta exceeds 8 mm between days 271 and 300, 10 mm between days 301 and 330, or 12 mm after day 330, it indicates placental failure and impending abortion.[208]

Monitoring of fetal viability if one is maintaining a high-risk pregnancy by altrenogest supplementationis important. Although most nonviable fetuses will be aborted, reports have been made of mares retaining mummified fetuses when the mare was maintained on long-term progestagen supplementation.[189]

16.8.7 Complications in Late Gestation

Once confirmed to be at least 45 to 60 days pregnant, most mares can be expected to carry the fetus to term. The incidence of fetal loss after 100 to 120 days of gestation is low and accounts for only a small percentage of total pregnancy wastage. Fetal death and maceration is uncommon in the mare. However, the author has managed a case of macerated twins in a Draft breed mare that suffered no ill effects systemically. The mare was presented for evaluation only when the owner noticed a foul-smelling vaginal discharge.[324] Ventral body wall ruptures and uterine torsions are uncommon, and hydropsof the fetal membranes is an especially rare condition. Accurate diagnosis and appropriate management of these clinical cases can prevent the development of a life-threatening situation for the mare. If a ventral body wall rupture or uterine torsion is present, then the birth of a viable foal may still be possible, provided that the case is managed correctly.[325]

16.8.7.1 HYDROPS OF THE FETAL MEMBRANES

Hydrops is a condition of the last trimester, with the pregnancy developing normally until somewhere between 7½ months through term. Hydrallantoisand hydramnios are rare conditions that involve a pathologic accumulation of fluid within the allantoic and amniotic compartments, respectively. Normal volumes of allantoic fluid in mares vary from 8 to 18 L at term. In documented cases of hydrops the allantoic fluid volume ranged from 110 to 230 L.[326] Just as with cows, hydrallantois accounts for most dropsical conditions in the mare.[327–329] The pathophysiology of hydrallantois in the cow has been related to an abnormality of placentation, whereas hydrops amnion has been associated with a fetal head anomaly that precludes

swallowing.[159] Dysfunctional placentation may cause an increased production of transudate or disruption of transplacental fluid absorption. No abnormality of the fetus or fetal membranes is an apparently consistent characteristic of the condition in the mare. Bain and Wolfsdorf have incriminated a mild diffuse placentitis or endometrial vasculitis in some cases.[330] In a report on 15 cases, all of the mares were pluriparous and ranged in age from 6 to 20 years.[326]

Generally, onset of abdominal distention is sudden, and walking becomes difficult. The mare exhibits variable degrees of colic. Loss of appetite is progressive, and the mare may experience some difficulty in defecation. The increasing pressure on the diaphragm causes dyspnea, and the mucous membranes may appear cyanotic, especially when the mare is recumbent.[326] On physical examination the rectal temperature is normal, but the pulse rate is elevated. Palpation per rectum reveals characteristic findings. One should use copious lubrication and extreme caution because pressure from the large fluid-filled uterus will impede passage of the forearm. The feces tend to be covered with mucus because of prolonged passage through the lower gastrointestinal tract. The gross distention of the uterus means that the fetus is usually not palpable. Failure to detect the fetus by external ballottement further supports the diagnosis. Transabdominal ultrasound confirms the presence of excessive amounts of hyperechoic fluid. One should perform a thorough examination from both sides of the abdomen to eliminate the possibility of twins.[325]

One should advise owners that the condition is progressive and that the mare likely will not be able to sustain the pregnancy and deliver a live foal. Bain and Wolfsdorf have used a partial drainage technique in an attempt to manage some cases that were diagnosed within 2 to 4 weeks of term.[330] The mares receive abdominal support (belly band), intravenous fluids, broad-spectrum antibiotics, and antiinflammatory medication. The technique of slow, repeated drainage involves a major time commitment and would not be cost-effective for many cases. Fetal death may occur because of placental separation. A considerable risk for iatrogenic fetal infection following contamination of the fetal fluids also is apparent, despite attempts to perform the drainage technique in an aseptic manner. Thus despite heroic attempts in valuable mares, the fetus is likely to be lost in cases of hydrallantois.

In most cases, induction of parturition may be advisable before the condition of the mare deteriorates further. Continued abdominal enlargement predisposes the mare to prepubic tendon rupture,[331] and uterine rupture also has been reported.[332] Induction is not without risk (shock, dystocia), but the prognosis for survival of the mare is good, provided that appropriate supportive therapy is instituted. The prognosis for the reproductive future of the mare also may be favorable, provided that no untoward sequelae (cervical lacerations, retained fetal membranes, metritis) occur. Application of PGE to the cervix before induction may facilitate atraumatic 1096
fetal extraction.[333] In one report, six of eight mares that had previously developed a hydrops pregnancy 1097
subsequently became pregnant and delivered normal, healthy foalsat term.[326]

Before a therapeutic induction of parturition, one should wrap the tail, clean the perineal area, and insert an indwelling intravenous catheter. Large volume intravenous fluid therapy may become necessary if hypovolemic shock develops on discharge of the allantoic fluid.[325] In some cases, controlled drainage may be beneficial before inducing delivery. An added complication in hydrops cases is that the thickened, edematous chorioallantoic membrane may be difficult to rupture.[327] If digital pressure alone does not rupture the membrane, then one can use an endometrial biopsy forcep to bite a piece out of the chorioallantois. Some authors report that thelack of pressure from the atonic uterine wall resultsin minimal release of fetal fluid from the punctured chorioallantoic sac, but in the few cases with which the author has been involved, the release of fluid was massive once the chorioallantoic membrane was ruptured.[327,329] If insufficient fluid

release occurs, then one can introduce a sterile nasogastric tube into the uterus to begin controlled siphoning of fluid. An alternative technique is to introduce a thoracic trocar catheter through the cervix and to use a sharp puncture of the chorioallantois.[330] This approach permits one to remove the excess fluid by controlled drainage. Administration of intravenous fluids together with gradual removal of the excess allantoic fluid permits the cardiovascular system of the mare to adapt. Oxytocin and prostaglandin injections have been used in an attempt to abort in these cases.[327–329] Although oxytocin is considered widely to be the most efficacious method for routine induction of parturition, in hydrops cases the distended uterine musculature may not be able to contract effectively.[327,334] This uterine inertia is common, and gentle manual dilation of the cervix—or perhaps prior application of PGE—may be warranted.[333] Bain and Wolfsdorf have reported a smooth induction following two doses of cloprostenol administered30 minutes apart.[330]

The abdominal musculature may be weakened by stretching, and thus the typical stage II abdominal press may be compromised. Malpositioning and malpostures are common. The fetus may need to be extracted by assisted vaginal delivery, but one should take care so as not to traumatize the cervix by overzealous traction.[325] The expelled fetus generally is alive, and humane euthanasia is warranted. In one report, at least 50% of fetuses had some malformation.[326] One should add more oxytocin (1.0 IU per minute) to the intravenous fluids to promote uterine involution. One should expect retention of the fetal membranes, and appropriate treatment for removal of these membranes and prevention of the metritis-laminitis complex is indicated. One should monitor uterine involution by transrectal palpation and ultrasonography.

16.8.7.2

VENTRAL BODY WALL HERNIAEAND PREPUBIC TENDON RUPTURE

Apart from those with pathologic pregnancies, mares with ventral body wall defects are generally close to term. Damage to the abdominal sling of the pregnant mare may involve rupture of the transverse abdominis and oblique muscles, the rectus abdominus muscles, andthe prepubic tendon. The prepubic tendon attaches to the cranial border of the pubis.[325] Although breed (Draft mares) and age (older mares) may predispose a mare to development of the condition, in most cases the predisposing cause is not apparent.[331] The extreme abdominal distention associated with the hydrops condition may cause rupture of the ventral musculotendinous support. Defects in the ventrolateral abdominal wall are more common than complete prepubic tendon rupture.[331,335,336] In extreme cases the rupture may lead to hemorrhage, shock, and death.[337]

The most obvious clinical sign of an impending ventral body wall rupture is a thick plaque of ventral edema extending a variable distance cranial to the udder. However, ventral edema may be a normal side effect of late pregnancy and can indicate external trauma. The author has managed one case in which a large ventral swelling was associated with a massive hematoma that appeared to have originated from a kick. Mares in late pregnancy often develop a thick plaque of ventral edema that can extend from the udder to between the forelimbs. The edema is associated with the compressive weight of the gravid uterus on the venous and lymphatic drainageof the ventral abdomen. The presence of a hemorrhagic secretion in the mammary gland supports a diagnosis of tissue trauma rather than pregnancy edema. Unilateral edema is more indicative of damage to the ventrolateral body wall but may be associated with partial rupture of the prepubic tendon.[325] The extreme pain associated with progressive enlargement of a ventral body wall rupture causes a significant tachycardia that may not respondto analgesics. Pregnant mares with a ruptured prepubic tendon or ventral abdominal wall show signs of colic and generally are reluctant to move. If the prepubic tendon ruptures completely, the pelvis will tilt such that the tailhead and tuber ischii are elevated, and a lordosis will be

present. The mammary gland often is displaced craniad and ventrad because of loss of the caudal attachment to the pelvis. A rent in the abdominal musculature may be complicated by bowel incarceration.[325,331]

Confirmation of the tentative diagnosis can be difficult. Because one cannot always be certain that a rupture has occurred, one should confine mares with severe ventral edema to a stall, with exercise restricted to 1097
walking in hand. Palpation of the defect per rectum is usually not possible because of the advanced stage of 1098
the pregnancy. External palpation is also generally unrewarding because of the thickness of the edema, although one may note some crepitation of the ventral abdominal wall. The mare generally is extremely sensitive and resists palpation of the area.[325] Ultrasonographic examination of the posterior aspect of the ventral abdomen may be useful insome cases and can detect the presence of a bowel segment.[312,325,337] One often cannot assess the dimensions of the defect accurately until the fetus and fetal fluids are expelled and the ventral edema has subsided.[325]

Termination of the pregnancy may be the most humane treatment for the mare, for further tissue damage is likely to occur to some degree until parturition. In extreme cases the mare may eventually become recumbent. One also should consider the possibility of a segment of intestine becoming incarcerated in the defect. However, the present fetus may well be her last, and owners often request that an attempt be made to maintain the pregnancy to term. In these cases the treatment is essentially supportive. Antiinflammatory drugs help to alleviate the discomfort of the mare. An abdominal sling (belly band) made of canvas or padded leather or a snug abdominal bandage helps provide support for the ventral abdominal wall. In the author's experience, abdominal bandages tend to be purely cosmetic because they soon stretch and thus provide minimal long-term support. If one uses a sling, the area over the back must be well padded to prevent pressure necrosis because the purpose of this support is to transfer the weight of the gravid uterus to the vertebral column.[325] Reducing the bulk of the ration and feeding a mild laxative may help reduce the degree of abdominal exertion associated with defecation.[337]

Assistance with parturition should be available, for the mare is likely to experience difficulty in mounting sufficient abdominal pressure to expel the fetus. One should make arrangements for an alternate source of colostrum because ventral edema may preclude the foal from suckling. One should inform the owner that although in some cases surgical repair of the defect may be possible by mesh herniorrhaphy, rebreeding the mare may not always be advisable.[331] Some mares with small, unrepaired defects may foal subsequently without assistance, but one should consider the potential risk of further pregnancies exacerbating the condition.[337] Embryo transfer offers a viable alternative if this procedure is condoned by the relevant breed society.

16.8.7.3 UTERINE TORSION

Uterine torsion accounts for 5% to 10% of all complicated obstetric conditions in the mare.[338,339] The causes of uterine torsion in the mare are not well defined. The condition is much more common in cattle, and in that species a large term fetus has been implicated as a major risk factor. Most uterine torsions in cows occur at term, and most are thought to result directly from fetal positional changes during late first stage and early second stage of labor.[340] A striking difference between the mare and the cow is that more than 50% of uterine torsions in mares occur before the end of gestation.[338] In the author's clinical experience, most torsions occur before term and may occur as early as 8 months of gestation.[325] In fact, one recent report documented a case as early as 126 days of gestation.[341] Although Ginther has shown that the fetus is locked

into a dorsopubic position during the final months of gestation, it still is possible for the entire pregnancy (uterus and fetus) to rotate approximately90 degrees on the lower maternal abdominal wall.[4] This rotation occurs because any rotational movement of the caudal half of the fetus (pelvis and hindlimbs) by necessity involves the close-fitting uterus. In extreme cases this rotating action seems likely to lead to a clinical uterine torsion.[342] Owners who work closely with their mares may mention that they have observed excessive fetal movements in the flank area in the past day or two. In a recent study, 80% of term fetuses were in dorsosacral position when the uterine torsion was corrected. This suggests that fetal righting reflexes may have played a role in creating the torsion.[339] The author believes that vigorous fetal movements during the latter stages of gestation are likely to be a significant factor in the cause of uterine torsion in the mare.

The clinical signs that attract the owner's attention result from abdominal pain[325,343–346] and include restlessness, sweating, anorexia, frequent urination, sawhorse stance, looking at flanks, and kicking at the abdomen. When the veterinarian is first summoned, the signs may have been present for two hours, but sometimes for up to 3 days or more, especially if they are intermittentand moderate.[325,344] In mares that are close to term, the owner may assume that the signs indicate impending parturition. In more extreme cases the signs are more severe and may be associated with concurrent involvement of the small or large colon.[344,345] Veterinarians always should consider the possibility of uterine torsion when presented with a mild, persistent colic in a mare that is in the last trimester of gestation. Delay in making a definitive diagnosis increases the likelihood of fetal compromise.[325] Occasionally the condition may remain undiagnosed for several weeks.[347] In these instances an owner may have attempted treatment with analgesics that they have used for previous mild colic episodes.[325,343]

Palpation per rectum is essential to determine whether a uterine torsion is present.[325,343] The author is of the opinion that all late pregnant mares that display signs of mild to moderate colic warrant a thorough rectal 1098
examination to rule out the possibility of uterine torsion. Although vaginal involvement in the torsion is 1099
common in the cow, uterine torsions in the mare seldom cause detectable changes in the vagina.[325,340,344,347,348] Thus vaginal examination is generally not diagnostically useful. On palpation per rectum the clinician should aim to advance the forearm carefully while palpating for a taut band on either side of the rectum. The ligament on the side of the torsion tends to be more caudal and is palpable as a tight vertical band. As one advances the arm further, the opposite ligament will be palpable as it is pulled horizontally across the top of the uterus before being displaced ventrally. An accurate examination of the broad ligaments confirms the diagnosis, determines the direction of the torsion, and gives some idea of the severity of the torsion. A transrectal ultrasound examination is useful to evaluate the condition of the fetal fluids and to note if any placental detachment has occurred. One can gauge the degree of uterine compromise by notingthe thickened uterine wall and distended vasculature. Compression of the veins and lymphatics occurs before occlusion of the arterial blood supply. Thus the initial changes are associated with pooling of fluid within the uterine wall.[325] The compressive forces of the displaced broad ligaments may cause variable amounts of constriction of the small colon.[344,349]

One may use transabdominal ultrasonographic imaging to assess fetal viability (heart rate and rhythm) and to evaluate the condition of the fetal fluid. Abdominocentesis can provide prognostic information and guide the clinician in choosing a mode of correction.[350] Because obtaining peritoneal fluid from a mare in late gestation may be difficult, transabdominal ultrasonography is sometimes useful in locating a pocket of fluid. Uterine rupture can be a complication of uterine torsion in the mare.[344,351] In the author's experience, mild uterine torsions or those of short duration do not alter the color, cellularity, or total protein content of the

peritoneal fluid. Severe torsions or misdiagnosed cases that are more chronic may develop significant uterine compromise that results in changes in the composition of the peritoneal fluid. Any alterations in the composition of the peritoneal fluid may indicate the presence of a compromised or ruptured uterine wall.[350] A flank laparoscopic examination can confirm the condition of the uterine wall.[352] This information facilitates an informed choice of surgical approach or perhaps supports a decision for euthanasia if economic considerations preclude surgical intervention.

If the mare is at term and the cervix is dilated sufficiently to permit passage of a well-lubricated arm into the uterine body, then one may possibly reach the fetus. One should grasp the fetus ventrolaterally and then rock it back and forth until sufficient momentum is achieved to continue up in an arc. This manipulation should roll fetus and uterus back into a normal position. Vandeplassche[353] has reported that more than 80% of term torsions can be corrected in this manner. Options for management of a preterm uterine torsion are rolling the mare, flank laparotomy, or a ventral midline celiotomy.

The anesthetized mare may be rolled in an attempt to rotate the body of the mare around the stationary gravid uterus.[354] Placement of the mare in lateral recumbency on the side of the torsion is essential. The aim of the procedure is to roll the mare such that the pelvis literally catches up with the displaced uterus. Correction by rolling the mare is controversial.[325,337,344,353] Citations in the literature report on a limited number of cases.[338,345,354] Concerns with this approach include unsuccessful attempts to correct the torsion prolong its adverse effects; misdiagnosis of the direction of the torsion means that rolling the mare may make the condition worse; inability to evaluate the condition of the uterus; and the potential for creating a displaced colon.[325] In addition, the risk of placental detachment and uterine rupture is reportedto be higher.[337,338,345] Another concern is that if general anesthesia is induced under less than ideal conditions, then maternal hypoxia may cause fatal complications in the already compromised fetus.[355]

In the standing flank approach, one makes a grid incision on the same side as the direction of the torsion.[344] One corrects the torsion by placing the forearm under the uterus and then rocking the uterus and contents back and forth to gain momentum. A combination of lifting and rotating movements generally results in easy correction of the torsion. The presence of a live fetus greatly facilitates the detorsion manipulations. One may experience more difficulty in mares that are close to term. In these cases an incision in the opposite flank permits a second surgeon to assist by gently pulling across the top of the uterus as it is elevated from below.[348] If the fetus is dead, the mare should abort naturally once the uterine torsion has been corrected, thereby avoiding hysterotomy and any associated complications.[217,325,326] However, one should monitor the mare closely, and obstetric assistance must be available to correct any malposition or malposture. One should operate on intractable mares under general anesthesia.[338,344] A ventral midline celiotomy also is indicated when one is concerned about significant uterine compromise or if one suspects another problem coexisting in the abdomen.[325]

The prognosis for mares with uterine torsion depends on the degree of vascular compromise. Severity and duration of the condition affect placental circulation and subsequent fetal viability.[325] In chronic cases in which uterine compromise is significant, performing an ovariohysterectomy to salvage the mare for nonbreeding purposes is feasible.[343,347] In the author's experience, if the fetus is alive and the uterine wall is 1099
not severely congested and edematous, then the prognosis for survival of the mare and for the birth of a live 1100
foal at term is good.[325,341] Progestin supplementation for 3 to 5 days after the manipulations involved in correcting a uterine torsion may be indicated to ensure myometrial quiescence and thus maintenance of the

placental attachment.[85] Although supplementation after a uterine torsion would be in the last 2 to 3 months of gestation, some authors report mares retaining a nonviable (died at 3 to 5 months of gestation) fetus while being administered progestins.[189] Thus if one administers progestin supplementation to a mare after correction of a uterine torsion, monitoring of fetal viability at regular intervals is prudent.[325] Continuation of the supplementation once the mare has been discharged from the hospital has little merit.

16.8.7.4 VAGINAL HEMORRHAGE

Visible blood on the tail hairs and hindlimbs of a pregnant mare warrants a careful vaginal examination. Three barriers protect the fetus: vulvar lips, vestibular sphincter, and cervix. Because one will enter two of these barriers when performing a vaginal speculum examination, one must ensure stringent hygiene. Often one can see blood clots in the vestibule on parting the vulvar lips. One should cover a sterile speculum with sterile lubricant and then gently insert it into the vagina. Often no blood is visible in the cranial vagina. If the cervix is closed, pale, and covered with tenacious mucus, then the blood likely is not associated with fetoplacental unit. Although the blood could be associated with cystitis or urolithiasis, in most cases the source of the hemorrhage is varicose vessels in the remnants of the hymen at the level of the vestibular sphincter. To miss these on insertion of the speculum is not unusual. Thus one should pay particular attention to this area while withdrawing the speculum. In some cases, introduction of a fiberscope permits easier visualization of the distended vessels. One can ligate, or in some instances cauterize, the varicosities.

16.8.8 Fescue Toxicity and Agalactia

A wide range of reproductive problems (thickened placenta, abortion, prolonged gestation, dystocia, dead or weak foals, agalactia) have been attributed to the effects of the fungal endophyte *Acremonium coenophialum*, now known as *Neothyphodium coenophialum*.[356–358] The endophyte produces a dopaminergic, vasoactive, ergopeptine alkaloid (ergovaline).[356] This alkaloid disrupts the fetoplacental production of progestagens, but the precise mechanism has not been established.[91,94,359,360] Umbilical vein progestagen levels suggest that the disruption isnot at the level of placental steroidogenesis, a remarkable observation when the fetal membranes are so edematous. Premature chorioallantoic separation and the failure of the membrane to rupture (“red bag”) are attributable to the edematous splanchnic mesoderm.[356] ACTH, thyroxine, triiodothyronine, progestagen, and cortisol concentrations are lower in foals born to endophyte-exposed mares, suggesting that the effects are actually at the level of the fetal hypothalamo-pituitary axis, thyroid, and adrenal cortex.[94,361] This is likely to be the basis for the prolonged gestation and fetal dysmaturity that are associated with fescue toxicosis.[94,359] The ergovaline also inhibits prolactin secretion in affected mares by acting as a dopamine agonist at the maternal pituitary level.[123,124,360] Prolactin secretion can be inhibited experimentally by administration of dopamine agonists such as bromo cryptine. Not only does such prolactin-inhibiting treatment of pregnant mares result in agalactia, but it also mimics the other symptoms of fescue toxicosis (thickened placenta, prolonged gestation, and dystocia).[125] Ryan PL, Bennett-Wimbash K, Vaala WE, et al.[237] have demonstrated that an effect of fescue toxicosis in pregnant mares is a lowering of the circulating relaxin levels. Clinical observations suggest that a one-time injection with fluphenazine improved pregnancy outcome by reducing the adverse effects of fescue toxicosis concomitant with a stabilization of plasma relaxin concentrations. These data support the hypothesis that systemic relaxin may be a useful biochemical means of monitoring placental function and treatment efficacy in the mare.

Because late pregnant mares are so susceptible to the toxic effects of ergopeptine alkaloids, they should not be permitted to graze endophyte-infected tall fescue pasture or hay derived from such pasture. Short-term exposure by mares at 300 days of gestation results in a significant decline in prolactin and total progestagen concentrations within 48 hours. Fortunately, removal of pregnant mares (300 days of gestation) from infected pasture results in a significant increase in prolactin and progestagen levels within 3 days and prevents the development of the typical symptoms associated with fescue toxicosis.[94,359] Even when alternate feed sources are limited, one should make every attempt to remove pregnant mares from endophyte-infected fescue by 30 to 60 days before the expected foaling date. When this is not possible, prophylactic administration of the dopamine receptor antagonist, domperidone (Equidone) can prevent the development of fescue toxicosis.[123–125,357,362–365]

16.8.9 Induction of Parturition

Induction of parturition may be indicated as a clinical management procedure for some high-risk pregnancies including mares with hydrops, ruptured prepubic tendon, or ventral herniation. These mares often require assistance with the delivery because the stage II abdominal press will be compromised.[325] This author does not 1100
believe that induction should be practiced for convenience alone.[366,367] One should advise owners that 1101
complications such as dystocia, premature placental separation, fetal hypoxia, and dysmaturity are common sequelae of the induction procedure.[368–370] The aim of a controlled foaling is not only to deliver a viable fetus but also to prevent any injury to the mare that may compromise future fertility.[325]

Induced foalings sometimes are indicated to ensure that optimal veterinary assistance is available when complications are expected and to optimize resuscitation attempts when a compromised fetus has been monitored in utero. When one detects fetal stress in a high-risk pregnancy, the clinician is faced with the dilemma of inducing delivery and attempting supportive care in a neonatal intensive unit or leaving the compromised fetus in utero. One should inform owners that delivery is indicated only if the probability of extrauterine survival exceeds that for continued maternal support. Experience suggests that an abnormal uterine environment often is more successful at maintaining the life of a fetal foal than a neonatal intensive care unit.[316] A fetus that has been exposed to an adverse uterine environment for some time may be more tolerant of premature delivery.[149] Many clinicians administer a dose of corticosteroids to the mare if premature delivery appears to be unavoidable. Corticosteroids may stimulate surfactant production and promote accelerated maturation of the fetal lungs.

The normal physiologic processes in the prepartum mare and fetus have been discussed in a previous section. Final maturation of the fetus results in increased ACTH release from the pituitary gland and subsequent stimulation of the fetal adrenal cortex.[87,90,91] Not until the maturing adrenal gland attains 17α-hydroxylase capacity are the high levels of pregnenolone metabolized into fetal cortisol.[93] These vital changes occur in the last24 to 48 hours before birth, and thus the equine fetus is at a substantially increased risk of dysmaturity/prematurity if one does not plan the induction carefully.[371] Planning traditionally has involved confirmation of gestation length, monitoring mammary development and milk/colostrum production, and ultimately evaluation of the amount of cervical softening.[372] The fetus usually is in dorsopubic position with neck and limbs flexed before induction. The incidence of posterior and transverse presentationsis rare, but detection of these abnormalities by palpation per rectum before induction would be reason to reevaluate the induction plans. Delivery by cesarean section maybe a more prudent course of action, especially with transverse presentations.

Gestation length is notoriously unpredictable in mares.[373,374] Although the frequently recommended minimum gestation length for successful induction is 330 days, one must remember that many mares carry a foal past 340 days and occasionally to 360 days and beyond.[3,372,374,375] In a retrospective study of Standardbred mares the mean duration of gestation was 343.3 days and was significantly greater for colt fetuses (344.4 days) than for filly fetuses (342.2 days). Sire has been associated with duration of gestation; gestation after mating with certain sires was consistently less than 340 days in duration, whereas gestation after mating with other sires was consistently more than 350 days.[376] Mares tend to carry foals longer if they are due to foal early in the season (shorter day length), whereas gestation length may be shorter if the foal is not due until longer days have arrived.[374,376–378] Placentitis and other pathologic conditions of the placenta often are associated with precocious mammary gland development and premature changes in mammary secretion electrolyte concentrations. Milk electrolyte changes are unreliable for assessing fetal readiness for birth in abnormal pregnancies (e.g., placentitis, impending twin abortion). In these cases, mammary secretion calcium concentrations may be elevated (>10 mmol/L; >400 ppm; >40 mg/dl) before day 310 of gestation.[97]

Although mammary development is a useful sign of approaching parturition in normal mares, monitoring changes in mammary secretion electrolyte concentrations is the most reliable guide to imminent parturition.[379–383] An inversion in the sodium-to-potassium ratio, followed by a rapid rise in calcium concentrations in the last 24to 48 hours has been correlated with fetal maturity. Exact values may vary with the type of chemistry analyzer used by the diagnostic laboratory. In a normal term pregnancy the combined mammary secretion levels of calcium(>40 mg/dl), potassium (>30 mEq/ml), and sodium (<30 mEq/ml) indicate fetal maturity.[381] A concentration of 10 mmol/L (40 mg/dl; 400 ppm) calcium in mammary secretions is a reliable indicator of fetal readiness for birth.[373,379,381,383,384] Several stallside tests are available that can measure Ca^{2+} concentration in mammary secretions based on a colorimetric change of pads on a test strip. Water hardness kits are also useful for determining the concentration of calcium in mammary secretions.[371,384–388] These kits involve titration of a diluted sample until an indicator dye changes color, and although they are more labor intensive than the test strips, they are reported to provide a more reliable guide for predicting the onset of parturition within the next 24 hours.[388] However, one must ensure that the water hardness kit is only measuring calcium levels if one is using it to decide when to induce parturition safely. Many kits merely test for divalent cations, which includes magnesium and calcium.[373] Because magnesium levels peak earlier than calcium, one may obtain misleading information about fetal maturity.[383,384] If the intent is merely to predict the onset of spontaneous parturition, then the type of test is not as critical.[373]

A recent publication has questioned the interpretation of $CaCO_3$ test kit data that has formed the basis of 1101
recommendations that 200 or 250 ppm $CaCO_3$ be used as the benchmark for readiness for birth.[373,388,389] 1102
Paccamonti[373] contends that because calcium in milk is not in the form of $CaCO_3$, any test that measures $CaCO_3$ levels in solution must be adjusted to account for this fact. Because the molecular weight of $CaCO_3$ is 100 and that of calcium is only 40, the conversion factor is 2.5 (i.e., divide the $CaCO_3$ parts per million by 2.5 to obtain calcium parts per million). Furthermore, because mammary secretions usually need to be diluted before a water hardness test can be used to measure calcium, Paccamonti recommends that 1 ml of secretion be diluted in 4 ml of distilled water. Thus one should correct the final reading by a factor of 5.[373] The division by 2.5 to convert $CaCO_3$ to calcium and the multiplication by 5 to correct for the dilution means that the test result need only be doubled to provide an accurate level of parts per million of calcium. Using this logic, Paccamonti contends that the reports using 200 to 250 ppm $CaCO_3$ as an indicationof readiness for birth actually are using

calcium values of only 80 to 100 ppm.[373,388,389] However, if these values are corrected for the reported test dilution (1:6) factor, then the $CaCO_3$ level being reported would have been 1400 to 1750 ppm (560 to 700 ppm calcium; 14 to 17.5 mmol/L). These corrected values are thus in excess of the 400 ppm (10 mmol/L) calcium concentrations reported by others.[373,379–381,383,384] Obviously, keeping these calculations in mind is important, because water hardness kits may vary, and technicians may use different dilutions. Inappropriate application of the math could lead to an erroneous conclusion about fetal maturity and subsequent induction of a premature foal.[373]

In general, mammary secretion calcium concentrations are more reliable for predicting when a mare is unlikely to foal spontaneously rather than for determiningwhen she is likely to foal.[373,384] Ley et al. used a water hardness kit and reported that $CaCO_3$ levels greater than 200 ppm (see the preceding discussion) indicated a54% probability of spontaneous foaling within 24 hours, 84% probability of spontaneous foaling within 2 days, and 97% probability of spontaneous foaling within 3 days. A small percentage of mares foaled within 24 hours despite a $CaCO_3$ level less than 200 ppm.[388,389] However, using Paccamonti's logic, this equates to a corrected value of less than 560 ppm Ca^{2+}.[373] Because a value of 400 ppm Ca^{2+} indicates readines for birth, that some of these mares foaled is not surprising. Although the fetus initiates parturition, the mare appears to be able to regulate the actual timing of delivery.[367] Thus any untoward changes in the environment of the mare may cause her to postpone the delivery and thus create disagreement with predictions based on mammary secretion electrolyte concentrations. Calcium levels can change rapidly during a single day. Thus testing secretions morning and evening may be useful. If one performs only a single test, checking the calcium levels late in the day is preferable.[373] Generally, the more rapid the rise in milk calcium levels, the more imminent is parturition. Primiparous mares can be especially difficult to monitor because no change in mammary secretion electrolyte composition may be detectable until immediately before foaling.[371]

The presence of cervical softening traditionally has been suggested as a prerequisite for optimal induction of parturition in the mare, and in a recent study mares with a relaxed cervix before induction had a more rapid delivery.[334,372] This study found that foals delivered from mares with a preinduction relaxed cervix stood and nursed sooner and had fewer signs of intrapartum asphyxia (hypercapnia, maladjustment) than foals delivered from mares with a nondilated cervix. Those mares that developed parturient complications (premature placental separation, dystocia) all had a closed cervix before induction.[334] The status of the cervix is controversial because earlier reports suggest that inductions may proceed successfully even though the cervix is tightly closed and covered with mucus.[368] A recent innovation has been the intracervical administration of PGE_2 (2.5 mg) before induction, but no difference was apparent in the mean interval from initial oxytocin treatment to rupture of the chorioallantois or to the delivery of the foal.[333] However, the affect on foal viability was positive in that foals delivered from PGE_2-treated mares suckled sooner. The application of PGE_2 intracervically may have some merit when terminating a pathologic pregnancy (e.g., hydrops) in which the induction is known to be premature and is aimed at salvaging the breeding prospects of the mare. Although no correlation exists between myometrial strip (in vitro) response to oxytocin treatment and gestational age, in the author's experience, premature induction with oxytocin can take much longer (1 to 2 hours) than an induction in mares at term.[148,390] This is consistent with the belief that a critical sequence of hormonal changes is required before fetal expulsion can occur.[148] Because the aborted fetus generally is alive and gasping for air, having some euthanasia solution ready to destroy the nonviable neonate humanely is advisable.

Several experimental protocols have been reported for induction of parturition in the term mare, including glucocorticoids,[370,391,392] prostaglandins,[368,370,381,393–398] and oxytocin.[*] High and repeated doses are required for glucocorticoid induction, because unlike with ruminants, this regimen has limited efficacy in the mare. Likewise, prostaglandin induction is not efficient in the mare. The synthetic products (fenprostalene, fluprostenol, and prostalene) are more effective than natural prostaglandin, but results can vary.[368,370,381,393–398] 1102
1103 Oxytocin is the preferred drug for induction of parturition in the mare. A wide range of protocols have been suggested over the years, including a bolus dose (20 to 75 units), low doses(2.5 to 20 units) repeated every 15 minutes to effect, and a slow intravenous drip of 60 to 120 units total(1.0 unit/min).[*] Recent work suggests that the choice of oxytocin regimen is less important for foal viability than appropriate case selection and adherence to criteria for induction.[334,371] A low-dose protocol has been recommended because it appears to work only in those mares that have a mature fetus. Mares were diagnosed as being ready for birth by mammary secretion calcium strip test measurements. A single intravenous injectionof 2.5 IU oxytocin was given between 5 P.M. and 7 P.M. and resulted in the delivery of a normal foal within120 minutes in 95% of mares. Twenty-four of 38 (63%) treated mares foaled in response to the first oxytocin injection. Another 9 of 38 (24%) foaled the following evening in response to the second injection, and 3 of38 (8%) foaled in response to the third treatment. The conclusion was that the major advantage of injecting a daily low dose of oxytocin appears to be that such a low dose only induces delivery in mares that are carrying a mature fetus and that are ready to foal. This low-dose, early-evening oxytocin protocol has been proposed to be used as a reliable method to induce parturition or to predict that the mare would not foal that night if parturition did not occur within 2 hours of treatment.[402] However, even this promising protocol has limitations because it is still possible occasionally to induce a mare to deliver a premature foal.

Because one performs most inductions based on an expectation of complications, the clinician should be well prepared before administration of the induction agent (low-dose oxytocin).[373] Even if the induction is not being performed by an intravenous drip, insertion of an indwelling catheter is recommended. The catheter facilitates rapid induction of general anesthesia if obstetric difficulties ensue. One should place a fully stocked obstetric kit and ample volumes of lubricant outside the stall. One should anticipate neonatal resuscitation efforts and should have appropriate supplies (e.g., oxygen delivery system) readily available. Premature separation of the placenta is a common complication of induced births. The clinician should rupture the exposed chorioallantois immediately and then assist with fetal delivery along with the expulsive efforts of the mare. Overzealous traction at this time may cause a laceration of the cervix if it has not yet dilated fully. If necessary, one can place an oxygen tube in the nostril of the foal during the minute or two that may be required to complete the assisted delivery.

* References 302, 333, 334, 368, 372, 399–403.

* References 62, 368, 369, 372, 399–401, 403.

16.8.10 Obstetrics

16.8.10.1 MANAGEMENT OF THE PREGNANT MARE

A series of publications that discuss the effects of nutrition on various aspects of equine reproduction have been published recently.[404–407] Regular exercise and routine hoof maintenance are important for broodmares. A regular anthelmintic program is essential to ensure the well-being of the mare and also reduces the exposure of the foal to parasite eggs in the feces of the mare and the transmammary transfer of *Strongyloides*

westeri larvae. Pregnant mares should be current for all vaccinations that are recommended for that particular geographic location.[408,409] In North America, advising owners about the importance of a regular vaccination program for equine herpesvirus is especially important. Owners and or farm managers should be aware of the need to isolate pregnant mares from transient horses to reduce the risk of infectious disease, especially respiratory viral infections. A tetanus booster may be indicated a month before foaling. Another recommendation is that the mare be transferred to the final foaling environment at least a month before the due date. Foals are born essentially agammaglobulinemic, and the neonate depends on passive transfer of colostral immunoglobulins to provide initial protection from environmental pathogens. If a vulvoplasty (Caslick) has been performed, one should make plans to open this approximately a week earlier than the expected foaling date. If the mare has a history of a previous hemolytic foal (neonatal isoerythrolysis), then one should make plans to prevent the neonate from suckling the mare until all the colostrum has been stripped out (see Chapter 19).

Those persons responsible for monitoring the foaling process should understand that mammary development, followed by distention and waxing of the teats, and then relaxation of the perineal area indicate approaching parturition in the mare. The use of mammary secretion electrolyte concentrations to predict foaling has been discussed in a previous section. Electronic monitoring systems are also available that can signal the start of parturition. One should counsel inexperienced personnel about normal foaling events and how to recognize when professional assistance is required. Inappropriate intervention by ill-informed individuals can jeopardize the life of the foal and potentially cause life-threatening complications in the mare. Separation of the fetal membranes deprives the fetus of oxygen, and this is the critical factor one must consider when assessing an obstetric case that involves a live foal. Although most references suggest that fetal survival rates are low if the foal is not delivered within 30 to 40 minutes of chorioallantoic rupture, one author reports having delivered live foals by cesarean section up to 90 minutes later.[410] These are mares that were presented 1103
promptly to the veterinary hospital and that had minimal, if any, vaginal intervention at the farm. The limited 1104
vaginal intervention likely is less disruptive to the placental attachment, and thus these foals are not deprived of their oxygen supply. Keeping the mare on her feet, and walking if necessary, may help to reduce straining while professional assistance is sought.

16.8.10.2 NORMAL PARTURITION

16.8.10.2.1 Terminology

The terms *presentation, position*, and *posture* describe the disposition of the fetus as it enters the vaginal canal. Often a fetus is described as having been malpresentedor malpositioned when the only anomaly present is postural, the most common cause of dystocia in the mare.[411] In an attempt to avoid confusion, Vandeplassche introduced the all-encompassing term *fetal maldisposition* to describe the combination of presentational, positional, and postural abnormalities that can contribute to a dystocia.[353] Presentation describes the aspect of the fetus that enters the vaginal canal first and also the orientation of the fetal spinal axis to that of the mare (anterior or posterior longitudinal; ventro- or dorsotransverse). More recently the tendency has been toward using the terms *cranial* and *caudal presentation*. Position describes the relationship of the fetal dorsum (longitudinal) or head (transverse) to the quadrants of the pelvis of the mare. The normal position for delivery is dorsosacral. A fetus that is still on its side would be right or left dorsoilial, and a fetus that is upside down would be dorsopubic. The terms *right* and *left cephaloilial* refer to the position of the fetal head relative to the pelvic walls of the mare and imply that a transverse

presentation is present. Posture is purely fetal and describes the relationship of the extremities (head, neck, limbs) to the body of the foal.[159,411]

16.8.10.2.2

Fetal Kinetics

Fetal mobility has been discussed concerning umbilical cord torsion and abortion. Fetal rotation within the amniotic cavity and amniotic sac rotation within the allantoic cavity result in the characteristic twisting of the umbilical cord.[286,290] Some highly efficient mechanism ultimately guides most equine fetuses into a cranial presentation.[4] Ultrasonographic studies have notedthe percentage of anterior, posterior, and transverse presentations at 5 to 6 months to be 52%, 29%, and 19%, respectively, but between 7 and 11 months the fetal presentation becomes predominately anterior.[286,287,289,290,296] Vandeplassche reported the incidence of anterior, posterior, and transverse presentations in the normal parturient mare population to be 98.9%, 1.0%, and 0.1%, respectively.[353,412] Ginther[286,289] and Ginther and Griffin[288] observed that muscular contractions close the lumen of both uterine horns between 5 and 7 months, and thus the allantoic fluid along with the fetus is confined to the uterine body. During this time, the fetus positions itself so that its head end points toward the cervix of the mare (cranial presentation).[287,288,296] The neurologic signals within the fetal inner ear have been proposed to respond to the slope of the ventral uterine wall and to guide the fetus to lie with its head elevated toward the cervix.[289] In most cases the noncord horn remains closed, whereas the cord horn gradually permits the entry of the hindlimbs between 7 and 9 months. The limbs can enter the horn only when the fetus is in dorsal recumbency because the angle is so acute between the horn and body by this stage of gestation. Thereafter the hindlimbs remain enclosed within the cord horn and the hooves extend to the horn tip by the 10th month.[286–289] Thus the selective closing and opening of the uterine horns, with subsequent trapping of the hindlimbs, is believed to be a key feature of the mechanism that ultimately directs fetal orientation into cranial presentation.[287,289] Entrapment of the hindlimbs within the uterine horn generally means that the caudal portion of the anteriorly presented fetus is lying in a dorsopubic, and occasionally dorsoilial, position.[289] Ginther's ultrasonographic investigations have substantiated the classic radiographic study that demonstrated that the full-term equine fetus initially is lying in a dorsopubic position with the head, neck, and forelimbs flexed.[413]

In early pregnancy the mesometrial attachments suspend the uterine horns so that they point cranially and dorsally, but by late gestation the horn containing the hindlimbs comes to rest on the dorsal surface of the uterine body, with the tip of the horn directed back toward the cervix.[4,287] The hooves and horn tip may be pushed so far caudally that they actually come to lie over the fetal head, meaning that when one performs a per rectum evaluation of a mare in late gestation, the fetal hooves that are palpable may be attached to the hindlimbs. In some mares the vigorous pistonlike thrusts of the hindlimbs in association with elevation of the fetal rump may push the hooves past the cervix into the rectogenital pouch.[4,287,289] This observation may explain the acute colic episodes that previously have been attributed to uterine dorsoretroflexion.[326] Although the caudal aspect of the fetus is associated intimately with the uterine wall, the cranial portion has room to rotate within the uterine body itself. Ultrasonographic studies on mares closeto term (>330 days of gestation) have shown that the cranial half of the fetus was in dorsopubic position approximately 60% of the time and in dorsoilial position about 40% of the time. The forelimbs and head usually were flexed (about 80%), but in the remainder the head or limbs were extended.[289] Postural changes are common, and thus palpation per rectum before the onset of first stage labor is not a good predictor of 1104

impending dystocia.[289,411] However, detection of a posterior or transverse presentation at this late stage is cause for concern, and one should make appropriate plans to manage the impending delivery. 1105

16.8.10.2.3
Stages of Parturition

Behavioral changes that characterize the first stage of parturition include the mare looking at her flank, frequently lying down and getting up, stretching as if to urinate, and passing small amounts of feces. Patchy sweating may develop, and some mares leak colostrum.[159,411] The restless behavior is similar to that of mild colic and is associated with the development of coordinated uterine contractions that increase uterine pressure and push the chorioallantoic sac (in the region of the cervical star) into the gradually dilating cervix. The increasing uterine tone during stage I of parturition has been proposed to stimulate the fetus to extend its head and forelimbs up into the dilating pelvic canal.[4] Once the head and forelimbs are extended fully, they are unlikely to return to a flexed posture unless the foal reacts to manual intervention on the part of a foaling attendant. However, the neck or a forelimb possibly may develop a malposture if not correctly aligned when the mare begins an expulsive effort. Passage of the urinelike allantoic fluid (waters breaking) concludes the first stage of labor. Rupture of the chorioallantois and passage of the allantoic fluid does not occur until the fetlocks, or sometimes the knees, are at the level of the external cervical opening. If the chorioallantois does not rupture, then further separation from the endometrium can result in a “red bag” delivery with the velvety, red membrane appearing at the vulvar lips. In a normal delivery, the chorioallantois is thought to remain attached to the endometrium until after the foal is delivered.[411]

Failure of rupture of the chorioallantois is a common complication of induced parturition.[334] If this happens, then continued separation from the endometrium will compromise transplacental oxygen exchange, and the likelihood of fetal hypoxia is high. Thus premature separation of the placenta is an emergency situation, and one should instruct foaling attendants to break the membrane and to provide gentle traction in unison with the expulsive efforts of the mare. Although the foal should be delivered as quickly as possible, injudicious traction at this time may cause a laceration in an incompletely dilated cervix.[411] Applying traction only with the expulsive efforts of the mare reduces the likelihood of creating cervical trauma.

As parturition progresses, passage of the fetus into the pelvic inlet initiates a reflex release of oxytocin from the posterior pituitary (Ferguson's reflex), thereby enhancing uterine contractility.[159] Stage II is characterized by strong abdominal contractions that provide the expulsive force necessary to expel the fetus. Most mares assume lateral recumbency once active straining commences. Many mares get up once or twice during second-stage labor in what is believed to be a further attempt to position the fetus correctly.[411] One can expect the translucent fluid-filled amnion to appear at the vulvar lips within5 minutes of rupture of the chorioallantois.[414] Any delay in the stage II expulsion process increases the likelihood of fetal asphyxia or neonatal problems associated with hypoxia because of placental separation. At least one hoof should be visible within the amniotic sac, and the other should be located a couple of inches behind it. If everything is progressing normally, the soles of the hooves should face down toward the hocks of the mare, and the head of the foal should be resting between the carpi.[411]

By the time the nose has reached the vulva, the cranial half of the torso should have rotated from a dorsopubic to a dorsoilial position.[4] The mare probably assists the fetus to reposition itself by the characteristic side-to-side rolling each time she becomes recumbent.[411] Some dystocias involving fetal

malposition and/or malposture likely are caused by the failure of a compromised fetus to participate actively in the foaling process. Many authors have suggested less vigorous or absent fetal righting reflexes early in the parturient process as a cause of fetal maldisposition.[159,339,413] The observation that ventral deviation of the head and neck is more likely to be present if the fetus is in dorsoilial position than in dorsosacral position further substantiates the hypothesis that the fetal righting reflexes are compromised early in these cases.[339]

The second stage of labor in the mare is rapid, with the most forceful contractions occurring as the chest passes through the pelvic cavity. Most foals are delivered within 20 to 30 minutes after the chorioallantoic membrane ruptures. Primiparous dams generally require longer to expel the fetus than do multiparous dams.[159,414] The amniotic sac usually ruptures during these expulsive efforts.[411] However, the equine amniotic sac is not attached to the chorioallantois as is the case with a ruminant placenta, and the foal sometimes may be delivered with a portion of the sac wrapped around its head.[414] One should instruct foaling attendants to free the head of the foal promptly from the amniotic sac to prevent suffocation. Active straining ceases once the hips of the foal are delivered, and the mare will rest in lateral recumbency.[411] An active foal will extract the hindlimbs from the vagina as it struggles to stand. The third stage of parturition involves expulsion of the fetal membranes, and this typically takes between 30 minutes and 3 hours.[159] One should advise owners to seek veterinary assistance if passage of the membranes is delayed, because life-threatening toxic metritis and laminitis are common sequelae of membrane retention.[411] 1105

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16.8.10.3 CAUSES OF DYSTOCIA

The incidence of dystocia in the general horse population varies among breeds: Thoroughbreds (4%), Shetland Ponies (8%), and Draft breeds (10%).[353] Dystocia is one of the few true emergencies in equine practice, where literally a matter of minutes can determine a successful outcome—birth of a live foal.[411] Perinatal asphyxia associated with dystocia is a major cause of equine reproductive loss.[223] The long fetal extremities (limbs, neck) predispose the mare to foaling problems.[159,353,411] Alert, informed foaling attendants are essential to ensure early recognition of abnormalities. Attendants should suspect that the mare is experiencing obstetric problems if the first or second stage of parturition is prolonged or not progressive.[411] Signs that a mare may be in dystocia include failure of any fetal parts or of the amniotic membrane to appear at the vulvar lips for a prolonged period of time after rupture of the chorioallantois, appearance of only one hoof at the vulva, hooves upside down at vulva, hooves and nose in abnormal relationship, nose but not hooves at vulva.[414] The most common impediments to delivery are malpostures of the fetal extremities (head and neck; limbs).[339] An experienced foaling attendant may be able to correct minor problems and facilitate a successful delivery. However, inappropriate intervention can have potentially fatal consequences for the mare. Furthermore, obstetric manipulations easily can damage the uterus and cervix to the extent that the reproductive future of the mare is jeopardized.[411]

16.8.10.4 MANAGEMENT OF DYSTOCIA

When attending to a mare in dystocia, the veterinarian should make a rapid assessment of the general physical condition of the mare, noting in particular mucous membrane color and refill time (hemorrhage, dehydration, shock).[411] A mare that is aborting in late gestationmay experience a dystocia because the dead fetus cannot participate in the delivery process. A malodorous discharge strongly suggests the presence of an

emphysematous fetus. One should inspect the perineal area for the presence and nature of any vulvar discharge and the presence of fetal membranes and to identify any fetal extremities. Excessive hemorrhage and vulvar swelling may indicate that nonprofessional intervention has caused trauma to reproductive tract. Occasionally, a mare has a rectal prolapse, an everted bladder, or intestinal loops protruding from the vulvar lips. The intestines may be of fetal origin if incomplete closure of the ventral abdomen has occurred, but a ruptured vagina is more likely. In the later scenario, the foot of the foal may have ruptured the floor of the cranial vagina, but one should not discount injudicious manipulations by an inexperienced attendant. If a rectal prolapse or the urinary bladder is present, then one should administer an epidural anesthetic to prevent further straining. Alternately, one may anesthetize the mare to facilitate hoisting the hindquarters.[411] The advantage of this approach is stopping the straining immediately, which is especially important if the prolapse involves an intussuscepted colon (type IV).[415,416] In these cases a palpable trench may extend several feet into the rectum, and avulsion of the mesentery can be a fatal complication that is not readily amenable to surgical correction.

This author considers stocks to be contraindicated when examining a foaling mare and prefers to perform the initial examination on a standing mare with no more restraint than a twitch or lip chain if her demeanor permits this to be performed safely. The behavior of a mare in stage II labor is unpredictable and may be violent. One must ensure that the examination area is clean and has good footing. Space should be ample for the mare handler, obstetrician, and assistants to move to safety if necessary. Although most veterinary tranquilizers readily cross the placenta and can compromise the fetus, adequate restraint is essential for the safety of all concerned.[417] Sedation with tranquilizers may be necessary for some uncooperative mares, and in extremely intractable cases, anesthetization of the mare with a short-acting combination may be preferable. In these cases, a hoist should be available because manipulating the foal when the mare is in lateral recumbency can be difficult. Although not essential, an initial rectal examination can rule out the presence of a term uterine torsion, determine the condition of the uterine wall (tears, spasm), and may provide useful information regarding the disposition of the fetus. Before any vaginal examination, one should wrap the tail of the mare and thoroughly clean the perineal area. The clinician should scrub the arms and hands with disinfectant soap.[411] In a hospital environment, the author also wears sterile rubber obstetric sleeves. Cleanliness and lubrication are the cornerstones of obstetrics.

Friction associated with vaginal manipulations easily traumatizes the vagina and cervix of the mare. Once the mucous membrane has been abraded, adhesions and fibrosis are likely to follow. Thus copious amounts of lubricant are vital to ensure that the soft tissues of the genital tract are not traumatized so as to preserve the future fertility of the mare.[418] Lubricants include methylcellulose, polyethylene polymer, white petrolatum combined with 10% boric acid, and mineral oil. Water-soluble lubricants are generally not as desirable because they rapidly lose their lubricating abilities in the presence of fluids.[159] If a large volume of lubricant is to be pumped around the fetus, then a preceding investigation of the possibility of a uterine laceration is essential. In a referral situation, the author routinely performs an abdominocentesis. Serosanguinous to 1106
sanguinous fluid that contains elevated total protein levels and an increased white blood cell count is highly 1107
suggestive of a uterine rupture.[350] If no likelihood of a uterine tear is apparent, the author prefers to mix a polyethylene polymer powder with warm water and then to use a clean stomach tube and pumpto instill large volumes of lubricant gently into the uterine lumen.[418] The author repeats this procedure as oftenas necessary to keep the fetus and reproductive tract coated with lubricant.

One should explore the vagina, cervix, and accessible parts of the uterus carefully to ascertain the source of any hemorrhage. One should note any lacerations and discuss their presence with owners or the attending personnel before attempting any veterinary manipulations. Occasionally, the cause of the dystocia is a pelvic

deformity (e.g., callus). One must determine the degree of cervical dilation.[411] If the mare has been in labor for some time, the uterus possibly is relatively dry and tightly contracted around the fetus, which makes intrauterine manipulations much more difficult, especially because safely repelling the fetus back into the uterus becomes difficult. If the uterus is contracted, warm lubricant tends to induce some uterine relaxation, and the volume expansion creates some additional space in which to perform manipulations.[337,418] One can control myometrial contractions (uterine spasm) by tocolytic drugs (isoxsuprine, clenbuterol) if they are available for veterinary use.[353] Although fetopelvic disproportion is uncommon in the mare, it can be a factor in some equine dystocias.[38,39,41,411] One should note the disposition of the fetus and determine fetal viability. One should exercise care because active fetal response to manipulations easily can complicate an initially simple dystocia. Placement of a rope snare behind the ears and into the mouth of the foal ensures that the clinician always has control of the head and facilitates easy correction of a potentially life-threatening development such as lateral deviation of the head and neck if the fetus pulls away from the clinician's manipulations.[411] If one places the snare around the mandible, application of only gentle traction to guide the fetal head through the vaginal canal is essential. Excessive force may cause a fracture of the mandible. When obvious fetal movement is absent, one may initiate digital withdrawal in response to pinching of the coronary band. Slight digital pressure over the eyelid onto the eyeball may arouse a response, as may stimulation of the tongue (swallowing). If one can reach the thorax, a fetal heart beat is a definitive sign of life. In posteriorly presented cases the digital and anal reflexes are useful as indicators of fetal viability. Occasionally, reaching the umbilical cord may be possible.[159]

The clinician should inform the owner of the various options, costs, and prognosis once the current status of the foal is known, and the cause of the dystocia has been determined. Ensuring that the owner is aware of the potential complications that may arise is especially important, because postpartum medical care can become expensive. If delivery of a live foal is anticipated, the clinician should consider the potential for fetal cardiovascular compromise before administering any tranquilizers to the mare.[411] Light sedation of the mare with acetylpromazine (2 to 3 mg/100 kg intravenously) has minimal effect on the foal and may be useful in some cases. Xylazine is preferable to detomidine if the fetus is viable because its depressant effects are of much shorter duration.[419] However, one should use neither xylazine nor detomidine on its own to sedate a mare with dystocia because some apparently sedated mares can become hypersensitive over the hindquarters.[419,420] The combination of xylazine and acepromazine provides good sedation in a quiet mare.[417] The author routinely uses a combination of xylazine(0.3 to 0.5 mg/kg intravenously) and butorphanol (0.01 to 0.02 mg/kg intravenously) for standing obstetric procedures if more sedation is required (e.g., fetotomy procedure). This provides good sedation and analgesia, and one may administer additional doses as necessary. The clinician instructs the attendants to keep the lip chain loose and to tighten it only when instructed to doso. This ensures that the lip chain retains its effectiveness when required to divert the attention of the mare.[411] LeBlanc[417] suggests a combination of xylazine (1.1 mg/kg) and morphine (0.1 to 0.2 mg/kg) to sedate mares for fetotomy but cautions that gastrointestinal stasis is a frequent complication. Although one can resolve most dystocias at the farm quickly by brief manipulation and assisted vaginal delivery, the practitioner should consider the alternatives if resolution is likely to take more than 10 to 15 minutes. Prolonged, unproductive vaginal manipulations are contraindicated in equine obstetrics. One should base decisions for the next recourse on the viability of the foal, the clinician's obstetric skills, the availability of equipment and facilities, and certainly the financial constraints imposed by the owner.

An epidural anesthetic does not prevent the myometrial contractions or the abdominal press of the mare, and the time involved in administering an effective epidural anesthetic may make this form of restraint impractical when a live foal is present.[411,419] However, if the foal is dead, epidural anesthesia does reduce

vaginal sensitivity and thus the perception of the mare of vaginal manipulations (Ferguson's reflex). Caudal epidural anesthesia should be used at the clinician's discretion, especially if general anesthesia or referral may become necessary. When an epidural is indicated, the author uses a combination of xylazine (0.17 mg/kg) and lidocaine (2 to 3 ml) diluted in saline, such that the final volume does not exceed 8 to 10 ml in order to reduce the likelihood of hindlimb weakness.[419,421] Excess volume can cause the mare to become ataxic. 1107
Short-term general anesthesia may be indicated when minor postural abnormalities are present and maternal 1108
expulsive efforts make correction difficult.[355] A combination of xylazine (1.1 mg/kg intravenously) followed by ketamine (2.2 mg/kg intravenously) provides a smooth, short-duration (10- to 15-minute) general anesthetic. Addition of the central-acting muscle relaxant guaifenesin (1 L of a solution of 5% guaifenesin in 5% dextrose) can provide an additional 10 to 20 minutes for fetal manipulation.[419] In specialist equine hospitals located close to well-managed broodmare farms, the fetus is often still alive when the mare arrives.[410] Common practice is immediately to anesthetize these mares and maintain them on halothane and oxygen with controlled ventilation. Halothane anesthesia has been shown to compromise umbilical circulation, and thus one should keep the concentration to a minimumif the foal is still alive.[355] Total intravenous anesthesia (the so-called triple drip of ketamine, xylazine, and guaifenesin) may be preferable until the foal has been delivered.[417] One should ventilate the mare with oxygen and administer fluids intravenously as required. Using the hindend elevation technique, one can resolve almost three fourths of such cases by controlled vaginal delivery. However, if the fetus is still alive and has not been delivered within 15 minutes, one must perform an immediate cesarean section, with a 30% foal survival rate possible, provided that the time from rupture of the chorioallantois to presentation at the veterinary hospital has been kept to a minimum.[410,422] In the field, if a hoist is available, one can place hobbles on the hind pasterns and elevate the hindquarters briefly 1 to 2 feet. The combination of a relaxed uterus and the effects of gravity can facilitate fetal repulsion and manipulation. If attempts at mutation are successful, one should lower the mare into lateral recumbency to permit extraction of the foal.[411] Prolonged dorsal recumbency results in compression of the aorta and vena cava and reduction in venous return, cardiac output, and blood pressure.[417] Hindlimb paresis may develop after prolonged hindquarter suspension and can complicate the recovery process. The use of pads to support the hindquarters helps to take some weight off the limbs while the mare is suspended.

Mutation is an obstetric term that describes manipulation of the fetal extremities, together with correction of any positional abnormalities so that assisted vaginal delivery can proceed.[159] Although extra space is available for manipulations when the fetus has been repelled back into the uterus, the clinician should remain cognizant at all times that overzealous obstetric manipulations are a major cause of uterine rupture.[159,337,349,350,423–427] Repulsion of the fetus from the maternal pelvis is contraindicated if the uterus is contracted down around the fetus. In some cases, pumping warm obstetric lubricant around the fetus induces some uterine relaxation. In those countries where they are legal, tocolytic agents are effective in relaxing a contracted uterus. If the fetus is dead, many cases may be amenable to correction by fetotomy provided that the clinician has the appropriate skills and equipment.[428–430] Poor technique and inappropriate cuts often lead to infertility. The alternative is cesarean section.[422]

One must apply traction with careful regard for maternal and fetal well-being. Often traction applied entirely by hand is all that is necessary. Obstetric straps or chains may provide a better grip. This author prefers to apply one loop above the fetlock, with a second loop encircling the pastern. In assisted vaginal deliveries, one should apply traction as an adjunct when the mare is exerting expulsive force and should release the traction when the dam stops straining, thereby permitting rest and recovery. This approach is critical to permit adequate dilation of the caudal reproductive tract.[418] Copious lubrication and slow traction with

continuous monitoring of cervical dilation are especially important to perform a controlled vaginal delivery on an anesthetized mare.[422] Excessive use of force may be associated with fetal fractures (ribs, vertebrae, and limbs) and maternal soft tissue trauma. A maximum of two to three persons (depending on size and strength) should apply traction to the fetus.[418]

16.8.10.4.1 Cranial (Anterior) Presentation

An anteriorly presented fetus in dorsosacral position with head and forelimbs extended should require minimal traction to complete the delivery, assuming that the vaginal canal is well lubricated. By ensuring that slightly more traction is applied to one limb than the other, one can reduce the width of the fetus across the shoulders and deliver most foals successfully. If one is not making progress, then all traction should stop and one must explore the vaginal canal fully.[418] The three likely possibilities for difficulty are elbow lock (incomplete extension of the forelimb), dog-sitting/hurdling posture, and occasionally fetal oversize. Absolute or relative fetopelvic disproportion is uncommon in the mare, even in those cases in which the foal has been carried several weeks past the expected due date. In fact, some cases of prolonged gestation may involve a smaller than normal, dysmature fetus.[159,431] In a referral hospital study, less than 2% of dystocias were attributed to this condition.[339] Of significance, however, is that approximately 30% of referral hospital dystocia cases are in primiparous mares.[339,412,414] Primiparous mares were represented disproportionately in a report on dystocia and neonatal asphyxia from the central Kentucky area.[181,432] Thus although fetopelvic disproportion is not common in the mare, obstetric assistance (traction) is required much more often in primiparous mares.[353,432,433] Dystocia in these mares is complicated further 1108
by a tight vaginovestibular sphincter, which may predispose primiparous mares to lacerations and 1109
rectovaginal tears.[432] If copious lubrication and gentle traction do not help, cesarean section or partial fetotomy are the only alternatives.

Incomplete elbow extension may be uni- or bilateral and should be suspected if the fetal muzzle lies at the same level as the hooves.[418] In this posture the fetal elbows are tucked back under the shoulder joint, causing increased depth and width of the fetus within the maternal pelvic inlet. Correction involves repelling the fetal trunk so that the forelimbs can be extended and thus raise the elbows up over the floor of the pelvic inlet.[337] In a dog-sitting/hurdling posture the fetus has bilateral (dog-sitting posture) or unilateral (hurdling posture) hip flexion. This causes the fetal hooves (hoof) to push against the pelvic brim during attempts at fetal extraction.[418] The unilateral posture is more common. One can inflict severe trauma on the mare if one does not recognize this malposture and applies inappropriate amounts of traction. Thus one must stop all traction and repel the fetus enough to sweep the floor of the pelvic inlet. In extreme cases, the hindlimb actually may extend under the fetus and up into the vagina.[339] Although one may repel the hindlimb successfully if the fetus is alive, the procedure is difficult and is associated with some risk of uterine laceration. Judicious use of a snare or fetatome may facilitate safe repulsion of the hindlimb by looping the pastern and using the instrument to repel the hoof away from the pelvic brim. One should not attempt repulsion on a standing mare if the fetus is dead, because the hindlimb may not return to its normal position.[428,430] In such cases the hoof of the flexed hindlimb can puncture the ventral uterine wall on extraction of the foal. If a dog-sitting/hurdling foal is dead, use of general anesthesia and hoisting of the hindquartersis recommended to reduce the risk of ventral uterine rupture.[434] In experienced hands, partial fetotomy is a viable alternative to cesarean section.[428–430] A surgical alternative to a cesarean section involves manipulation of the hindlimb through a ventral midline celiotomy incision. An assistant

may be able to extract the fetus through the vagina once the hindlimb has been grasped throughthe ventral incision. If successful, this technique reduces the potential for contamination that may be associated with cesarean section. If a cesarean section is necessary, some surgeons prefer to remove that portion of the foal that is protruding through the vulvar lips before withdrawing the hindend out through the surgical site.[435]

In a foot-nape posture, one or both of the forelimbs will be displaced over the head of the foal and pushed against the roof of the vagina. To correct this malposture, one must repel the fetus into the uterus by applying pressure to the head. Once the forelimbs have been replaced under the head, fetal extraction can proceed uneventfully.[418] If the malposition is not corrected immediately, the straining of the mare can cause the fetal hoof to lacerate the vaginal roof and in extreme cases can result in a rectovaginal fistula. A fistula is all that occurs if the foal withdraws its hoof from the rectum before delivery. A third-degree perineal laceration occurs if the strong expulsive efforts of the mare cause the limb that has penetrated the rectum to dissect through the caudal rectovaginal shelf and rupture through the anal sphincter, thereby creating a cloaca.[436–438]

Carpal flexion may be uni- or bilateral, and typically the affected carpus is located at the pelvic inlet.[418] To correct this malposture safely, one first must repel the fetal body into the uterus. If the dystocia is prolonged, the amount of uterine contraction may preclude meaningful repulsion of the fetus back into the uterus. Because the fetus is likely to be dead in these cases, one can make a simple fetotomy cut at the level of the distal row of carpal bones. This permits safe delivery of the foal without the need to traumatize the reproductive tract and generally facilitates extraction within minutes.[428,430] If the foal is alive, then repulsion back into the uterus permits one to grasp the flexed limb at the level of the fetlock and pastern. By rotating the wrist, one can rotate the carpus laterally while bringing the flexed fetlock medially and caudally into the birth canal. This maneuver allows maximal use of available space by obliquing the extremity through the pelvic inlet. The obstetrician should be aware that flexural deformities are considered to be the most common congenital anomaly of foals and that the rigid deformity often means that one must perform a cesarean section or fetotomy.[181,223,412,428–430] Limb contractures are generally bilateral. Contracture is more common in the forelimbs than in the hindlimbs but can involve all four limbs.[412,418] One cannot straighten severely affected limbs, and one can inflict needless trauma on the genital tract by unrewarding attempts to correct the malposture manually. The clinician must cup a hand over the bottom of the fetal hoof at all times while attempting to straighten the limb. Failure to do so may result in injury to the reproductive tract. Application of an obstetric chain or rope to the distal limb can be a useful aid and permits application of traction to the distal limb while the hand covers and guides the hoof.[418]

The single most common abnormality in referral hospital dystocia populations is a reflected head and neck.[339,353,412] Unfortunately, these malpostures are often iatrogenic in that a viable fetus has pulled back from the initial vaginal intervention that aimed to correct a minor postural problem. If the mare strains while the head of the foal is pulled back, the muzzle possibly may engage the wall or floor of the pelvic inlet. The forceful expulsive efforts of the mare then may drive the head and neck ventrally, or laterally
along the thorax, while pushing the forelimbs further into the vaginal canal. Ventral or lateral 1109
displacements of the head and neck can be difficult to correct. The length of the neck of the foal often 1110
makes reaching the head impossible. Inexperienced clinicians should consider referral on diagnosing the condition, because prolonged unrewarding manipulations easily can jeopardize the future fertility of the mare.[418] A simple fetotomy cut can resolve these cases atraumatically if the fetus is dead when the veterinarian arrives. The author believes that this approach is preferable to prolonged, and often

unsuccessful, attempts at manual correction of this difficult malposture.[428] If the fetus is alive, one can attempt to place eye hooks or to loop a snare around the mandible. Some obstetricians even suggest applying a clamp on an ear to pull the head back enough to place a snare. Whenever possible, the author prefers to use a snare. If one can apply traction to the head, one can repel the body of the foal carefully while attempting to bring the head and neck around into a normal posture for delivery. Factors influencing the successful outcome include uterine tonicity, clinician arm length and skill, and the presence or absence of torticollis and facial scoliosis. As with contracted tendons, the practitioner must consider the possibility of a wry neck. This condition is not amenable to correction by mutation, and one can inflict needless trauma on the genital tract by unrewarding attempts at correction. Ventral deviation of the head is easy to correct if the fetal nose is just below the brim of the pelvis (poll posture). Generally, rotating the head laterally before attempting to bring the muzzle up over the pelvic brim is easier. In more severe cases the neck is tucked down between the forelimbs and the head often cannot be reached (nape posture).[412,418] If attempts to reposition the head and neck are unsuccessful, then cesarean section or fetotomy is indicated.[422,428–430]

Shoulder flexion posture may be unilateral (swimming posture) or bilateral (diving posture).[412,418] To gain access to the retained limb, one usually must repel the head and neck back into the uterus. An immediate cesarean section may be preferable if the foal is alive because correction of this malposture can be difficult and time-consuming. If cesarean section is not an option, then placing a soft rope snare on the fetal head (behind ears and into mouth) is recommended so that one can retrieve the head readily after correction of the shoulder flexion. If one can reach the limb, one corrects this malposture in two stages. Initially, one converts the shoulder flexion to a carpal flexion by grasping the limb in the area of the humerus and working down to the distal radius. One then pulls the limb caudally and medially while repelling the fetal body. One then hooks the carpus over the brim of the pelvis to create a carpal flexion, which one then corrects as directed previously. One must remember that repulsion of the head sufficient to gain access to the retained forelimb is not always possible. In these cases cesarean section is the only option for delivery of a live foal. If the fetus is dead, a fetotomy cut to remove the head and neck may provide sufficient room to correct the malposture.[428–430]

16.8.10.4.2

Caudal (Posterior) Presentation

A foal in posterior presentation has the soles of the hooves facing upward. Although the author has seen dystocias in which an anteriorly presented foal was in dorsopubic position with both forelimbs extended, this is an unusual complication. One should instruct foaling attendants to wash the perineum of the mare and to use a clean arm to check for the hocks somewhere in the vaginal canal. Gentle traction on the hindlimbs along with the expulsive efforts of the mare may facilitate delivery of a live foal. However, approximately half of the fetuses may be malpositioned as well and often require veterinary assistance to permit an atraumatic delivery. Foals in caudal (posterior) presentation are more likely to be in dorsoilial position than are foals in a cranial (anterior) presentation.[339] Although a normally positioned fetus in caudal presentation may not be that difficult to deliver, the foal is more likely to suffer hypoxia because of compression of the umbilical cord under the fetal thorax or because of premature rupture of the umbilical cord.[418] Although only about 1% of foals are presented posteriorly, this malpresentation accounts for 14% to 16% of referral hospital dystocia cases because any postural abnormalities create a major complication. Typically both hindlimbs are involved, and these types of dystocia cases (hock flexion, hip flexion) are difficult to correct under field conditions. Hock flexion malposture accounts for about one fourth of referred posterior cases.[339,353,412] Correction of a hock flexion is dangerous because of the risk of

perforation of the dorsal aspect of the uterus. One must repel the fetus into the uterus while pushing one hock dorsolaterally and directing the distal limb medially. The procedure for obliquing the extremity into the birth canal is similar to that previously described for correction of a carpal flexion. One can use an obstetric chain or strap to apply traction to the limb while cupping the hoof in the hand. Straightening a flexed hock entails considerable risk because the hock invariably is forced against the dorsal uterine wall. In cases in which the uterus is contracted, a real possibility exists of causing a laceration or perforation. Cesarean section may be preferable for delivery of a live fetus. This author strongly believes that fetotomy is a safer procedure than attempts at mutation if the fetus is dead.[422,428–430] Approximately half of referred posterior presentation cases are breech (bilateral hip flexion posture).[339,353,412] A cesarean section is indicated if the fetus is alive because the manipulations involved in correcting this malposture are time consuming and difficult. The comments for managing a hock flexion apply because if one attempts 1110
mutation, one must first convert the hip flexion into a flexed hock posture.[418] A key point is to remember 1111
to flex both hocks before attempting to straighten a limb. If one limb is extended into the vaginal canal while the other hip remains flexed, the fetal body will move back into the pelvic canal, and this will make accessing the retained limb difficult.[431] If the fetus is dead, the author recommends attempting to convert the bilateral hip flexion into a hock flexion posture, followed by correction with two fetotomy cuts through the distal row of tarsal bones. This procedure may be safer and cause less trauma than attempting to straighten the limbs, provided that the clinician is experienced in using a fetatome.[429] Attempts to correct a bilateralhip flexion by fetotomy, without first creating a bilateral hock flexion, are often unrewarding because of difficulty in correctly placing the fetotomy wire. Referral for cesarean section often provides the best prognosis for future fertility in these cases.

16.8.10.4.3 Transverse Presentation

Only about 1 in 1000 foals present transversely. Successful resolution of these dystocia cases requires a significant amount of obstetric experience, which explains why these rare presentations account for 10% to 16% of referral hospital dystocia cases.[339,412] Most transverse presentations are ventral transverse with the abdomen and limbs of the fetus presented toward the birth canal.[339,353,412] Although the widespread adoption of ultrasonography has reduced the likelihood of a twin birth greatly, one always must explore this possibility when more than two limbs are present in the birth canal.[181,223] In some instances, one possibly may repel the head and forequarters of the fetus while extending the two hindlimbs into the pelvic canal. If the manipulations are successful, the transverse presentation thus is converted into a posterior presentationfor vaginal delivery. The likelihood of successfully resolving one of these cases improves if one has anesthetized the mare and elevated the hindquarters.[418] Transverse presentations may be associated with flexural limb deformities, angular limb deformity, and spinal deformity. If thefetus is alive, the delivery method of choice is cesarean section.[422] Dorsal transverse presentations, with the spinal column of the fetus presented toward the birth canal, are rare. These cases warrant an immediate referral for cesarean section, even if the foal is dead.[339,353,412] Although an experienced obstetrician may be able to deliver a transversely presented fetus by fetotomy, the owner should be advised that this is a difficult and time-consuming procedure, with a high risk of trauma that likely will impede the future fertility of the mare.[418,428–430]

16.8.10.4.4 Fetal Anomalies

Hydrocephalus is not uncommon in equine fetuses, especially in pony breeds.[353,412] The condition occurs when increased intracranial pressure causes the bones of the skull to enlarge, sometimes almost doubling the size of the head.[412] The skull is often thin, and many affected foals can be delivered after incising the soft portion of the skull with a finger knife, allowing the skull to collapse. The trunk of the hydrocephalic fetus is generally smaller than normal and seldom interferes with delivery.[418] If the enlarged cranium is bony, then a fetotomy cut may be necessary to reduce the size of the head.[428–430]

16.8.11 Care of the Postpartum Mare

One should examine the fetal membranes as a matter of routine to ensure that they have been passed intact and to check for any placental anomalies that may forewarn of impending problems in the neonate. Often the chorioallantois has tears that can be misleading, especially ifthe mare has trodden on the membranes repeatedly. Examining the allantoic side of the membrane may be helpful in that one can piece together the blood vessels, which will give some idea as to whether a portion actually is missing.[292] Ideally, all foaling mares should receive a brief physical examination within 24 hours post partum. If the attitude of the mare is normal and she displays typical maternal behavior toward the foal, then one should check the udder and inspect the perineal area for evidence of trauma.[439] A detailed reproductive examination is usually unwarranted because it unnecessarily may disrupt the normal mare-foal bond that is developing at this time. All mares should receive a thorough reproductive examination at the foal heat.[337] Occasionally, one may detect an enlarged ovary, which may be a granulosa-theca cell tumor that has enlarged during the course of the previous pregnancy. Prompt diagnosis and surgical intervention may permit the mare to resume normal cyclicity and to conceive during the current breeding season.

Abdominal discomfort in the peripartum mare may be due to uterine contractions, especially if the mare has been treated with oxytocin to promote passage of the fetal membranes. However, one should not discount other causes of abdominal pain.[350,440–442] When a postpartum mare displays abdominal discomfort, this author believes that abdominocentesis is indicated. The normal foaling process does not alter the composition of the peritoneal fluid from within the normal range. Even a dystocia does not necessarily cause significant changes in the peritoneal fluid. If an experienced obstetrician performs the vaginal manipulations and/or fetotomy, the fluid should remain normal. If the peritoneal fluid is normal, then one should monitor the mare closely for signs of clinical deterioration. Repeated abdominocentesis may be indicated in cases in which clinical signs suggest that a parturient-related abdominal lesion may be present, because the peritoneal fluid constituents can change 1111
within hours. A single, elevated peritoneal fluid value (total protein, white cell count, or percent neutrophils) 1112
may be an incidental finding. Elevation of two or more values often signals the onset of clinical abnormalities. The author's experience has been that if a postpartum peritoneal fluid sample has total protein greater than3.0 g/dl along with a white blood cell count greater than 15,000 cells/μl and white blood cell differential count of greater than 80% neutrophils (especially if degenerative changes are present), then the presence of a potentially life-threatening lesion is likely.[350] One should not view the peritoneal fluid analysis in isolation and must consider it along with the history and clinical signs exhibited by the mare. In this author's experience, detection of changes in the peritoneal fluid almost invariably indicates the presence of foaling-related trauma in the reproductive or gastrointestinal tract. An early diagnosis followed by appropriate medical or surgical intervention often results in a favorable outcome. If treatment is not implemented until the affected mare has

become depressed and febrile, with accompanying signs of shock and toxemia, the prognosis may be more guarded.

16.8.11.1 PERIPARTURIENT HEMORRHAGE

The arterial supply to the uterus is supported by the mesometrium (broad ligament). The major blood supply to the uterus is from the uterine artery, a branch of the external iliac artery. The uterine artery forms a cranial branch that supplies the proximal uterine horn and a caudal branch that supplies the distal uterine horn and uterine body. The smaller ovarian artery gives off a uterine branch that anastomoses in the proximal horn with the cranial vessels from the uterine artery. The urogenital artery is a branch off the internal pudendal artery and gives rise to the caudal uterine artery along with vessels to the rectum, ureter, bladder, urethra, and vagina. The caudal uterine artery supplies the lateral side of the cranial vagina and continues past the cervix to ramify on the uterine body where it anastomoses with the caudal branch of the uterine artery.[3,443,444] Hemorrhage from these vessels, especially the large-diameter uterine artery, is a significant cause of periparturient colic signs and death in older, multiparous mares.[*] The condition occasionally may occur before foaling. In a study of 98 postpartum deaths, almost 40% were caused by uterine artery rupture. The rupture may be anywhere along the vessel and is typically 2 to 3 cm long and oriented parallel to the long axis of the vessel. Generally, no evidence of a predisposing aneurysm is apparent.[442] An association with low serum copper levels has been proposed as a reason for vessel fragility in aged mares.[448]

A predilection appears to exist for right-side uterine vessel rupture. The extent of cecal displacement of the gravid uterus to the left has been suggested to be sufficient to place increased tension on the vessels in the right broad ligament.[442,447] Although the added stress of dystocia may increase the chances of arterial rupture, many cases occur in mares that appeared to have an uneventful delivery.[423,442] Hemorrhage from the hypertrophied vessels that supply the gravid uterus may be rapidly fatal, especially if the artery ruptures directly into the peritoneal cavity. The mare may be found dead or moribund with pale mucus membranes, tachycardia (up to 140 beats/min), and tachypnea. Heroic attempts to administer blood transfusions, plasma expanders, and associated fluid therapy may save the life of some valuable mares, but costs are often prohibitive.[242,337,423] If the bleeding is contained within the broad ligament, the mare may be trembling and exhibiting signs of extreme pain (anxiety, sweating, colic), presumably because of the stretching of the broad ligament as the hematoma develops.[337,425,442] The color of the mucous membranes may not change initially because of vascular compensation, and often these initial colic signs are mistaken for the typical discomfort experienced by postpartum mares as the uterus contracts. However, if significant hemorrhage is present, the color of the mucous membranes eventually becomes pale, and capillary refill is delayed.[425] One must monitor these mares closely because the hematoma subsequently can rupture out of the mesometrium and lead to rapid exsanguination.[447]

If one suspects a ruptured artery, then one should not disturb the mare any more than necessary to perform an examination.[449] In many cases, postponing or even forgoing palpation per rectum may be prudent. Although an internal examination reveals valuable diagnostic information, transabdominal ultrasound, abdominocentesis, and a hemogram may be all that is necessary to confirm that an acute hemorrhagic episode has occurred.[337,439] Transabdominal ultrasonographic evaluation reveals free blood in the abdominal cavity if the hematoma has torn the broad ligament.[242] If broad ligament tears following a uterine artery rupture, then invariably a bloody tap results, with an elevated red blood cell count in the peritoneal fluid.[350] The centrifuged sample has a pink or hemolyzed appearance if hemoperitoneum is present. A smear that

reveals phagocytosed erythrocytes indicates hemorrhage rather than contamination during sampling. Even if a clot has contained most of the hemorrhage within the broad ligament, often considerable amountof blood is lost into the peritoneal cavity. The initial hemogram during an acute hemorrhagic episode can be confusing because the loss of erythrocytes and plasma may not alter the hematocrit immediately. Splenic contraction also temporarily raises the hematocrit.[242] In the author's opinion, one should not transport these mares 1112
because movement could destabilize the clot and prove to be fatal. One should administer any supportive 1113
therapy at the stall until the mare has stabilized. One should keep the foal safely nearby so that the mare does not become unduly distressed.

Most recommendations for managing postpartum hemorrhage in mares are based on the collective wisdom of experienced clinicians and from methodologies that have been extrapolated from the human trauma literature. The approach one takes is governed by the facilities and expertise available and especially the economics of the case at hand. In some instances an extreme hypotensive state may actually offer the best chance for survival (conservative approach), whereas in other cases an attemptto restore intravascular pressures and circulatory volume could be indicated. The conservative approach is to confine the hypotensive mare to a dark, quiet stall with minimal disturbances. In some cases a platelet-fibrin plug seals the rent in the vessel once the arterial pressure falls. One should use tranquilizers (especially acetylpromazine) with caution because any induced drop in blood pressure may exacerbate the hypovolemic shock. Some clinicians use “hypotensive resuscitation” by administering a vasodilating agent along with intravenous fluid therapy. The idea in this instance is to provide life-saving volume replacement while maintaining a low mean arterial pressure. In life-threatening situations, anything that may stabilize the mare is worthwhile, but the clinician should consider the possibility of impeding resolution of the hematoma by rapid expansion of blood volume and elevation of blood pressure. The need to support cardiac output and ensure oxygen delivery must be balanced against the prospect of the increased arterial pressure promoting further hemorrhage.[242]

Although the costs can soar rapidly, an aggressive therapeutic approach occasionally can save the life of a valuable mare.[242,442,450] If a valuable mare presents in shock and appears to be deteriorating rapidly, then one should insert a large intravenosus catheter and begin substantial fluid therapy. One must give whole blood transfusions slowly and thus they are of little benefit for resuscitative purposes when rapid volume expansion is required. One option is rapid administration of 2 to 3 L of hypertonic saline, followed with 10 to 20 L of lactated Ringer's solution over a period of 2 to 4 hours. An alternative to the hypertonic saline is the high oncotic pressure exerted by colloids (e.g., 3 L hetastarch). Synthetic oxygen-carrying fluids are commercially available but extremely expensive. One can provide supplemental oxygen via nasal insufflation at a flow rate of5 to 10 L/min. If the hematocrit continues to drop to under 15%, then whole blood transfusions (6 to 8 L over several hours) may be warranted.[242] Benefits include provision of oxygen-carrying cells, clotting factors, and oncotic pressure (albumin).

A shock dose of corticosteroid is indicated. Because hemorrhagic shock can cause ischemic-reperfusion damage to the gut and lungs (multiple organ failure), broad-spectrum antibiotics, antioxidant drugs, and antiinflammatory medication may be warranted if the mare survives the initial hemorrhagic crisis. One administers flunixin meglumine (1.1 mg/kg) to reduce the inflammatory cascades activated by ischemia, and this may help to alleviate the discomfort of the mare. Low-dose (10 to 20 IU) oxytocin therapy may be useful to promote uterine involution and thereby reduce the weight supported by the ligaments. One should avoid higher doses becausean induced colic episode may precipitate a fatal hemorrhage. Antifibrinolytic drugs (aminocaproic acid; tranexamic acid) may assist with clot stabilization. Pentoxifylline increases erythrocyte flexibility and may increase oxygen delivery to ischemic tissues.[242] One should remember that little in the refereed veterinary literature validates the use of some of these medications in the horse. For instance, a

conjugated estrogen product has been proposed, based on its ability to shorten prolonged bleeding times in human beings. However the benefit, if any, would not be realized until several days after the crisis has passed. Likewise, anecdotal reports suggest that naloxone (8 mg) may be efficacious, but the concept has been extrapolated from small animals, and controlled equine studies are lacking.[242,337,423] A controversial historical therapy to promote hemostasis in the horse is the use of intravenous buffered 10% formalin. Advocates suggest that dilute formalin solutions could enhance the activation of the clotting cascade. However, recent controlled studies were not able to demonstrate an effect on coagulation parameters or template bleeding times in normal horses.[451] One should administer broad-spectrum antibiotics to prevent infection of the hematoma.[445]

If hemorrhage is contained within the wall of the uterus, then the intramural hematoma may be an incidental finding at the foal heat examination. However, some mares may exhibit variable signs of abdominal discomfort, even to the extent of warranting an exploratory celiotomy.[423,436,452–454] If an endometrial laceration severs an artery in the uterine wall, then substantial hemorrhage may ensue, often with blood escaping from the vagina. One should confine the mare to a stall and institute low-dose oxytocin therapy. Uterine irrigation is contraindicated because it disrupts clot formation and prolongs the hemorrhagic episode. The internal pudendal artery, one of the terminal branches of the internal iliac artery, gives rise to the umbilical artery and the urogenital artery before terminating in branches to the perineal area and the vestibular bulb. The small cranial vesicular artery supplies the apex of the bladder before the remainder of 1113
the umbilical artery terminates into the cordlike round ligament of the bladder. The urogenital artery gives 1114
rise to a caudal uterine branch that runs cranially on the side of the vagina and ramifies with the caudal branch of the uterine artery on the body of the uterus. The urogenital artery also supplies branches to the rectum, ureter, caudal bladder, and urethra and continues as the vaginal artery to the caudal portion of the reproductive tract.[3,443,444] A hematoma arising from these vessels may dissect along the fascial plane within the pelvic cavity and present as a large unilateral vulvar swelling.[425] Affected mares typically experience violent colic. Abscessation of a retroperitoneal hematoma can become a life-threatening complication following a dystocia, and thus prophylactic broad-spectrum antibiotic coverage is warranted.[442] Mares with an infected retroperitoneal hematoma develop signs of toxemia. In these cases the peritoneal fluid has an increased total protein content (3.0 to 5.0 g/dl) with a massive increase in the white blood cell count (often exceeding 100,000 cells/μl).[350]

* References 159, 423, 425, 442, 443, 445–447.

16.8.11.2 UTERINE PROLAPSE (EVERSION)

Uterine prolapse is an uncommon complication of equine parturition that may occur up to several hours (and occasionally several days) after fetal delivery.[423] The condition may be complicated by bladder eversion or prolapse, uterine rupture, or intestinal herniation and may be rapidly fatal if the uterine artery ruptures.[*] If the mare is standing and personnel are available, one should give instructions to place the uterus in a large plastic bag and elevate it to the level of the vulva. This may prevent further damage to the endometrium and more importantly will relieve the tension on the uterine vessels. Fluid therapy may be indicated, and any calcium deficit must be corrected. Epidural anesthesia may reduce the amount of reflex straining provoked by vaginal manipulations but will not eliminate the strong abdominal press of the mare.[419] General anesthesia may be necessary if the mare exhibits violent discomfort or if straining is excessive. One should close any uterine lacerations with absorbable sutures. One then “kneads” the well-lubricated uterus back through the vagina. The finger tips easily can damage the edematous tissue, and manipulating the uterus

through a plastic bag reduces the likelihood of a finger rupturing the wall.[337,455] Ultrasonography is useful to evaluate any suspicious contents. One may aspirate a trapped bladder through a large-diameter needle, but a loop of bowel may require a ventral midline celiotomy.[445]

One should distend the replaced uterus with sterile saline to ensure that the tips of both horns are extended fully. One should administer repeated low doses (10 to 20 IU every 2 hours) of oxytocin to promote uterine involution. Failure to ensure complete extension of the uterine horns into a normal position within the abdomen may result in discomfort, straining, and recurrence ofthe prolapse.[337] Vulvar retention sutures should not be necessary, provided that the uterus has been returned completely to its normal position, that the calcium deficit has been corrected, and that low-dose oxytocin therapy has been administered. Broad-spectrum antibiotics, nonsteroidal antiinflammatory drugs, and tetanus prophylaxis are indicated. One should monitor the mare closely for evidence of internal hemorrhage. Affected mares may exsanguinate after the uterus has been replaced. Ischemic damage to trapped bowel is a potential complication. The clinician should be cognizant of the potential risks of endometritis/metritis, septicemia, endotoxemia, and laminitis. Two to 3 days of intrauterine therapy may be warranted, depending on the condition of the exposed endometrium.

* References 159, 349, 426, 439, 442, 445.

16.8.11.3 PARTIAL INVERSION (INTUSSUSCEPTION) OF THE UTERINE HORN

Injudicious traction on a retained fetal membrane remnant may invert the tip of the uterine horn, and this may progress to complete uterine prolapse.[337] If only the horn is affected, then compromised circulation and pressure on nerve endings may produce signs of abdominal discomfort. Thus one should palpate the tips of both uterine horns per rectum when evaluating a postpartum colic case. The affected horn is shorter than normal and extremely thickened.[439,445,456] Manual reduction by pressure from within the uterine lumen may be possible in some cases, and infusion of several liters of saline solution usually ensures extension of the affected horn.[439] One then should administer oxytocin (10 to 20 IU) and drain the fluid from the uterus as it contracts. One should confirm resolution of the problem by palpation per rectum.

16.8.11.4 UTERINE RUPTURE

In any dystocia case a risk for iatrogenic tears exists, and one always should check the uterus for any obvious lacerations immediately after extraction of the fetus. Early recognition is important because the prognosis is worse once peritonitis develops.[350,424,425,442] However, obstetric intervention is not always the cause of uterine tears. Occasionally, a hoof of the foal may be forced through the dorsal uterine wall during the expulsive efforts of the mare, and the mare may be found with a loop of bowel protruding through the vulvar lips.[349,423] One should rinse the exposed bowel with sterile saline and replace it, but a ventral midline celiotomy may be warranted to evaluate intestinal damage fully and to repair the uterine laceration. A more 1114
common lesion in unassisted deliveries is a tear toward the tip of the gravid uterine horn. Although the fetal 1115
hooves are covered with hard gel-like pads that presumably protect the placenta and uterine wall, the vigorous pistonlike thrusts of the hindlimbs occasionally may cause a rupture.[4,287,457] Affected mares generally experience bouts of colic and become depressed, febrile, and anorectic as peritonitis develops. The interval from occurrence of the tear to diagnosis and initiationof therapy has a significant affect on the prognosis for survival.[337,350,425,442,458] Ascertaining the uterine integrity by vaginal palpation alone may not be possible.[425] The tips of the horns may be especially difficult to palpate from within the postpartum uterus. The changes in the peritoneal cavity depend on the duration of the condition, but generally one can expect to

see serosanguinous to sanguinous fluid containing elevated total protein, increased white blood cell counts, and often extracellular and intracellular bacteria.[350,427,458] Laparoscopic evaluation of the uterus may confirm the diagnosis and provide useful information to determine whether surgery is indicated.[459] Complete perforation of the uterine wall is not necessary for peritonitis to develop if traumatic obstetric manipulations have damaged the uterine wall.[460] However, recent research has proved that even a fetotomy procedure does not alter the composition of the postpartum peritoneal fluid if it is performed correctly.[350]

If one suspects a uterine laceration (partial or full), the mare should receive systemic broad-spectrum antibiotic coverage. Nonsteroidal antiinflammatory medicationmay prevent the development of endotoxemia. Oxytocin therapy (10 to 20 IU every 2 hours) promotes uterine involution. One can increase the dose if the mare does not become uncomfortable. One should administer fluid therapy as necessary, ensuring that calcium levels are within the normal range. Intensive medical management may suffice for small dorsal uterine tears, but most warrant suturing if costs are not a limitation.[458,459] Opinions vary on the need for and usefulness of peritoneal lavage.[459] Large, full-thickness tears warrant surgical intervention. In some instances, one can suture a laceration in the uterine body blindly in situ, but often a ventral midline celiotomy is the preferable approach.[337,351,424,458]

16.8.11.5 RETAINED FETAL MEMBRANESAND TOXIC METRITIS

Once the umbilical cord ruptures, blood flow through the capillary network in the placenta suddenly ceases.[159] This causes a reduction in the tissue volume of the microcotyledons, and the rhythmic tubocervical contraction waves cause the membrane tips to separate and invaginate into the horn. The ongoing tubocervical detachment process causes the membranes to be passed inside out, with the allantoic surface exposed. The membranes should be expelled within 3 hours post partum, and the incidence of retention has been reported to range from 2% to 10% of foalings.[338,445,461,462] Membrane retention tends to be associated most commonly with the tip of the nongravid horn, and appears to be associated with dysfunction of the initial separation process.[23] In circumstances in which tissue inflammation is common (abortion, dystocia, cesarean section), membrane retention is more likely to occur. In these cases the endometrial edema may trap the microcotyledons within the endometrial crypts. Mares with membrane retention may have a significantly lower serum calcium level.[463] A recent study noted that the number of endometrial mast cells observed during the puerperal period is significantly lower in the endometrium of mares with retained fetal membranes.[464] Some dysfunction of the normal endocrine-related maturational processes within the microcotyledons likely is involved.

Appropriate management of a mare with fetal membrane retention varies depending on the time since foaling.[465] Although some mares, especially those foaling in a natural environment, may not experience any complications, prophylactic medication is recommended under intensive husbandry conditions.[462,466] Bacterial contamination in this environment is highly likely. If a severe metritis develops, inflammation of the uterine wall permits bacteria and toxins to enter the systemic circulation, producing septicemia and endotoxemia.[460,467] Laminitis is a frequent sequela.[337] The approach to treating retained fetal membranes varies considerably, depending on the duration of membrane retention and the presence or absence of metritis with septicemia. In normal, unassisted foalings, one or two treatments with oxytocin may be all that is required to facilitate passage of the retained membranes. One should tie the protruding placental remnants in a knot above the hocks of the mare. An initial, low dose (10 to 20 IU) of oxytocin is recommended because some postpartum mares can be especially sensitive to this hormone and may experience a severe bout of

colic within minutes of treatment. Higher doses are likely to be counterproductive because myometrial spasm occurs instead of the desired rhythmic, tubocervical contractions. If colic does occur, one should sedate the mare so that she does not roll and possibly injure the neonate.[337] In these cases, one should reduce the next dosage of oxytocin. The response of each mare to the initial treatment governs the subsequent dose recommendations: 10 to 20 IU incremental increases every 2 hours.

The author routinely distends the chorioallantoic sac with fluid after any obstetric procedure. This procedure, known as the Burn's technique, has promoted membrane expulsion (5 to 30 minutes) in most postdystocia mares that the author has managed. A major advantage is that expulsion of the intact fetal membranes removes any contaminants that may have been introduced by the obstetric procedures.[468] The technique only 1115
works if one can pass a sterile nasogastric tube beyond the torn distal fragments and is best performed while 1116
the membranes are still fresh. In more protracted cases the rapidly autolyzing chorioallantois becomes friable and generally tears once the fluid pressure increases. To perform the procedure, one tightly holds the exposed fetal membranes around the tube while infusing 12 to 15 L of solution. One then ties off the opening with umbilical tape. The exact mechanism is unknown, but expansion of the uterine lumen may dilate the endometrial crypts such that the weight of the membranes can pull the microcotyledons free atraumatically. One can supplement endogenous oxytocin release to enhance uterine contractions.

Because the uterine response to oxytocin wanes during the postpartum period, the dose may be increased in small increments every 2 hours in those mares that retain their fetal membranes despite the initial therapy. If a hospitalized mare is receiving intravenous fluids, then one can add each oxytocin treatment to the fluid line. Another option is to add oxytocin to the fluid bag ata dose that is calculated based on the flow rate (1.0 IU/min).[337] However, a disadvantage of this approach is that one must discard these fluids if the mare becomes uncomfortable yet still requires rehydration. The calcium ion plays a vital role in myometrial contractility, and one must ensure that calcium levels are within the normal range.[383,469] Supplemental calcium can expedite the rate of passage greatly, suggesting that uterine hypomotility is a component in some of these cases.[463] Controlled exercise is often beneficial in promoting uterine involution but is not always feasible if the mare is hospitalized or is being kept in a stall while a compromised neonate is being medicated.

Excessive traction on the fetal membranes is accepted widely as being contraindicated, but a recent study suggests that cautious manual removal of the membranes may not be as deleterious as previously thought.[470] When one extracts the membranes by force, inevitably disruption of the epithelial barrier occurs, making the traumatized uterine lining more susceptible to bacterial invasion and the development of metritis.[467] Endometrial trauma is also likely to contribute to the development of periglandular fibrosis. For the membrane tip to tear off and remain firmly attached within the nongravid horn is not uncommon.[23] Injudicious traction on the membranes also can cause an inversion of the tip of the uterine horn, and this can progress to a complete uterine prolapse.[337] If the fetal membranes have not been expelled after a couple of days of supportive therapy, the autolytic tissue becomes less firmly embedded and a gentle twisting technique, with minimal traction, applied within the attached horn often results in successful removal of the entire chorioallantois. In the author's experience, this procedure works best while the uterus is being distended during a uterine lavage. Recent studies suggest that a safe and potentially effective treatment for retained fetal membranes in mares may be intraplacental injections of collagenase.[471,472]

If the membranes have been retained for 6 to 8 hours when one first examines the mare, then systemic antibiotic therapy is indicated.[337] Drugs that have been recommended for systemic administration include ampicillin, gentamicin, kanamycin, penicillin, ticarcillin, and trimethoprim-sulfamethoxazole.[473] If a

remnant is missing when one examines the membranes, then the approach to therapy should proceed as if the entire membranes were still present. A mare with toxic metritis is characterized by fever, depression, anorexia, tachycardia, and injected mucus membranes.[467] The foal does not receive adequate milk intake, and many of these mares have bounding digital pulses and evidence of laminitis. Palpation per rectum reveals a large, thin-walled, atonic uterus that contains moderate to large amounts of fetid fluid.[337] A large volume of toxic, red-brown, watery fluid can accumulate within the pendulous postpartum uterus before any obvious vaginal discharge becomes evident. Often the history reveals that the fetal membranes were discarded without being checked that they were passed intact.

Because the endometrium is likely to be necrotic, therapy should include broad-spectrum antibiotics, antiinflammatory drugs, and intravenous fluids if indicated. Tetanus prophylaxis is advisable. A combinationof penicillin and gentamicin is used widely to provide broad-spectrum systemic coverage, especially against the coliforms that frequently contribute to endotoxemia and laminitis.[474] One should administer flunixin meglumine to ameliorate the effects of endotoxemia. The drug commonly is administered intravenously at a reduced dose (0.25 mg/kg) 3 times daily.[337] Phenylbutazone (2 to4 mg/kg) and provision of deep, soft bedding are useful to alleviate pain when laminitis appears to be imminent. Radiographs can be useful to monitor changes in the position of the pedal bone. One may administer vasodilators such as acetylpromazine maleate by intramuscular injection (0.02 to 0.04 mg/kg every 4 to 6 hours).[474]

One should lavage the uterus with sterile saline or very dilute povidone-iodine solution and should exercise extreme caution to avoid puncturing the inflamed uterine wall with the tube. If povidone-iodine solution is used, the final concentration should not exceed 0.1%; this is equivalent to 10 ml of 10% povidone-iodine solution (e.g., Betadine) in one liter of saline. One should repeat the lavage until the returning fluid is clear. The goal of therapy is to eliminate toxins and to prevent the rapid proliferation of bacteria, especially coliforms and possibly anaerobes. The administration of intrauterine antibiotics in the postpartum mare is 1116
controversial because little scientific validation has been performed. Intrauterine administration of antibiotics 1117
and antiseptics may depress the phagocytic activity of uterine neutrophils, and many chemicals are known to irritate the endometrium in mares being infused for endometritis.[473] The pharmacokinetic properties of each drug influence its efficacy in the postpartum uterus. Most of the studies regarding intrauterine therapy in mares have addressed therapy for endometritis in nonparturient animals. The efficacy of antibiotic formulations in the presence of the mixed bacterial population and tissue debris associated with fetal membrane retention in the mare remains to be established. One should add the antibiotic of choice to a large infusion volume (2 to 3 L) to ensure uniform distribution across the inflamed endometrial surface once a lavage has removed the toxic fluid and necrotic debris. Unpublished microbiologic studies in the author's laboratory have demonstrated that many organisms cultured from metritis fluid are sensitive to amikacin.[262] Infusion of 2 g of this antibiotic after a uterine lavage has proved to be clinically effective. Polymixin B may have some merit because of its endotoxin-binding ability. Powdered and propylene glycol–based oxytetracycline formulations are known to be irritant when infused into the involuted uterus and should be avoided.[475,476] Other antibiotics that have been suggested for postpartum intrauterine therapy include ampicillin (3 g), ticarcillin (1 to 3 g), and gentamicin (2 to 3 g).[477] In the author's unpublished studies, less than 60% of isolates from metritis fluid were sensitive to ampicillin.[262]

16.8.11.6 GASTROINTESTINAL COMPLICATIONS

Prolonged straining during a dystocia can lead to variable amounts of rectal mucosa being forced out through the anal sphincter (type I rectal prolapse). The tissue then becomes subject to trauma, contamination, and

vascular compromise. If one does not correct the condition promptly, pressure from the anal sphincter causes venous congestion and swelling, which promotes more straining, and the condition can deteriorate rapidly. A type II prolapse involves all or part of the ampulla recti.[438,478] An epidural anesthetic may help to decrease straining. One may apply topical glycerin or dextrose to the prolapsed tissue to reduce edema.[337,478] A purse-string suture can cause additional straining and impedes defecation.[478,479] One should administer fecal softeners and should modify the diet (pellets, pasture) to help produce soft feces. Chronic prolapses may warrant surgical resection of the devitalized mucosal mass.[478,480] In a type III prolapse a full-thickness rectal prolapse plus an intussusception of the peritoneal rectum or small colon occurs.[478] In a type IV prolapse the intussuscepted bowel protrudes through the anus such that a palpable trench that may extend several meters into the rectum, depending on the length of the intussusception.[415,416,478,479] Midventral celiotomy is usually necessary to reduce the intussusception, although some smaller prolapses reduce after an attendant has extracted the foal.[478] The short mesentery that supports this section of bowel often is torn fromthe colon. Thus these cases have a guarded prognosis, depending on the vascular integrity of the affected small colon. The author has seen this condition develop when as little as 6 to 10 inches of bowel appears to be prolapsed. The avulsion most likely occurs when the intermittent straining of the mare forces an extra 4 to 6 inches of bowel in-and-out of the rectum. Thus to prevent straining as soon as possible is imperative. If the foal has not yet been extracted, then immediate anesthetization of the mare may be best, followed with elevation of the hindquarters before attempting to correct the cause of the dystocia.

In rectal prolapse cases that have been managed conservatively, one of the first postpartum clinical signs may be discomfort attributable to impaction colic. If avulsion of the mesocolon has occurred, then ischemic necrosis of the affected bowel causes a delayed peritonitis. Because early intervention is essential, sequential abdominocentesis is indicated when one is managinga type III or IV rectal prolapse conservatively. Initially changes in the composition of the peritoneal fluidmay be negligible. However, if an avulsion has occurred, then the compromised segment of bowel soon looses its integrity and a massively increased white blood cell count can occur within 24 to 48 hours as peritonitis ensues.[350] Laparoscopic evaluation of the abdomen can provide an immediate assessment of bowel integrity and permitan accurate prognosis to be given to the owner.[352] The affected colon is not readily accessible for resection and anastomosis, so the prognosis in most cases is guarded.[480]

Variable degrees of uncomplicated impaction are not uncommon in the postparturient mare, possibly because of localized perineal pain causing reluctance to defecate.[337] Astute managers note an absence of fecal matter in the stall. Treatment with fecal softeners (e.g., mineral oil) and analgesics generally corrects the problem. Laxative feeds (e.g., bran mash) are effective in reducing the incidence of constipation in foaling mares.[439] Postpartum mares appear to be at an increased risk for developing a large colon torsion.[221,442,481] This condition presents as an especially violent colic with readily discernable abdominal distention. Extensive ischemic damage affects the prognosis, but early surgical intervention can increase the survival rate.[425,482] Bruising of the abdominal viscera can occur during foaling, with subsequent development of moderate to severe signs of impaction colic and peritonitis.[423,425] Occasionally the mesentery may be torn from a 1117
segment of intestine, leading to ischemic necrosis and peritonitis. An early diagnosis and prompt surgical 1118
intervention may save the life of the mare.[350,352,440–442,483] A rent in the mesentery or broad ligament at the time of foaling may permit a segmentof bowel to become incarcerated even weeks later.[218,436,441,442,484] One should advise owners that surgical correction is only feasible if the segment of devitalized bowel is accessible.[218,441,483–485]

Although mares tend to reduce their feed intake in the days leading up to foaling, ensuring a reduction in the amount of available roughage may help reduce the incidence of bowel rupture.[439] The tip of the cecum is the most likely site of a foaling-related rupture in the alimentary tract. On palpation per rectum the inflamed serosal surfaces feel roughened with a discernable crepitus. Abdominocentesis reveals dark green-brown gastrointestinal fluid that contains plant material and massively increased neutrophil numbers. Humane euthanasia is indicated because the leaking ingesta incites a severe peritonitis with accompanying septic shock, and the condition is likely to be rapidly fatal.[423,442,486–490] Diaphragmatic herniation has been reported as a rare parturient complication in heavily pregnant mares.[349,491–493] Colic symptoms are attributable to strangulating obstruction or tension on the mesentery. Some mares may exhibit respiratory distress. Transthoracic ultrasonography can help confirm the presence of bowel within the thorax.[494] Surgical repair of the defect may not be possible, and assisted ventilation is required.[492,493]

16.8.11.7 VAGINAL LACERATIONSAND BLADDER PROLAPSE

Primiparous mares are especially susceptible to vaginal trauma. Vaginal lacerations are most likely to occur during injudicious attempts to relieve dystocia. Although most lacerations are retroperitoneal, they still may contribute to severe vaginitis, fibrosis, and possibly abscessation. If ventral trauma is present, then one should pass a urinary catheter to check for urethral integrity. In some instances ligation of a severed artery is necessary. Emollient creams, tetanus prophylaxis, broad-spectrum antibiotics, and antiinflammatory drugs are indicated. A major concern is the possibility of herniation of intestine into the vagina if the tear is located just caudal to the cervix in the vicinity of the urogenital pouch.[442,495] If eventration has occurred, one should cleanse the bowel and examine it for evidence of vascular compromise. If the involved intestine appears to be grossly normal, then one should rinse it with sterile saline and return it to the abdominal cavity. If one detects vascular compromise at the time of the initial examination, then the prognosis is guarded and a ventral midline celiotomy to facilitate resection is warranted. A bladder prolapse occurs when the bladder is forced up through a vaginal laceration. The viscus rapidly becomes distended because of continued accumulation of urine from the ureters and an inability to void urine because of kinking of the urethra. The edematous serosal surface of the bladder may protrude through the vulvar lips.[337] One should clean the exposed organ thoroughly and then gently return it the abdominal cavity. Administration of an epidural and aspiration of urine to facilitate replacement may be necessary. If possible, one should suture the vaginal laceration once any viscera have been returned to the abdominal cavity. In some cases the severity of the trauma precludes successful closure, and the wound must heal by second intention.[495] Placement of a Caslick suture reduces the possibility of bacterial aspiration. Mares may be cross-tied for several days to decrease the risk of eventration brought about by increase of intraabdominal pressure as the mare lies down.[337] One should treat the mare for impending peritonitis (broad-spectrum antibiotics; nonsteroidal antiinflammatory drugs). Tetanus prophylaxis is indicated. If severe colic symptoms develop, then one should suspect bowel compromise.[350]

16.8.11.8 EVERSION OF THE URINARY BLADDER

The urethra of the mare has a large diameter, and occasionally the bladder may be everted up into the vagina following severe straining.[496] If the everted bladder protrudes through the vulvar lips, the exposed mucosal surface rapidly becomes edematous, and urine may drip from the ventral surface. Closer inspection reveals that the urine is dribbling from the exposed papilliform openings of the ureters on the dorsal surface of the

neck of the bladder.[497] A lip chain and epidural may provide adequate restraint to facilitate replacement. One should clean the mucosal surface thoroughly and repair any defects and then apply sterile lubricant and gently massage the friable organ back through the urethra. In some instances, incising the urethral sphincter may be necessary if the bladder mucosa is especially thickened.[497] One should close this incision after replacing the bladder. One can insert a Foley catheter to lavage the bladder lumen and to ensure complete repositioning. Broad-spectrum antibiotic coverage, nonsteroidal antiinflammatory drugs, and tetanus prophylaxis are indicated.

16.8.11.9 RUPTURE OF THE URINARY BLADDER

Occasionally the bladder may rupture as a consequence of increased intraabdominal pressure in the foaling mare or because of direct trauma during parturition.[498–500] Clinical signs are delayed and are associated with electrolyte imbalances. Affected mares may be depressed and inappetant, with failure to void urine. Clinical examination reveals tachycardia, tachypnea, and decreased gastrointestinal activity. Blood chemistry reveals elevated serum levels of creatinine, blood urea nitrogen, and potassium with decreased sodium and chloride 1118
levels. Evaluation of a peritoneal fluid sample helps to confirm the diagnosis. The fluid contains elevated 1119
urea and creatinine levels and calcium carbonate crystals.[498,499] Cystoscopy is useful to evaluate the size and extent of the bladder injury. Once one has stabilized the medical condition of the mare, surgical repair is indicated.[436,498–500] A standing vaginal approach eliminates the need for general anesthesia and allows excellent observation and repair of bladder tears in adult mares.[500]

16.8.11.10 RECTOVAGINAL FISTULAEAND PERINEAL LACERATIONS

A first-degree perineal laceration involves the mucous membrane of the vestibule and the skin of the vulvar lips. In second degree perineal lacerations the deeper tissues of the perineal body are involved. Both of these conditions may be associated with unassisted delivery of a large foal or may be sequelae of dystocia. The lacerationmay be amenable to immediate repair and placement of a Caslick suture, or the clinician may elect to wait until the wound has granulated. One should treat the mare with broad-spectrum antibiotics, antiinflammatory medication, and tetanus prophylaxis. Provision of a bran mash diet and administration of mineral oil may facilitate defecation during the initial inflammatory period.

Third-degree perineal lacerations generally occur during unassisted foalings when the fetal hoof catches on the vaginal roof at the vestibulovaginal junction. Forceful straining by the mare can drive the hoof through the rectovaginal shelf such that the fetal hoof comes to lie within the rectum. If the fetus is viable, it may remove the affected limb so that delivery proceeds unimpeded, and a rectovaginal fistula results. If the limb remains within the rectum, then continued passage of the fetus causes the trapped limb to tear out the perineal body and anal sphincter. The resulting defect is called a third-degree perineal laceration. These injuries do not respond well to immediate surgical intervention, and the general recommendation is to wait 4 to 6 weeks before attempting reconstructive surgery.[436,496] In the interim, one should treat the mare with broad-spectrum antibiotics, antiinflammatory medication, tetanus prophylaxis, and fecal softeners.

16.8.11.11 PERINEAL BRUISINGAND VULVAR HEMATOMAs

Much of the swelling after prolonged obstetric manipulations is edematous. Fecal softeners such as orally administered mineral oil or a bran mash are recommended to ease the passage of feces through the swollen

and bruised perineal area.[349,439] Hematomas in the vaginal wall and vulvar lips are not uncommon, especially in primiparous mares and in those mares that have delivered a large foal. One must differentiate a bulging vestibular hematoma from an everted or prolapsed bladder.[439,443] Needle aspiration of vulvar hematomas is not recommended because of the risk of abscessation. Broad-spectrum antibiotics and tetanus prophylaxis are indicated. Most hematomas resolve uneventfully, but some vulvar, vaginal, or pelvic hematomas may warrant drainage in7 to 10 days.[349]

16.8.11.12 POSTPARTUM ECLAMPSIA(LACTATION TETANY)

Postpartum eclampsia is rare in mares but may occur in animals that are lactating heavily. The highest incidence is reported to occur in Draft breeds, but the author has encountered a case in a pony mare. Equine eclampsia generally is associated with some type of stress (e.g., change in surroundings). Early signs include restlessness, tachypnea, staring eyes, twitching, trembling, and clonic spasms (especially diaphragmatic). The clonic spasms gradually become more tonic, and eventually the mare may be unable to stand. The differential diagnosis is tetanus, but the nictitating membrane is not prolapsed. The condition responds well to intravenous administration of calcium gluconate.[159]

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16.9 16.9—Assisted Reproductive Techniques

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16.9.1 Assisted Reproductive Techniquesfor the Mare

Until recently, use of assisted reproductive techniquesin the mare has been limited; however, during the last decade, new methodologies have been developed and proven. New assisted reproductive techniques allow production of offspring from mares that are infertile using standard breeding techniques or embryo transfer.

16.9.1.1 OOCYTE TRANSFER

Although the first successful oocyte transfer was performed in 1988, the technique was not used for commercial transfers until the late 1990s.[1–3] Oocyte transfer involves the transfer of an oocyte from a donor into the oviduct of a recipient; the recipient is inseminated within the uterus. Fertilization, embryo development, and fetal development occur within the recipient, thereby avoiding problems associated with

ovulation or the tubular genitalia of donors. The incidence of ovulatory failure increases with age and during the autumn months.[4,5] Prolonged exposure to an abnormal follicular environment results in aging and death of the oocyte. One can detect some types of ovulatory failure with ultrasound as an atypical morphology of the follicle or ovulatory site. Mares that repeatedly fail to ovulate can provide oocytes for transfer successfully if oocytes are collected before deleterious changes occur within the follicle.[2]

Historically, the uterus has been considered the primary cause of reduced fertility in the mare. Mares with pyometras or persistent endometritis are expensive to treat and frequently do not provide embryos. Mares with problems such as cervical lacerations, cervical or uterine adhesions, or urine pooling often fail as embryo donors. However, oviduct dysfunction has been shown to be a major impediment to fertility, especially in aged mares. When the oviducts of old mares (>20 years) and young mares (2 to 9 years) were flushed between 1 and 4 days after ovulation, collection rates of recently ovulated oocytes or oviductal embryos were significantly higher in the young versus old mares (26 of 27, 96%, versus 17 of 29, 59%, respectively).[6] In subfertile mares, pathologic changes of the oviducts were imaged using scanning electron microscopy, and significantly fewer sperm were detected in the caudal isthmus in subfertile mares than in fertile mares. Few sperm found in the oviducts of subfertile mares were motile, whereas oviducts of the normal mares contained highly motile sperm.[7] Obstructions of the oviductal lumen have been postulated to be the cause of subfertility in some mares. Globular masses composed of type I collagen were found more frequently in older than in younger mares.[8] In another study, oviductal masses were found in the oviducts in 73% (16 of 22) of mares between 2 and 22 years of age; in a small number of mares (3 of 43), the masses occupied and distended the oviductal lumen and could have resulted in infertility. The equine embryo remains in the oviduct for 5 to 6 days before entering the uterus; therefore, oviductal problems such as inflammation could affect embryo viability.[9]

16.9.1.2 PROCEDURES FOR OOCYTE TRANSFER

Requirements for oocyte donors are minimal. If one detects intrauterine fluid collections or vulvar discharges in donors, one should culture the uterus and treat the mare to prevent introduction of a pathogen into the abdominal cavity during transvaginal oocyte collections. Donors should have regular estrous cycles with growth of a preovulatory follicle. The age of the donor affects success rates. When oocytes were collected from the follicles of young donors (6 to 10 years) and old donors (20 to 26 years) and transferred into the
oviducts of young recipients (3 to 7 years), significantly more oocytes from young than old donors developed 1130
into embryonic vesicles (11 of 12, 92%, versus 8 of 26, 31%, respectively).[10] A higher incidence of 1131
morphologic anomalies were observed in oocytes from old than from young mares.[11] Although younger mares are better candidates for oocyte donors, older (>20 years) mares frequently are presented to commercial oocyte transfer programs, and pregnancies are obtainable through repeated transfers.[2]

16.9.1.3 OOCYTE COLLECTION

Currently, most oocytes are collected from preovulatory follicles between 24 and 36 hours after the administration of human chorionic gonadotropin (hCG; 1500 to 2500 IU intravenously) to the donor (between 14 and 0 hours before anticipated ovulation, respectively). Therefore oocytes are probably at metaphase I or II. Criteria for hCG administration are (1) a follicle greater than 35 mm in diameter, (2) relaxed cervical and uterine tone, and (3) uterine edema or estrous behavior for a minimum of2 days. Some mares, especially old mares, do not consistently respond to hCG. In these cases, the authors use a

combination of gonadotropin-releasing hormone agonist (Ovuplant, deslorelin acetate, 2.1 mg subcutaneously) and hCG (2000 IU intravenously), with hCG administered between 4 and 5 hours after the gonadotropin-releasing hormone agonist. Oocytes have been collected from the follicles of mares using laparotomies,[12] colpotomies,[13] flank punctures,[14,15] and ultrasound-guided, follicular aspirations,[16,17] Currently, most laboratories collect oocytes through the flank or with ultrasound-guided punctures.

For the collection of oocytes using flank punctures, one places a trocar through the flank ipsilateral to the preovulatory follicle at approximately the position of the ovary. One manipulates the ovary per rectum to position the preovulatory follicle against the end of the cannula. While one stabilizes the ovary per rectum, one places a needle (12 to 17 gauge) through the cannula and into the follicular antrum and removes the follicular fluid and oocyte by gentle suction and lavage of the follicle. Transvaginal, ultrasound-guided follicular aspirations require use of an ultrasound machine. Linear, curvilinear, and sector transducers have been used. The transducer is placed in a casing containing a needle guide. Rectal contractions have been minimized through administration of propanthelene bromide (0.04 mg/kg intravenously)[2] or intrarectal use of lidocaine. One applies a nontoxic lubricant to the transducer and positions it within the anterior vagina lateral to the posterior cervix and ipsilateral to the follicle to be aspirated. One carefully positions the follicle through transrectal manipulations with the follicular apex juxtaposed to the needle guide and advances the needle through the needle guide to puncture the vaginal and follicular walls. In the authors' laboratory, a 12-gauge, double-lumen needle is used (Cook Veterinary Products, New Buffalo, Michigan). One aspirates the follicular fluid from the follicle usinga pump (Cook Veterinary Products) set at 150 mm Hg. After removal of follicular fluid, one lavages the lumen with between 50 and 100 ml of flush, typically modified Dulbecco's phosphate buffered solution or an embryo flush solution (EmCare, ICP, Auckland, New Zealand) containing fetal calf serum (1%) or bovine serum albumin (0.4%) and heparin (10 IU/ml). Oocytes were collected successfully from between 70% and 80% of the follicles in client donors.[2]

16.9.1.4 OOCYTE CULTURE AND TRANSFER

Oocytes are sensitive to temperature changes; therefore one should warm media and equipment for handlingthe oocyte to 38.5° C. On collection, one pours the flush into large search dishes and examines the flush under a dissecting microscope to locate the oocyte. One transfers oocytes collected 36 hours after hCG administration to the donor immediately into a recipient's oviduct. One cultures oocytes collected 24 hours after administration to the donor in vitro between 12 and 16 hours before transfer. Most oocytes are cultured in medium similar to that first described by Carnevale and Ginther.[10] The time of oocyte collection (24 versus 36 hours after administration of hCG to donors) did not affect pregnancy rates.[18] A modification of these procedures was to collect oocytes 24 hours after hCG and immediately transfer them into the recipient's oviduct. Oocyte maturation was completed within the oviduct, and recipients were inseminated after oocyte maturation should have been completed at 16 hours after transfer. Pregnancy rates were not statistically different for oocytes matured within the oviduct or within an incubator (43% versus 57%).[19]

Because the reproductive tract of the recipient provides the environment for sperm transport, fertilization, and embryo development, these mares should be young (optimally 4 to 10 years) and have normal reproductive tracts. Cyclic and noncyclic hormone-treated mares have been used as oocyte recipients. When cyclic mares are used, recipients are synchronized with the donor, and the recipient's own oocyte is removed by transvaginal or flank aspiration before transfer of the donor's oocytes.[20] Anestrus and early transitional mares were used as recipients during the nonovulatory season.[2,21] During the breeding season, a high dose of a gonadotropin-releasing hormone agonist (4.2 mg deslorelin acetate)[22] or injections of progesterone and

estrogen (150 mg progesterone and 10 mg estradiol)[3] have been administered to reduce follicular development in potential recipients. The endocrine environment of the cyclic mare is imitated in the noncyclic recipient with administration of estradiol (2 to 5 mg daily for 3 to 7 days) before transfer and progesterone (150 to 200 mg daily) after transfer. Pregnancies were maintained through the administration of exogenous progesterone or progestins.[2] 1131

1132

Because one transfers oocytes surgically, adequate exposure of the oviduct is essential, and mares with short, thick flanks or short, broad ligaments are not good candidates for recipients. Most oocyte transfers are performed through a standing flank laparotomy. Tranquilization, preparation, closure, and aftercare of recipients are similar to previously described methods for embryo transfer.[23] The authors generally use a fire-polished glass pipette to transfer oocytes. One locates the oviductal os by following the outline of the oviduct along the external surface of the infundibulum. One identifies the end of the structure and inserts the pipette containing the oocyte into the os and carefully advances the pipette 2to 3 cm. One deposits the oocyte and a minimal amountof medium (<0.1 ml) into the ampullar region of the oviduct, gently returns the ovary to the abdominal cavity, and closes the surgical site.

16.9.1.5 INSEMINATION OF RECIPIENTS

In a commercial oocyte transfer program, use of stallions with good fertility is essential for success; however, cooled, transported semen frequently is provided from different stallions of variable fertility.[2,24] The equine oocyte remains viable for approximately 12 hours after a natural ovulation.[25] Because of this limited life span, one must inseminate recipients before or directly after oocyte transfer or both. Pregnancies have occurred when recipients were inseminated only before[26,27] or after[19] the transfer of oocytes. However, for most experimental transfers, recipients were inseminated before transfer (approximately12 hours) and after transfer (approximately 2 hours) with a total of 2×10^9 motile sperm. In a commercial program using older donors and cooled semen from numerous stallions of variable fertility, pregnancy rates when recipients were inseminated before or before and after oocyte transfer were significantly higher than when recipients were inseminated only after transfer (18 of 45, 40%; 27 of 53, 51%; and 0 of 10, respectively).[24] The results suggest that insemination of a recipient only before transfer with at least 1×10^9 progressively motile sperm from a fertile stallion is sufficient. However, if fertility of the stallion is not optimal, insemination of the recipient before and after transfer could be beneficial.

After insemination and transfer, one examines the uterus of the recipient with ultrasound to detect intrauterine fluid collections. One treats recipients with intrauterine fluid collections as one treats ovulating mares, with oxytocin or prostaglandins to stimulate uterine contractions or with uterine lavage and infusion.

16.9.1.6 SUCCESS OF OOCYTE TRANSFER

Pregnancy rates for commercial transfers, using older donors and semen of variable quality, ranged from 27% to 40% per transfer.[2,24] In contrast, experimental transfers under similar conditions using oocytes from young mares and fertile stallions resulted in pregnancy rates between 54% and 83% per transfer.[24] However, one or more pregnancies were obtained for more than 80% of donors during the breeding season in a commercial oocyte transfer program.[2] All mares in the program had histories of reproductive failure in breeding and embryo transfer programs, with a mean of 7 years (range of 3 to 15 years) from the last successful pregnancy or embryo collection.[2]

16.9.1.7 IN VITRO MATURATION OF OOCYTES

Collection of oocytes from small follicles during diestrus results in significantly reduced collection rates compared with collection from preovulatory follicles.[28] In vitro fertilization is not repeatedly successful in the mare, with only two foals born after in vitro fertilization.[29,30] In a recent study,[26] oocytes were collected from small follicles and from preovulatory follicles. Oocytes collected from small follicles were matured in vitro for 36 to 38 hours before transfer, whereas oocytes collected from preovulatory follicles were transferred immediately into a recipient's oviduct. Embryo development rates after transfers were 9% for in vitro maturation and 82% for in vivo. In the past few years, a medium has been developed specifically for the equine oocyte,[31] and fertilization has been achieved by intracytoplasmic sperm injection (ICSI).[32,33] Research involving oocytes from excised ovaries is aimed at developing a method to salvage gametes from the ovaries of valuable mares that have died or have been euthanized. One can collect ovaries from mares immediately after death and ship them to a facility for oocyte recovery, maturation, and transfer. This technique was first attempted in 1999[2]; a pregnancy was established, which later underwent embryonic death. At this time, a late-term pregnancy has been established after shipment of ovaries from a mare that was euthanized for medical reasons.

16.9.1.8 CRYOPRESERVATION OF OOCYTESAND EMBRYOS

Cryopreservation of the equine oocyte results in the preservation of female genetics, whereas cryopreservation of the embryo results in the preservation of the female and male genome. The first foal was produced from a cryopreserved embryo in 1982.[34] Procedures for embryo cryopreservation have been reviewed.[35] Cryopreservation of small embryos (morulae or early blastocysts) has resulted consistently in acceptable pregnancy rates close to 50%.[36,37] Cryopreservation of larger embryos (>300 μm) is usually unsuccessful. Therefore embryo donors are examined twice daily for ovulation; and embryo collection is recommended on day 6 or 6½ after ovulation.[35] Although cryopreservation of the oocyte is difficult, 1132
successful fertilization of cryopreserved oocytes has been documented.[31,38,39] In 2001, the first foals were 1133
born after cryopreservation of oocytes.[27]

16.9.2 Assisted Reproductive Techniquesfor the Stallion

Maximum fertility was obtained when fertile mareswere inseminated every other day during estrus with500 × 10^6 progressively motile sperm.[40] Insemination of low numbers of sperm would be beneficial for frozen semen that is of limited supply, semen from subfertile stallions with low sperm numbers, and insemination of sex-sorted sperm. The following discussion summarizes current techniques for low-dose inseminations.

16.9.2.1 DEEP INTRAUTERINE INSEMINATION

Uterine contractions move sperm into the tips of the uterine horns within 20 minutes of routine artifical insemination.[41] The aim of deep uterine insemination is to increase the number of sperm entering the oviduct ipsilateral to ovulation.[42–44] One passes a flexible insemination pipette through the cervix and into the uterine horn ipsilateral to the preovulatory follicle. One then uses rectal manipulation to position the catheter at the tip of the uterine horn where the sperm are deposited. Fresh, cooled, and sex-sorted sperm in volumes

ranging from 0.2 to 1.0 ml of glucose milk extender have been used for deep intrauterine inseminations. Pregnancy rates after deep intrauterine inseminations with 5×10^6 progressively motile sperm were between 30% and 50%,[45,46] and inseminations with 25×10^6 progressively motile sperm ranged from 57% to 63%.[45,47] However, in the study by Woods, Rigby, Brinsko, et al.,[47] control mares were inseminated with 25×10^6 progressively motile sperm in the uterine body, and pregnancy rates were not significantly different between standard and deep uterine inseminations. Because control inseminations were not done in many studies, the true benefit of deep uterine insemination has not been determined.

16.9.2.2 HYSTEROSCOPIC INSEMINATION

Hysteroscopic insemination entails deposition of sperm directly onto the papilla of the uterotubal junction. A minute volume of extended sperm (approximately 0.05 to 0.25 ml) is desired for hysteroscopic insemination. Sperm are centrifuged through a density gradient to select a sperm population with a high percentage of motility. Numbers of fresh sperm that were inseminated ranged from 1 to 10×10^6 progressively motile sperm, with pregnancy rates from 40% to 75%.[46,48–50] Studies have been conducted using higher volumes[51] or lower sperm numbers[48]; however, fertility was reduced.

One aspirates semen into an equine gamete intrafallopian transfer (GIFT) catheter (Cook Veterinary Products) protected by an outer polypropylene cannula and loaded into the working channel of the videoendoscope. With a sterile gloved arm in the vagina of the mare the operator guides the flexible endoscope (1.6 m in length) through the cervix and uterine lumen; directs the endoscope along the uterine horn ipsilateral to the preovulatory follicle; and on imaging the papilla of the uterotubal junction, extrudes the outer cannula and then the inner GIFT catheter containing the sperm suspension from the working channel of the endoscope. When the tip of the GIFT catheter touches the papilla, the operator bubbles the inseminate onto the surface of the papilla.[48]

Low-dose insemination with frozen-thawed sperm maximizes the use of a conventional dose of frozen sperm (800 to 1000×10^6 progressively motile sperm) by reducing the number of sperm needed for insemination. Using 5 or 10×10^6 frozen-thawed progressively motile sperm, different investigators obtained pregnancy rates between 33% and 47%.[49,52,53] Alvarenga, Trinque, Lima, et al.[54] inseminated client mares with 100 to 150×10^6 frozen-thawed sperm from 15 Warmblood stallions and obtained an overall pregnancy rate of 57%, demonstrating that hysteroscopic insemination can be applied immediately in the horse industry. Current rates for sorting sperm into X or Y chromosome–bearing populationsare approximately 10 million sperm per hour, meaning that low-dose inseminations are necessary for sex-sorted sperm. Several studies have been conducted using hysteroscopic insemination of sex-sorted sperm, resulting in pregnancy rates between 25% and 44%.[49,52]

16.9.2.3 GAMETE INTRAFALLOPIAN TRANSFER

Gamete intrafallopian transfer involves transfer of oocytes and sperm into the recipient's oviduct. In comparison to oocyte transfer, GIFT requires low numbers of sperm. The first successful GIFT in the horse was reported in 1998.[21] After collection, one must centrifuge raw sperm through Percoll density gradient to select a population with high percentage of motile sperm, free of debris and seminal plasma. One places between 2 and 5×10^5 progressively motile sperm in medium containing the oocyte and places both gametes

into the oviduct of a recipient mare. Pregnancy rates obtained with GIFT ranged from 27% to 82%.[19,55] GIFT is a potentially valuable technique to produce pregnancies from subfertile stallions, frozen semen, and sex-sorted sperm. Recent studies in the authors' laboratory using cooled and frozen semen for GIFT resulted in pregnancy rates of 25% and 8%, respectively.[56]

16.9.2.4 METHODS FOR FERTILIZATION IN VITRO

Only two foals have been produced after in vitro fertilization of equine oocytes matured in vivo.[29,30] 1133
Increased fertilization rates have been observed following the application of ICSI in which one sperm is 1134
aspirated into a fine-bore needle and injected into a mature oocyte. The injected oocyte is activated and placed in culture to allow the completion of fertilization and cellular division in vitro. Squires, Wilson, Kato, et al.[57] reported the first successful ICSI of an equine oocyte that was maturedin vitro, and foals have been produced by ICSI using oocytes matured in vivo or in vitro.[58,59] However, pregnancy rates per transferred oocyte were low (13% and 6%, respectively). Recently, several investigators reported the in vitro production of blastocysts with higher efficiency,[32,60–62] increasing the expectations of establishing ICSI as a practical assisted reproductive technique for the horse.

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16.10

16.10—The Stallion

Juan C. Samper

16.10.1

Anatomy and Physiologyof the Stallion

A breeding stallion is often the most significant financial asset of an equine breeding operation. A variety of factors may influence the future breeding potential of a colt as it is maturing. An understanding of the anatomy and physiology of the stallion assists a veterinarian in providing optimal monitoring, diagnostic, and therapeutic services to a farm.

16.10.1.1 REPRODUCTIVE PHYSIOLOGY

16.10.1.1.1 Testicular Descent

Normal testicular descent into the scrotum occurs between the last 30 days of gestation and the first 10 1135
days post partum. In some colts the testes may descend into the inguinal region and remain there for some 1136
time before fully descending. Androgen production by the developing fetal testis probably plays an important role,[1] as may mllerian inhibiting factor.[2] Traction of the gubernaculum, which attaches the caudal pole of the testis to the inguinal region, is believed to draw the developing testicle and epididymis into the inguinal ring.[3]

Failure of the testis to descend into a normal scrotal position is termed *cryptorchidism*. The left testis is retained more commonly in stallions. One diagnoses cryptorchidism by manual palpation of scrotal contents. Rectal palpation and careful inguinal palpation may assist in identification of an abdominally or inguinally retained testis. In some instances, heavy sedation of the stallionis necessary for one to examine the area carefully. Ultrasonography has been recommended as a useful diagnostic tool for such examinations as well.[4] In horses with bilaterally retained testicles or apparent geldings with stallionlike behavior, hormonal profiles maybe useful in diagnosis of a retained testis. Testosterone levels have been suggested as a method to diagnose retained testicular tissue in an apparent gelding. A stimulation test using human chorionic gonadotropin increases the chances of detecting testosterone. For this, one injects 5000to 10,000 IU of human chorionic gonadotropin intravenously. One determines testosterone concentrations before injection and 60 to 120 minutes later. A fivefold or greater increase in hormone indicates a retained testicle. However, false negatives are possible. A single measurement of blood estrone sulfate concentration is a reliable indicator of the presence of testicular tissue, especially in colts over 3 years of age.[5–7]

16.10.1.1.2 Puberty

Puberty is defined as the age at which a colt is able to mount, copulate, and successfully impregnate a mare and occurs during the second spring after the year of birth. Puberty should not be confused with sexual maturity, which occurs after the age of 5.

Puberty is probably regulated by the reactivationof the hypothalamic pulse generator, a group of cells located in the arcuate nucleus of the hypothalamus.[1] The pulsatile secretion of gonadotropin-releasing hormone (Gn-RH) from the hypothalamus stimulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. Season, age, breed, nutritional status, and external hormones affect puberty, but in general puberty is complete by 18 to24 months in the horse.[8]

16.10.1.2 ENDOCRINOLOGY

The pineal gland plays a significant role in the seasonality of the horse. The retina captures photoperiod information and transports it via nerve fibers to the pineal gland, which in turn inhibits the production of melatonin during long days. Low levels of circulating melatonin are consistent with higher levels of Gn-RH and gonadotropins. Stallions, in contrast to mares, do not undergo a complete reproductive quiescence and

continue to produce sperm during the short photoperiodic days. The cause for this partial refractoriness of the stallion to changes in photoperiod is not well understood.

The hypothalamus, pituitary, and testes (hypothalamic-pituitary-gonadal axis) must work in synchrony fora stallion to be able to start and sustain sperm production. The primary role of the hypothalamus, locatedon the base of the brain, is the production of a10-amino-acid peptide known as Gn-RH, which is secreted in multiple daily pulses and then transported via the hypothalamic-pituitary portal system to the anterior pituitary. In addition to melatonin-mediated stimulus, the hypothalamus responds to tactile, olfactory, and visual stimuli.[9]

The pituitary, which is connected to the hypothalamus by neural fibers, has two lobes. The anterior lobe possesses Gn-RH receptors. Gonadotropin-releasing hormone binds to these receptors and induces secretion of FSH or LH. The anterior pituitary also produces prolactin, the role of which is unclear in the stallion. Follicle-stimulating hormone and LH act on the Sertoli's and Leydig's cells, respectively, stimulating production of steroids and other protein hormones. The peptide hormones inhibin and activin regulate FSH at the pituitary level. Testicular steroid hormones, mainly estradiol and testosterone, in turn have positive or negative feedback actions at the level of the pituitary on FSH and LH, respectively.

The complexity of the interaction between hormones has precluded clinicians and researchers in the field of stallion andrology from being able to develop a test or a series of hormonal tests to predict or diagnose infertility or subfertility. Until such diagnostic methods become available, hormonal supplementation is strictly empirical and potentially can worsen or elicit a reproductive dysfunction in otherwise normal stallions.[9]

16.10.1.3 TESTICULAR CELLS

The endocrine role of the testes is to produce testosterone and estrone sulfate, whereas the exocrine role is to produce spermatozoa. The testicle is composed of 85% to 90% testicular parenchyma, of which seminiferous tubules comprise 70%. In turn the seminiferous tubules are formed by Sertoli's and germinal cells. The interstitium, formed primarily by Leydig's cells and myoid cells, occupies close to 15% of the parenchyma.[10]

16.10.1.3.1 Sertoli's Cells

Sertoli's cells, also known as supportive cells, contain the most testicular receptors for FSH. Some of the 1136
most important functions of the Sertoli's cell in the process of sperm maturation include the following: 1137

1. Isolation of the advanced (haploid) stages of spermatogenesis by tight gap junctions forming the blood-testis barrier.

2. Production of androgen binding protein, activin, and inhibin. Androgen binding protein binds the bioactive form of testosterone, dihydrotestosterone, to maintain high levels of these products in the seminiferous tubules and epididymis. Activin and inhibin stimulate or suppress the release of FSH from the pituitary. The Sertoli's cells contribute to the regulation of Leydig's cell function and establish feedback mechanisms to the anterior pituitary primarily through the production of activin and inhibin. Sertoli's cells synthesize other proteins, such as SGP-2, ceruloplasmin, and transferrin, that are necessary to support spermatogenesis. Ceruloplasmin and transferrin act as carrier proteins for copper and iron, respectively, which are important regulators of spermatogenesis.

3. Germ cells begin the process of differentiation as large round cells on the basal compartment of the seminiferous tubule and approximately 55 days later finish as elongated cells in an adluminal position. This change in size, shape, and position occurs between two adjacent Sertoli's cells. The number of spermatogenic cells and ultimately the total sperm production of a given stallion are determined by the number of cells that can be accommodated between the tight gap junctions between two Sertoli's cells. Day length appears to be one of the most important factors in determining the number of Sertoli's cells per testis in adult stallions. Numbers of Sertoli's cells increase in young animals:1 billion cells at 2 years of age, 2.8 billion at 3 years, and 3.6 billion after 4 to 5 years of age. The number of Sertoli's cells then decreases with advancing age.[11]

16.10.1.3.2
Leydig's Cells

The interstitial or Leydig's cells contain most of the testicular receptors for LH and are the main site of testosterone production. Testosterone concentration in the testicular microcirculation is at least 10 times higher than that in the general circulation. Through steroid production, Leydig's cells provide the feedback mechanisms on the pituitary necessary to maintain spermatogenesis, secondary sex characteristics, and libido. Season and not age appear to affect the testosterone production in adult stallions, which is mediated through a changein total number of Leydig's cells rather than totalvolume of cells per testis. Unlike Sertoli's cells, Leydig'scell numbers do not increase dramatically with age(1.4 billion at 2 years and 4.7 billion as a mature stallion). However, a significant increase in cell volume occurs that is regulated primarily by season.[12]

16.10.1.3.3
Myoid Cells

The myoid cell has profound effects on germ cells and Leydig's cells primarily through the action of paracrine modulating factors. A paracrine modulating factor in the rat known as P-Mod-S is thought to stimulate or modulate some of the androgen binding protein functions. In addition, the myoid cells are responsible for the round architecture of the seminiferous tubule and probably for the intratesticular movement of sperm.

16.10.1.3.4
Germinal Cells

Sperm is the final product of a 57-day process that starts at the base of the seminiferous tubule. In a normal stallion, testicular volume has a direct correlation with the number of sperm that particular animal should be able to produce.[13]

16.10.1.4
SPERMATOGENESIS

Spermatogenesis is the series of chronologic changes that occur in the seminiferous tubule, transforming a large, round spermatogonia into a spermatozoa. This process in the stallion takes approximately 57 days and is not affected by frequency of ejaculation or season.[14]

The process starts when an A_1 stem cell spermatogonia undergoes mitosis giving rise to (1) a second A_1 spermatogonia to maintain a constant population of stem cells and (2) an A_2 spermatogonia. In turn, the A_2 spermatogonia gives rise to A_3, A_3 to B_1, and B_1 to B_2. The entire process of spermatogenesis has been

divided into three phases of similar length known as spermatocytogenesis, meiotic divisions, and spermiogenesis. *Spermatocytogenesis* is characterized by the mitotic divisions ofA and B spermatogonia. *Meiotic divisions* result in the formation of primary spermatocytes from B_2 spermatogonia and the subsequent formation of secondary spermatids. Leptotene spermatocytes that form immediately after the first meiotic division are hidden from the immune system by the hematotesticular barrier. *Spermiogenesis* is characterized by the transformation of round spermatogonia to elongated spermatids and ultimately to spermatozoa. During spermiogenesis, the formation of the acrosome arises from the Golgi complex and the compaction of DNA, partly because of the expression of protamine genes at the spermatid stage. The end of spermatogenesis is characterized by *spermiation*, the release of the elongated spermatids or spermatozoa into the lumen of the seminiferous tubule.[15]

16.10.1.5 SEASONALITY

Although a reduction in testicular size, testicular volume, and daily sperm production during the non–breeding season occur, the seasonal effect varies significantly between stallions and is not as significant as 1137
the effect on ovarian function in the mare. Stallions from which semen is collected year-round decrease their 1138
sperm output on the average by 1 to 2 billion during the non–breeding season. In many of these stallions libido is not affected adversely. Although the total number of sperm is affected, sperm morphology and motility remain unaltered, provided stallions are maintained on a regular collection schedule. The effect of day length on sperm production can be explained by a decrease in serum LH during the non–breeding season. This lack of stimulus to the Leydig's cell has a direct effect on intra- and extratesticular testosterone levels. Decreased testosterone concentration affects the number of Sertoli's cells and in turn the total number of germ cells that can be allocated between two Sertoli's cells.[16]

The effect of external lighting programs on spermatogenesis is uncertain. Burns and Douglas[16a] reported increased sperm production and testicular size in stallions subjected to a lighting program but observed that this improvement was transitory, and in some cases detrimental, to stallion performance at the peak of the breeding season (April to May). Other investigators have foundno detrimental effect on photostimulated stallions.[16,17] Several Thoroughbred breeding farms provide lightto their stallions before the breeding season without significant reduction in conception rates.

16.10.1.6 TESTICULAR CELL INTERACTIONS

A well-established fact is that the Sertoli's or nursecells have a direct interaction with the germ cells andthat Leydig's cells interact with Sertoli's and germinal cells through hormonal production. In addition, the myoid cell produces at least one paracrine factor that interacts directly with the Sertoli's cell. Paracrine and autocrine modulating factors, products of the peritubular cell, appear to have an effect on androgen binding protein function. In turn, the myoid cell is under regulatory influence of transforming growth factorsα (stimulatory) and β (inhibitory). Other factors involved in cell-to-cell communication of the testicular cells include collagen, plasminogen activator, vitamin A, pyruvate, and carbohydrates.[18] Most of these products and mechanisms have not been investigated extensively in the stallion.

16.10.1.7 EPIDIDYMIS

The specific absorptive and secretory functions of each segment of the stallion epididymis remains the subject of considerable debate and investigation. The histologic structure of the epididymis changes as it

continues through its different regions, with epithelial height being greatest proximally and smooth muscle components greatest distally.[19] As spermatozoa are transported from the excurrent ducts into the head, along the body, and into the tail, they undergo a number of morphologic and physiologic changes that ultimately render them motile and fertile. Specific maturational changes include (1) the capacity for progressive motility, (2) shedding of the cytoplasmic droplet, (3) plasma and acrosomal membrane alterations, (4) DNA stabilization, and (5) metabolic changes.[20] The tail of the epididymis generally serves to store the matured spermatozoa. All of these changes occur primarily at the level of the mid to distal corpus.[20,21]

Throughout the epididymis, fluid resorption occurs at a steady rate and results in a significant increase in sperm concentration.[22] Whether stallions with high-volume ejaculates and poor sperm morphology have epidydimal dysfunction remains to be investigated.

16.10.1.8 EXTERNAL GENITALIA

16.10.1.8.1 Penis and Prepuce

The penis of the stallion is composed of a root, a body, and a glans penis and is musculocavernous. The penile base arises at the ischial arch in the form of two crura that fuse distally to form the single dorsal corpus cavernosum penis enclosed by a thick tunica albuginea. The corpus cavernosum, corpus spongiosum, and corpus spongiosum glandis are the three spaces that make up the erectile tissue of the penis. Engorgement of these spaces with blood from branches of the internal and external pudendal arteries and obturator arteries is responsible for erection. The cavernous spaces within the penis are continuous with the veins responsible for drainage. The corpus spongiosum originates in the pelvic area and surrounds the penile urethra within a groove on the ventral side of the penis and forms the corpus spongiosum glandis at the distal end of the penis.[23] The corpus spongiosum glandis is responsible for the distinct bell shape of the stallion penis following ejaculation.

The urethral process is distinctly visible at the center of the glans penis and is surrounded by an invagination known as the fossa glandis. Accumulations of smegma secretions, known as “beans,” are predisposed in one or all of the diverticulae of the fossa glandis and urethral sinus. Careful examination and cleaning of this area are imperative during the reproductive evaluation of a stallion or before breeding.

The bulbospongiosus muscle located in the ventral aspect of the penis provides rhythmic contractions or pulsations to assist in moving the penile urethral contents (semen and urine) distally during ejaculation. Two retractor penis muscles also run ventrally along the length of the penis and are responsible for returning the penis to the sheath following detumescence.[24]

The prepuce is formed by a double fold of skin thatis hairless and well supplied with sebaceous and sweat glands. The prepuce functions to contain and protectthe nonerect penis. The external part of the prepuce, or 1138
sheath, begins at the scrotum and displays raphae that are continuous with the scrotal raphae. The internal 1139
layer of the prepuce extends caudally from the orifice to line the internal side of the sheath and then reflects cranially toward the orifice again before reflecting caudally to form the internal preputial fold and preputial ring. This additional internal fold allows the considerable lengthening (approximately 50%) of the penis during erection. During erection the preputial orifice is visible at the base of the penis just in front of the scrotum, and the preputial ring is visible approximately midshaft of the penis.[24]

One can best examine the penis and prepuce of a breeding stallion following teasing with an estrous mare, when one can observe the stallion drop the penis and attain a full erection.[25] The prepuce and penis shouldbe free of vesicular, proliferative, or inflammatory lesions such as those found in cases of coital exanthema, squamous cell carcinoma, or cutaneous habronemiasis. Removal of smegma accumulations may be required for a complete examination of the skin surfaces.

16.10.1.8.2 Scrotum

The scrotum is slightly pendulous and forms two distinct pouches that contain, protect, and thermoregulate the testes and epididymides. The testes are located in the scrotum to maintain testicular temperature at 3° to 5° C below the normal body temperature, which is a requirement for normal spermatogenesis.[26]

The scrotal wall consists of four layers: the skin, tunica dartos, scrotal fascia, and parietal vaginal tunic.[22,27] The scrotal skin is thin, generally hairless, and slightly oily, containing numerous sebaceous and sweat glands that assist in thermoregulation. The tunica dartos adheres to the scrotal skin and consists of muscular and fibroelastic tissue. The tunica dartos lines both scrotal pouches and extends into the median septum, which appears externally as the median rapha of the scrotum. The scrotal fascia is between the tunica dartos and parietal vaginal tunic and allows the testis and associated parietal tunic layer to move freely within the scrotum. The parietal vaginal tunic is the innermost layer and is an evagination of the parietal peritoneum through the inguinal rings that forms during testicular descent. This layer forms a sac that lines the scrotum and is apposed closely to the visceral vaginal tunic, the outer layer of the testis itself. The vaginal cavity isthe space between the parietal and visceral layers of the vaginal tunic and normally contains a small amount of viscous fluid to allow some free movement of the testis within. The vaginal cavity is a potential space within which considerable fluid may accumulate.

The scrotum of the normal stallion should appear slightly pendulous, globular, and generally symmetric. One may observe normal variations in the positioningof the testes if one testis is anterior to or ventral tothe other. The skin should have no evidence of trauma, scarring, or skin lesions. Palpation of the scrotum of a normal stallion reveals a thin and pliable covering that slides loosely and easily over the testicles and epididymides within.

16.10.1.8.3 Testicles

The testes of a normal stallion are palpable as two oval structures of nearly equal size lying horizontally within the scrotal pouches. One ascertains normal orientation of the testis by palpation of the body of the epididymis, which is always dorsolateral to the testicle proper, and the tail of the epididymis and the ligament of the tailof the epididymis, which should be in a caudal position. The ligament is palpable as a fibrous nodule 5 to 19 mm in size that attaches the tail of the epididymis to the caudal pole of the testis. The ligament is particularly large in newborn colts and on palpation may be mistaken for a testis within the scrotum. Examination of a normal stallion may identify rotation of one or both testes, upto 180 degrees. Such rotations can be permanent, or the testis may rotate back and forth, usually with the stallion showing no outward signs of discomfort. A rotated testicle may have as much as a 40% reduction in blood flow.[26] Although this condition does not interfere with normal breeding by the stallion, it may be considered a criterion for failing a stallion during a breeding soundness examination in some breed regulations. One should note the presence of the condition clearly on the record of the stallion and communicate such

clearly to the owner. One must differentiate testis rotation from true testicular torsion in which stallions demonstrate signsof colic and for which palpation reveals a painful and swollen testicle.

Within the scrotum the testis is encapsulated by the tunica albuginea, a layer of tough collagenous tissue and smooth muscle that sends supportive trabeculae into the testicular parenchyma, dividing the testis into lobules. The muscular content of the tunica albuginea is thought to play a role in intratesticular sperm transport and determination of testicular tone.[22]

Testicular tone is described as the degree of turgidity of the testicle, which should be firm to turgid but resilient on palpation. Deviation from the normal toward a softer or firmer testis may be associated with degenerative, neoplastic, or traumatic conditions of the testis. Testicular degeneration is an acquired reversible or irreversible condition in which damage to the germinal epithelium results in eventual atrophy of the epithelium and an initial loss of testicular tone. As the disease progresses, the degenerating testicle becomes small and firm as fibrous tissue replaces testicular parenchyma. Because testicular conditions 1139
may afflict only one testis, comparison of the size and consistency of the two testes of any individual 1140
stallion is imperative. One can determine changes in testicular tone or consistency best by sequential examinations of the stallion that allow the clinician to monitor the severity and rate of change as the disease progresses. Regular physical examinations of breeding stallions are an important part of routine stallion management and may allow early detection of problems that may affect fertility.

16.10.1.8.4 Testicular Size and Volume

Testicular size in stallions increases from the pubertal period to reach maximal size at the age of 5 to 6 years and is affected by breed, season, and age. Each testisof an adult stallion weighs between 150 and 300 g and measures 50 to 80 mm in width, 60 to 70 mm in height, and 80 to 140 mm in length, with breed being the biggest factor in determining size. Testicular volume correlates highly with daily sperm production and therefore is a useful predictor of the sperm production potential of a stallion.[28]

To calculate the testicular volume, one should obtain measurements of the length, width, and height of each testicle by calipers or ultrasound. Ultrasonographic measurements may be more accurate, although proper placement of the probe across the testis to ensure one obtains a cross-sectional image is critical. Because a testis approximates the shape of an ellipsoid, the following formula converts length, width, and height measurements into testicular volume:

$$\text{Testis volume} = \tfrac{4}{3}\pi(\text{length}/2)(\text{width}/2)(\text{height}/2)$$

or,

$$\text{Testis volume} = 0.5333 \times H \times L \times W$$

Love, Garcia, Riera, et al.[28] also recommend using this volume to predict the expected daily sperm output (DSO) of the stallion, using the following formula:

$$\text{Predicted DSO} = [0.024 \times (\text{volume of left} + \text{volume of right})] - 0.76$$

One can compare predicted daily sperm output with actual daily sperm output as estimated by semen collection during the routine breeding soundness examination. A stallion in which actual daily sperm

output falls below that predicted for his testicular size requires further evaluation for disease conditions of the testes, epididymides, and accessory glands.

Testicular measurements are a useful and important part of the physical examination of any breeding stallion and can be used to predict sperm output and determine the size of the book for a given stallion. A stallion with small testicles will have lower sperm production and may need modification of the management strategies that result in optimization of the fertility of such a stallion.

16.10.1.8.5 Epididymides and Excurrent Duct System

The epididymis is a single, highly convoluted duct approximately 70 m in length that has a grossly distinct head, body, and tail. The head of the epididymis is a flattened structure that lies dorsomedially along the cranial border of the testis and is attached closely to the testis. The body, or corpus, lies along the dorsolateral aspect of each testis and continues as the tail, or cauda, the large, prominent structure attached to the caudal pole of the testis. The deferent duct, the excretory duct for sperm, attaches to the tail of the corresponding epididymis, runs along the medial aspect of the testis, and ascends via the spermatic cord through the vaginal ring into the pelvis. Each deferent duct widens into its corresponding ampullary region and eventually terminates at the colliculus seminalis of the pelvic urethra. The colliculus seminalis is a rounded prominence situated on the dorsomedial wall of the urethra about 5 cm caudal to the urethral opening from the bladder. The colliculus is the common opening to the ampullae and the seminal vesicles. With care one can palpate all sections of the epididymis through the scrotal wall. However, the head of the epididymis may be difficult to ascertain because of its flattened nature and the close apposition of the cremaster muscle overlying it.[29]

16.10.1.8.6 Spermatic Cord

Each spermatic cord is enveloped in the parietal layer of the vaginal tunic, which extends distally from the internal inguinal ring. Within each cord are the corresponding deferent duct, testicular artery, testicular veins, lymphatic vessels, and nerves. The cremaster muscle is situated in the caudolateral borders of each spermatic cord. The testicular artery, a branch of the abdominal aorta, descends through the inguinal ring into the cranial border of the spermatic cord in a tortuous manner and divides near the testis into several branches to supply the testis and epididymis. These small branches, embedded in the tunica albuginea, enter the parenchyma via the trabeculae and septae of the testis. A corresponding network of veins leaves the testis and surrounds the testicular artery in a tortuous manner, forming the pampiniform plexus. This arrangement of artery and veins is responsible for much of the thermoregulation of the testis in the stallion when heat from the testicular arterial blood is transferred to the venous side, resulting in testicular arterial blood being several degrees cooler than systemic blood temperature. Abnormal distention of the veins of the pampiniform plexus is termed a *varicocele* and is an uncommon condition in stallions. Palpation of the spermatic cord of an affected stallion reveals the dilated and often tortuous vessels. Varicoceles are usually not painful but can result in fluid accumulation around the vaginal tunics, most often involve only one side 1140
of the spermatic cord, and most often can diagnosed by the observation of the dilation of the vessels from 1141
the pampiniform plexus with ultrasonography. The condition has been identified in stallions with normal semen parameters.[29]

16.10.1.8.7 Ultrasonographic Examinationof the Testicles and Epididymis

Ultrasound examination of the testis and epididymis is a useful ancillary diagnostic tool that enables the clinician to assess palpable changes and to identify nonpalpable changes. Ultrasound is particularly useful in cases of generalized scrotal enlargement in which specific structures become difficult to palpate.

Examination is usually easier following semen collection when the stallion is relaxed. One uses a 5.0-, 7.5-, or 10.0-MHz linear array transducer.[30] One usually beings the examination at the cranial end of the testis and slowly moves the probe caudally in a vertical position. Visualization of the scrotum reveals a thin, echogenic, uniform layer. Minimal, if any fluid is visible between the scrotal skin and testicular parenchyma in the normal stallion. In the cranial third of the scrotum, the head of the epididymis, testicular parenchyma, blood vessels of the spermatic cord, and central vein are visible. As one moves the probe caudally, the central vein and spermatic cord vessels disappear and the head of the epididymis continues into the body of the epididymis. The head and body of the epididymis appear as heterogeneous areas just below the spermatic cord when the probe is positioned as described. As the probe continues further caudally, the body ofthe epididymis becomes indistinct.[31] With the exceptionof the central vein, the testicular parenchyma appears uniformly echogenic and homogenous. The central vein appears as a small anechoic area within the testicular parenchyma at the cranial third of the testis and should not be mistaken for a pathologic lesion. Dilation of the central vein may be visible in cases of varicocele or spermatic cord torsions and usually is accompanied by detectable dilations of the vessels of the spermatic cord. Well-defined and hypoechoic lesions within the parenchyma suggest testicular tumors.

When one reaches the most caudal aspect of the testis, one rotates the probe to face cranially in a vertical position to allow examination of the tail of the epididymis. This structure appears as a heterogeneous area, described as having a Swiss cheese–like appearance. Identification of the epididymal tail may assist in diagnosis of testicular rotations. In cases of 360-degree torsions, the tail of the epididymis, although in its caudal position, is more dorsal because of the tension on the ligament of the tail of the epididymis by the deferent duct.

One can visualize the spermatic cord most easilyby placing the probe horizontally across the cord, just proximal to the body of the testis. The arrangementof the pampiniform plexus results in the mottled, heterogeneous appearance of the spermatic cord, and the testicular artery and veins are identifiable in cross-sectional images.[31]

16.10.1.9 INTERNAL GENITALIA

16.10.1.9.1 Accesory Sex Glands

The bulbourethral glands, prostate gland, and seminal vesicles collectively are referred to as the accessory sex glands. Their secretions produce the seminal plasma that comprises most of the ejaculate volume. The ampullae, which are dilations of the vas deferens before openingin the colliculus seminalis, are considered a storage place for sperm.

Although a short exposure to seminal plasma appears to be important for sperm function, long-term exposure to seminal plasma components may be detrimental to spermatozoa survival for some stallions. Artificial insemination programs deal with this potential detrimental effect by dilution of semen with

extenders in fresh or fresh-chilled programs and by centrifugation to remove seminal plasma in frozen semen programs and some chilled-shipped programs. Seminal plasma appears to suppress the inflammatory response of the endometrium of the mare to sperm following insemination or natural mating. Although the functions of the specific components of the seminal plasma remain rather obscure, the fluid suspends the ejaculated sperm and also is thought to be a source of energy, protein, and other macromolecules required for sperm functions and metabolism.[32–34]

16.10.1.9.2 Examination and Ultrasonographyof the Accessory Sex Glands

In some cases the reproductive examination of stallions should include rectal palpation and ultrasonography of the accessory sex glands.[35,36] Most stallions tolerate this procedure well with adequate restraint in stocks, and tranquilization is not usually necessary. Glands on sexually stimulated stallions are easier to palpate and to visualize.

16.10.1.9.3 Bulbourethral Glands

The bulbourethral glands, although not usually palpable per rectum because of the urethralis and bulboglandularis muscles close to the ischiatic arch, are easy to evaluateby ultrasonography. Multiple ductules from the bulbourethral glands enter the medial aspect of the urethra distal to the prostatic ductules. Bulbourethral gland secretions comprise most of the presperm or first fraction of the ejaculate and serve as a cleanser and pH stabilizer in the urethra before ejaculation. Using ultrasonography, one locates the bulbourethral glands 3 to 4 cm insidethe anus off the midline, and in a stimulated stallion, the 1141
glands appear as two distinct ovoid structures with multiple small hypoechoic spaces throughout the 1142
parenchyma.[35]

16.10.1.9.4 Prostate Gland

In the stallion the prostate is formed by a central isthmus and two lateral lobes located on the caudolateral borders of each vesicular gland. Although not always palpable per rectum, the prostate is lobulated or nodular and firm, distinguishing it from the smooth, thin-walled vesicular glands lying next to it. Each prostatic lobe measures5 to 9 cm long, 2 to 6 cm wide, and 1 to 2 cm thick. Multiple ductules from the prostate enter the lumen of the urethra lateral to the colliculus seminalis. The secretions of the prostate contribute to the sperm-rich fraction of the ejaculate. The lobes of the prostate are easily identifiable with ultrasonography with the two symmetric and homogeneously echogenic lobes distinctly visible lateral to the area in which the penile urethra merges with the neck of the bladder. Hypoechoic dilations within the gland parenchyma of each lobe are evidentin a teased stallion.[35]

16.10.1.9.5 Ampullae

The ampullae are the enlarged distal portions of the deferent ducts measuring 1 to 2 cm in diameter and 10 to 25 cm in length. Palpable along the midline of the pelvic floor over the neck of the bladder, they converge caudally and pass beneath the prostate gland but lie dorsal to the pelvic urethra. At their distal ends they continue through the wall of the urethra, opening into the colliculus seminalis alongside the excretory ducts of the seminal vesicles. The ampullae, in addition to serving as a sperm storage area, have many branched tubular glands located within the thickened wall.[36]

Because of the longitudinal orientation of the ampullae, sometimes they are easier to find on rectal palpation. One can identify them by ultrasonography by their hypoechoic central lumen surrounded by a uniformly echogenic wall and a hyperechogenic outer muscular layer. Orienting the transducer in a transverse position inside the rectum can provide a good cross-sectional image of the ampullae. The ampullae can be a common site for blockage because of sperm stasis. In these cases, dilation of the lumen may or may not be visible. Stallions with such blockage usually have a history of infertility or subfertility and often display severe oligospermia or, in severe cases, complete azoospermia. When sperm are present, they have a variety of morphologic abnormalities with predominantly tailless heads; in some instances the ampullae are palpably enlarged. This condition may render a stallion virtually infertile if undiagnosed. Recommendations for treatment include ampullary massage per rectum and repeated daily semen collection following injection of low doses of oxytocin or prostaglandin.[37]

16.10.1.9.6 Seminal Vesicles

The seminal vesicles or vesicular glands are paired, pyriform, and thin-walled structures lying lateral to the ampullae. On occasion, they may extend far craniallyto hang over the brim of the pelvis. Sexual stimulation results in dilation and elongation of the vesicularglands, up to 12 to 20 cm long and 5 cm in diameter. The distal ends of the glands converge, passing under the prostate as they lie parallel the ampullae toward their termination at the urethra. The excurrent ducts of the vesicular glands open lateral to the excurrent ducts ofthe ampullae at the colliculus seminalis of the urethra. Secretions of the vesicular glands comprise the gel fraction of the ejaculate. Higher gel volumes are collectable with pronounced sexual stimulation and season. The specific function of the gel fraction is unclear, and one should remove it when processing semen for evaluation or artificial insemination. Palpation of the vesicular glands may be easier following considerable teasing of the stallion with an estrous mare. The glands also are readily palpable in instances of pathologic enlargement.

With ultrasonography the vesicular glands appear in longitudinal section as flattened oval to triangular sacs, depending on the degree of sexual stimulation. A thin echogenic wall surrounds a generally uniformly anechoic lumen.[35] Increased echogenicity of vesicular gland fluid is associated with the highly viscous gel fraction produced by some stallions. The seminal vesicles are the glands that are most prone to bacterial infections. Diagnosis is based on the cytologic evaluation of the semen with presence of white blood cells.

16.10.1.9.7 Evaluation of the Pelvic and Penile Urethra

Although one can evaluate the pelvic urethra by transrectal ultrasonography, in most cases such evaluation is unrewarding. Endoscopic examination provides better information of the anatomic integrity of the urethra and its accessory structures. One performs the procedure by gently passing a 1-m endoscope with an outer diameter of 8 to 9 mm into the urethra of a sedated horse, so that the penis is relaxed. One applies gentle and constant pressure so as to pass 70 to 80 cm of the endoscope into the urethra.[38,39] One must take care not to inflate the bladder with too much air because of a slight risk of rupturing the bladder.

The bulbourethral gland ductules are grouped about 2.5 to 3 cm distal to the prostatic openings and are visible as two rows of 6 to 10 small openings dorsal and close to the midline. The prostatic ductules are arranged in a similar way to the bulbourethral and visible as two groups of small openings lateral to the ejaculatory orifices about 5 cm deeper. Just ventral and cranial to the colliculus, the openings of the 1142

urethral glands are visible laterally on the widened pelvic portion of the urethra, at the level of the prostatic gland openings.[38] 1143

One can identify the colliculus seminalis as a rounded prominent structure found on the medial aspect of the dorsal wall of the urethra approximately 5 cm caudal to the internal opening of the urethra from the bladder. On either side of the colliculus is an ejaculatory duct orifice, a small slitlike diverticulum within which the ampullary ducts and ducts of the seminal vesicle open. By passing the endoscope into this orifice, one can visualize and evaluate the seminal vesicles.[38,39] One can take samples for culture with endoscopic culturettes if one suspects seminal vesiculitis.[40]

Endoscopic examination of the urethra is indicated in cases of hemospermia or in cases in which one suspects a pathologic condition of the accessory sex gland. In cases of hemospermia, one may visualize the bleeding area with the endoscope. One can identify these lesions most readily in the region of the ischiatic arch and distal urethra. One should take care to assess the urethral mucosa as the endoscope is passed forward, because some irritation and erythema of the mucosal lining often results from the endoscopic examination.[40] A false diagnosis of urethritis may result if one assesses the mucosa while withdrawing the endoscope.

16.10.2 Evaluation of the Breeding Stallion

Equids in the wild are considered to be long-day seasonal breeders that live in a stable social group or harem. Free-running stallions interact with a female for hours or even days before copulation. In many management situations the domesticated stallion is restricted severely from its sociosexual activity. In general, breeding stallions are confined to a paddock or a box stall and do not have social interactions with other horses. In addition, mating and/or ejaculation often is permitted only under two conditions: *Hand mating* at the convenience of the farm manager, allowing only a few minutes for stallion and mare interaction, or *mounting of a mare or a phantom* for artificial insemination purposes.

Perhaps the most remarkable difference in the breeding pattern, particularly with performance horses such as Thoroughbreds and Standardbreds, is the fact that most breedings are done during February to June, well in advance of the natural breeding season (May to September).

16.10.2.1 BEHAVIOR

16.10.2.1.1 Normal Behavior

Stallions display several behavioral responses during teasing and breeding. However, the intensity of the response, also known as *libido*, and the type of response depend greatly on breeding experience, management, and in some cases, season. Olfactory, visual, and auditory stimuli also influence libido. Typically, a normal stallion that has never bred a mare takes a longer time to mount but displays good libido. However, a stallion that has had a negative previous experience might show no interestin the mare or in mounting. Some of the typical normal responses by stallions when exposed to an estrous mare include vocalization, flehmen response, striking, nipping/biting, and sniffing/licking. A normal stallion should show interest in the mare and drop the penis within 1 to 2 minutes of exposure to a quiet mare in standing heat and should try to mount within the first 3 minutes. Once stallions are allowed to mount, they give several (fiveto eight) intravaginal thrusts, followed by three to five short thrusts immediately before

ejaculation. Signs of ejaculation are rhythmic and frequent urethral pulsation, flagging of the tail, and a head relaxation. A single stallion tends to be consistent in its breeding behavior, provided that the conditions under which he usually breeds are the same.[41]

16.10.2.1.2
Abnormal Behavior

Stallion behavioral dysfunction in many instances is difficult to define and is relative to the expectations of the breeding manager. A stallion that takes 30 minutes or more to mount and ejaculate or takes several mounts may be considered a problem in some intensive management situations. However, a stallion that takes several hours to achieve an erection, mount, and ejaculate may be considered normal if he only breeds two or three mares during the entire breeding season in pasture conditions.

A review of the incidence of problems in 250 stallions over a period of 5 years indicated that more than 50% of the cases had complaints related to poor libido or excessive aggressiveness. Of those, nearly half were described in stallions with no previous sexual experience. The rest were divided evenly between experienced stallions with low sexual interest and unruly and overly aggressive breeders. Mounting and erection dysfunction accountedfor 11% of complaints, whereas ejaculatory problems accounted for 25% of the total cases. Other problems, such as self-mutilation and severe stereotypies that reportedly could be detrimental to fertility, accounted for 11% of the reported cases.[42]

16.10.2.1.3
Diagnosis of Abnormal Behavior

A normal stallion exposed to a mare in standing estrus should vocalize, sniff or nuzzle the mare, display the flehmen response by curling his upper lip, drop his penis, and achieve an erection within the first 3 minutes after initial exposure.[43] These precopulatory responses should be followed by mounting, 1143
intromission, and ejaculation. A normal, experienced stallion that is hand bred should require no more than 1144
5 minutes from initial exposure to a mare until ejaculation. The frequency and intensity of the precopulatory responses is affected by management, breeding experience, and external stimuli. Stallions with no previous breeding experience are expected to be slower in mounting. However, interest in the mare and time to erection should be within normal limits. Once the novice stallion mounts, he soon gains confidence and ejaculation should occur following a normal thrusting pattern. After the first positive experience, time to mounting and to ejaculation should decrease. One must treat novice stallions with patience, positive reinforcement, and perseverance. During this time, unnecessary punishment and rough handling can aggravate a problem and may result in profound breeding disinterest. One should investigate any aberration in courtship or copulatory behavior carefully, always keeping in mind that sexual behavioral dysfunction is a problem with many possible causes involving management and the endocrine, cardiovascular, musculoskeletal, and nervous systems.[44]

16.10.2.1.4
Lack of Libido

Libido, defined as sexual drive or interest in breeding, is high in most stallions. Sexual stimuli and environmental factors profoundly affect libido in stallions. One may observe low libido in improperly stimulated stallions. The best stimulus is a mare in standing heat. If this is not possible, one may use an estrogenized ovariectomized mare. Some stallions might have preference or aversion for a particular color or type of mare.[44] Experienced stallions frequently are aroused by exposure to a breeding phantom. One

should not expect a novice stallion or one that has never mounted a breeding phantom to be stimulated positively by a dummy.

One may observe lack of libido in experienced stallions toward the end of the breeding season, particularly in heavily used or overused animals. One can correct this problem easily by decreasing the frequency of service or collection.

Stallions that have been kicked by mares or negatively reinforced for displaying sexual behavior in shows or while performing at the track may have reduced sexual desire. Unfortunately, circulating levels of steroids or gonadotrophins are often poor predictors of libido.

Treatment of a stallion with low sexual drive is directed best at correction of the underlying problem. However, assessment of the nature of the problem frequently is difficult. One may try several alternatives, such as the following:

1. Change of stimulus mare or environment.
2. Breeding or collecting another stallion in the presence of the low libido animal.
3. Intravenous administration of Gn-RH, 50 μg, 2 hours and 1 hour before breeding or of LH (human chorionic gonadotropin), 5000 to 10,000 IU, 1 hour before breeding.
4. Single injection of a short-acting testosterone.
5. Intravenous administration of diazepam at a dose of 0.05 mg/kg (maximum 20 mg) 10 to 15 minutes before breeding to reverse mild shyness in some stallions.[45]

The efficacy of most of these treatments is empirical and awaits further investigation; however, chronic administration of steroids, particularly androgens, is well documented to affect spermatogenesis negatively.[46] Therefore injection of stallions with exogenous steroids and particularly androgens to improve libido is not a recommended practice.

16.10.2.1.5 Erection Failure

The inability of a stallion to develop and maintain a normal erection despite normal libido suggests an anatomic rather than a psychogenic problem. The most common problems are vascular damages associated with traumatic injuries or neurologic problems associated with other penile or lumbosacral compromise.[47] Therapy of either problem, extrapolated from the human literature, may be medical with injection of vasoactive drugs directly into the corpus cavernosum or surgical with penile implants. To date, no reports indicate either procedure has been used in horses. No reports of dose or efficacy exist to support the use of Viagra in these stallions; anecdotal reports of its use suggest that results are inconsistent.

16.10.2.1.6 Ejaculatory Dysfunction

Some stallions show normal precopulatory behavior, mount, and copulate but fail to ejaculate. These stallions often attempt to ejaculate and may become exhausted or frustrated, becoming aggressive with the mare or handler. Before attempting to treat an ejaculatory dysfunction, one must examine the horse for evidence of degenerative joint disease in the hocks, spine, vertebrae, and pelvis and for lesions or

malformations in the hoof or foot abscesses. Recently, circulatory problems leading to iliac thrombosis have been reported to be associated with ejaculatory dysfunction.[48] Although difficult to diagnose, one always should consider psychologic problems that can lead to ejaculatory dysfunction when no organic causes can account for the problem.[47] Often typical behavior provides hints to the clinician about the psychogenic nature of the dysfunction. In most instances, psychogenic ejaculatory dysfunction results from traumatic accidents associated with breeding. If the role of musculoskeletal pain in ejaculatory dysfunction is uncertain, one may treat the horse with 1 g of phenylbutazone every 12 hours for
approximately 2 weeks. If the stallion refuses to ejaculate only under specific circumstances such as into 1144
an artificial vagina or when breeding a mare, the systematic approach of a patient, knowledgeable, and 1145
creative person is important to determine the problem. A variety of behavioral and managerial aids have been used to assist the stallions toward ejaculation.[49] One should adapt these aids according to the physical condition of the stallion, that is, a stallion that cannot achieve a full erection, stallions with difficulty mounting, or stallions that refuse to ejaculate after normal erection, mounting, thrusting, and belling of the glans. Neither mounting nor full erection are necessary for ejaculation. Stallions with difficulty mounting can be taught to ejaculate on the ground by stimulating the penis manually or with an artificial vagina. Stallions with erection problems ejaculate, provided that proper stimulation is given to the penis. One can achieve proper stimulation to the penis by raising the temperature of the artificial vagina or by applying hot towels to the base of the penis during thrusting. One also should consider changes in footing and surroundings, stimulus mare, handler, etc. before implementing pharmacologic therapy.[50]

Therapeutic regimes for ejaculatory dysfunction are empirical and include those already mentioned for the treatment of low libido stallions. In addition prosta-glandins, oxytocin, and xylazine have been used to aid stallions in the process of ejaculation. Oxytocin and prostaglandin also have been used to treat azoospermia caused by ampullary blockage.[51] The tricyclic antidepressant imipramine has been used orally to lower the threshold for ejaculation in stallions.[52] The doses for these products are discussed next.

16.10.2.1.7 Other Behavioral Problems

In addition to the previously described problems, stallions may have other abnormal behavior and vices that could eventually limit their fertility. These problems include overaggressiveness or stereotypies such as weaving, cribbing, wall kicking, and stomping.

Overaggressiveness is managed best by a good stallion handler and patience; in most cases the problems can be corrected. Stable vices often can be solved or somewhat alleviated by introducing a toy (rubber tire) or a companion animal (goat or sheep) into the stall with the stallion.

One of the most complex behavioral syndromes observed in stallions is self-mutilation. Although purely speculative, this problem has been proposed to have a genetic component. The stallion compulsively nips or bites the chest, shoulder, or flank or aggressively kicks the walls. Although self-mutilation is limited to postpubertal horses, it is not limited to confined animals. In some animals the problem is exacerbated on presentation of a mare to a confined stallion or breeding in the presence of another stallion.[53] The compulsive behavior seems to be more dramatic during the breeding season. Self-mutilation may be a problem exacerbated by olfactory stimuli. In most horses this behavior is triggered by smelling their own manure. The stallions in many instances recognize themselves as a threat, triggering the compulsive behavior. One must ensure that the horse does not develop the behavior out of frustration because of chronic pain such as an inguinal testicle or chronic gastrointestinal ulcers.

Therapy of this complex syndrome includes regular exercise, stall toys, or companion animals. One can use products to reduce the olfactory sense along with a reduction in the level of energy in the diet. Treatment with L-tryptophan in the grain also may be helpful for some horses. Physical restraint such as head cradles or muzzles most likely will lead to development of an alternate self-mutilating technique.[53] In extreme, inhumane, and refractory cases, castration of the stallion has eliminated the problem.

16.10.2.2 SEMEN COLLECTION

In addition to appropriate libido and behavior, a stallion must have good mating ability and be able to deliver an ejaculate. Mating ability and semen quality may be influenced by hereditary or environmental factors or learned patterns that are influenced greatly by management. An integral part of the diagnostic workup on a stallion with known or suspected infertility is the collection of semen. The collection process is critical because improper technique may result in poor fertility or inferior semen quality.

16.10.2.2.1 Semen Collection Area

The area used for semen collection should be spacious, dust free, clean, and free of distracting noises, animals, and person. The size of the breeding shed should be designed with awareness for the space needed for animal and human safety in the event of an uncooperative mount mare or unruly stallion. Stallions with low libido or reluctance to mount frequently are encouraged to mount a mare in estrus if the mare can be walked slowly forward or led in a large circle. There should be adequate space to permit safe handling of the stallion and mare. Additionally, the footing surface should afford the stallion good traction even when the flooring is wet.[54] Many stallions paw, strike, or kick out while teasing a mare, being washed, or after dismounting. One should remove loose dirt, stone dust, and shavings because some stallions paw debris and dust onto the washed, damp penis just before mounting. If the collection area is dusty, the area should be wetted regularly.

Collection of semen in an outside area is acceptablein most cases but on occasion may compromise semen collection because of distractions by other animals, persons, and vehicles. Ambient temperature also may 1145
have a great effect because it alters the rate at which the temperature of the artificial vagina declines during 1146
cold weather or adversely effects semen quality during hot weather. Semen collection in an outside, grassy area affords the stallion, mare, and handlers the best footing; is usually free of dust; and allows for plenty of space for safety. The distance from the semen collection area to the laboratory should be minimal.

16.10.2.2.2 Semen Collection Techniques

One can collect semen from stallions by using natural breeding with a condom; pharmacologic stimulation of ejaculation; manual manipulation of the penis; or an artificial vagina on the ground or on a mount. Under certain circumstances, one may find it necessary to use any one of these methods. However, for routine collection of semen for commercial use, an artificial vagina (AV) or manual stimulation of the penis of the stallion are the methods of choice.[52,55]

16.10.2.2.2.1

Condom

One fits the stallion with a latex condom and allows him to breed the mare naturally. Immediately after ejaculation and when the stallion has withdrawn the penis from the vagina, one retrieves the condom. Semen collected using the condom method is contaminated heavily by bacteria and debris. This method also requires that a mount mare be in estrus and increases the riskof mare contamination of the penis by vaginal entry, urination, and defecation during natural breeding. Many stallions do not tolerate breeding while wearing a condom. Condom and semen loss are also common. However, a stallion accustomed to natural service occasionallymay be intolerant of semen collection with an AV until adequately trained for this method of breeding.

16.10.2.2.2.2

Pharmacologically Induced Ejaculation

Numerous schemes have been published for the ex copula ejaculation of stallions using xylazine, imipramine, xylazine and imipramine, and prostaglandin.[42,56–58] Semen collected in this fashion is of low volume and high concentration. One can use the resulting ejaculate for cryopreservation or artificial insemination of mares in a cooled semen shipment program. Fertility with the fresh, cooled semen is normal. However, the inability to obtain ejaculates on a predictable schedule limits the commercial usefulness of these methods. In experimental ponies, semen was collected in 10 of 24 attempts using imipramine and xylazine.[56] Under selected cases of physical inability of the stallion to mount and copulate, one possibly may obtain semen specimens with the aid of pharmacologic agents. Under farm conditions, one obtains semen in 25% to 30% of the attempts. Keeping the stallion quiet and undisturbed is important. One should give intravenous treatment quietly. One such successful schemeis to administer 2.0 mg/kg imipramine hydrochloride intravenously. If the drug does not induce erection and ejaculation within 10 to 15 minutes, one administers xylazine intravenously at the rate of 0.2 to 0.3 mg/kg. With imipramine and xylazine, ejaculation occurs in association with erection and masturbation. If one uses xylazine alone to induce ejaculation, masturbation and erection do not occur in association with ejaculation. Ejaculation usually occurs as the stallion enters a period of sedation or when he is recovering from the sedation. This method of semen collection was used in a cooled, shipped semen program for a stallion with severe tenosynovitis of a rear leg.[57] Although successful about 25% of the time, the procedure was time consuming and unpredictable for mare owners. Success rate may increase if the dosages are altered for individual stallions.

16.10.2.2.2.3

Manual Manipulation of the Penis

Ejaculates collectedby manual manipulation of the penis are similar to ejaculates collected in an AV. This method of collection has not received widespread acceptance because of the training and dexterity required by the person collecting semen from the horse. Many stallions fail to ejaculate unless trained for this method of collection.[49,55] A major advantage of this method of collection is that only one or two individuals are necessary for semen collection. The stallion is usually not in direct contact with a teaser mare. Specialized equipment or facilities are not necessary for semen collection by the manual stimulation method.

With manual stimulation of the glans penis for semen collection, the stallion remains standing on the ground or is trained to mount a phantom. The stallion may be trained for collection in his stall, an open

barn aisle, or a corner of the breeding shed. An estrous mare is usually nearby, but mare stimulation for the stallion may need to be altered based on stallion response. The horse is teased until erection occurs. The operator washes the penis of the stallion with warm water. Once full erection is achieved, the operator places a plastic sleeve or bag over the penis. The operator uses one hand to cup and stimulate the glans penis to achieve favorable thrusting and glans engorgement by the stallion. The operator uses the other to stimulate the base of the penis and urethra. The operator sometimes places a warm towel at the baseof the penis to increase stimulation. Training a stallion for this method of collection may require considerable patience, whereas other stallions readily accept the procedure. Stallions trained for this method of semen collection become habituated to the routine of sights, sounds, and activities surrounding semen collection. These stallions may require little stimulation by a mare.

16.10.2.2.2.4 Ground Collection

Ground collection may be particularly beneficial in stallions with tarsal arthritis, rear fetlock or tendon injury, laminitis, or hindlimb weakness associated with neurologic disease. The need for an estrous mare 1146
usually is eliminated, risk of injury to the horse by the mare is prevented, and one less handler is needed. 1147
This method of semen collection has been most useful on small farms that stand a stallion for artificial insemination and do not have adequate personnel and facilities for mare and stallion handling and collection.[54]

Collection can be done in the breeding shed or barn aisle or in the stall. One exposes the stallion to another horse that can stimulate the stallion to achieve an erection. The teaser animal may be free in a stall or 5 to 10 m away, being held on a lead shank. One washes the penis of the stallion with clear, warm water. With the stallion positioned against a smooth wall to prevent lateral movement or in front of a solid wall to prevent his forward movement, one places the warm, lubricated AV on the erect penis and encourages the stallion to search and thrust into the AV. Once the stallion has engaged the AV, the collector uses the right hand to stimulate additional urethral pulsations while holding the AV against the abdomen of the stallion with the left hand. The stallion handler may help support the stallion by pushing against the shoulder of the stallion with the right hand. For safety reasons, the person collecting the semen always should maintain shoulder contact with the stallion.

Stallions may stand on their rear legs or walk forward slowly while ejaculating or continue to stand with all four feet on the ground. The handler should not discourage the horse from walking forward or standing up. Once horses are trained to the procedure, they usually stand flat-footed with arched back and a head-down posture. At first application of the AV to the standing stallion, a few stallions may kick out or want to nip or bite at the handler. The veterinarian should inform the handler of the stallion and mare of likely responses before initiating this method of semen collection. After a successful collection, one repeats the procedure in 1 to 2 days, preferably in the same location with the same handler and collection person. A lightweight model of AV is recommended for this procedure.

The author has had good success by placing the chest of the stallion against a phantom when a stallion is not trained to mount. The thrusting into the AV in most cases results in the stallion elevating his front quarters, resulting in the collection on the mount.

16.10.2.2.2.5 Artificial Vagina

Semen collection using an AV is the most widely used method of semen collection from stallions. Many models of equine AVs are available. The AV is fitted with a water jacket that allows for the passive

control of the internal temperature of the liner, usually at 44° to 48° C. In most cases, one can modify the internal diameter of the AV by the addition of water or air to the water jacket. One adds a lubricant manually to the innermost liner of the AV to alter the degree of friction during breeding. One should avoid lubricants containing bacteriostatic or spermicidal compounds because they are likely to be detrimental to sperm motility and fertility.[59] One may use Vaseline or petroleum jelly safely. One can modify most commercially available AVs to allow incorporation of a filter into the semen collection system, if desired, so that one can remove dirt, debris, and gel from the semen sample. Otherwise, one can filter the entire ejaculate after collection or can aspirate the gel from the sample using a syringe. Most sperm losses during collection are accounted for by the filter and in the gel fraction of semen. Twenty-five percent to 30% of sperm in an ejaculate can be lost in the gel and filter. Types of filters one can use are paper, polyester, or nylon, with paper retaining the most sperm.[60]

Ideally, the AV should be constructed to maintain the desired temperature for a significant period of time, allow the direct ejaculation into the semen receptacle, and allow for ease of handling and manipulation by the operator. If the AV is large and heavy, the operator may have difficulty positioning the AV for tall stallions or holding it in place in cases where the mount mare moves during collection. For the collector to be able to hold the AV in one hand at the appropriate position while using the other hand to deflect the base of the penis to the side of the phantom or mount mare is best. The arrangement is particularly helpful in stallions that thrust with significant force. Deflecting or stabilizing the base of the penis is stimulatory to most stallions and may help prevent penile accidents during collection.

Semen collection failures frequently are associated with inappropriate AV positioning for the particular stallion; an AV that has dropped in temperature below a critical point for the stallion; and the use of excess pressure in the AV. One should hold the AV parallel to the ventral abdomen of the stallion and directly aligned with the base of the penis. In this manner one avoids ventral or lateral bending of the penile shaft. In addition, one must ensure that the forceful thrusting of the stallion does not result in the forward movement of the AV, for this will result in the stallion searching for the end of the AV and in most cases failing to ejaculate. In certain circumstances, stallions having difficulty ejaculating into theAV necessitate elevating the internal temperature of theAV to 50° C. However, one should make an effort to have the horse ejaculate directly into the semen receptacle or coned portion of the AV liner to avoid heat shock to the sperm. Sperm cells exposed to excess heat from the AV liner exhibit a circling type of motility, have reduced sperm longevity in raw and extended semen, and may be rendered infertile. Exposure of semen to elevated temperatures for as little as 10 to 20 seconds is sufficient to cause heat shock damage.

16.10.2.2.2.6

Selection of an Artificial Vagina

All AVs used for semen collection from stallions are basically similar in that they have a water jacket 1147
that allows variation in the internal temperature and pressure of the AV liner. The specific 1148
characteristics of individual AV types vary in the overall length of the AV, its diameter, ease of filling the water jacket, ease of handling, weight of the AV, and location of ejaculation within the AV by the stallion. Commonly used AV models include the Missouri, Colorado, Hanover, Nishikawa, HarVet, and Polish models.[43,54]

16.10.2.2.2.7

Missouri.

The Missouri AV is used commonly in the United States. The Missouri AV is the least costly and is easy to clean. This AV does not need to be assembled for each use because the water jacket is formed by two molded layers of latex rubber. A single rubber cone leads from the water jacket for attachment of a semen receptacle. The AV is held by a leather case with leather handle. Addition of water or air to the water jacket allows for adjustment in AV temperature and pressure. In most instances, the glans penis of the stallion is beyond the warm water jacket at the time of ejaculation so as to avoid heat shock damage to sperm. One can attach a clean plastic or glass bottle, a Whirl-pack bag, or a disposable baby bottle liner to the AV for use as a semen receptacle. One also can incorporate a filter into the semen receptacle.

16.10.2.2.2.8

Colorado.

The Colorado model of AV is substantially longer, larger in diameter, and heavier than other AVs when ready for use. The AV consists of a solid outer plastic casing and is assembled by adding two layers of rubber liners to the casing to form the water jacket. This AV maintains the working temperature for stallions for a significantly longer period of time. Because of the weight and size of this AV, some operators have difficulty holding the AV in the most appropriate position for some stallions. A significant shortcoming of the Colorado model AV is that most stallions ejaculate midway along the length of the warm-water liner, exposing sperm cells to high temperatures. The operator needs to be extremely cautious when using this AV to avoid heat shock to sperm. Disposable filters and liners are available for the Colorado model AV to remove gel and reduce bacterial contamination from the rubber liners.

16.10.2.2.2.9

Hanover.

The Hanover model AV is used commonlyin Europe, is shorter and smaller in diameter than the Colorado AV, and is made of a hard rubber casing and inner rubber liner. This AV should work well for most stallions. Ejaculation occurs at or near the end of the water jacket.

16.10.2.2.2.10

Nishikawa or Japanese.

Although the Nishikawa or Japanese AVs are no longer available in the United States, replacement latex liners are still available. The aluminum casing makes this a lightweight, easy to handle model, and most stallions ejaculate directly into the semen receptacle.

16.10.2.2.2.11

HarVet.

The HarVet AV closely resembles the Nishikawa AV in its light weight and similar size with a plastic casing. This AV is designed to be used with disposable AV liners that form a semen receptacle atits distal end and therefore avoiding the water leakage problem of the Nishikawa AV.

16.10.2.2.2.12

Polish or Open-Ended Artificial Vagina.

The Polish model is substantially different from other models on the market. Using the open-ended AV, one can visualize the process of ejaculation and can collect individual jets of presperm, sperm-rich, or gel fraction of semen. This AV has been valuable in the diagnosis of hemospermia, urospermia, internal genital tract infections, and ejaculatory failure.[61] Additionally, this AV has been useful in obtaining semen for commercial use from stallions with hemospermia and urospermia, because most of these affected stallions ejaculate the blood or urine after the initial jets of sperm-rich semen. The open-ended AV also has been useful in cryopreservation programs to obtain sperm-rich and bacteria-free ejaculates from stallions. One also can use this method of collection to obtain "clean" ejaculates from stallions that are untrained and intolerant of penile washing. The Polish AV also allows the use of high internal AV temperatures without the risk of sperm cell damage because the ejaculate usually is emitted directly into a funnel with an attached receptacle held by a second person.

Open-ended AVs are not currently available in the United States but can be homemade from plastic or polyvinyl chloride tubing or by removing the coned portion of the Missouri model AV and using only the innermost rubber liner to form a water jacket.

To reduce the risk of chemical residue exposure ofthe semen from the AV liner cleaning process or to allow the use of the same AV by multiple stallions, sterile, plastic disposable liners have become commercially available for most types of AVs. However, many stallions object to these liners, and the number of mounts per ejaculation increases. Breakage of the plastic liner may occur during thrusting, and complete eversion of the liner may occur during dismount. If stallions ejaculate on first entry into an AV fitted with a disposable liner, the bacterial contamination of semen is reduced sharply. However, as the number of entries into the AV or the number of thrusts in the AV increases, the bacterial contamination of semen also increases.

One should clean the AV immediately after each use, should rinse it thoroughly with hot water, and should wipe away dirt, debris, and smegma. If one does not use disposable liners, one should immerse the rubber liners in 70% alcohol for 1 hour or more, rinse the liner thoroughly with hot water, and hang the liner in a dust-free, dry environment. One should not use soaps and disinfectants on the rubber equipment to avoid accumulation of chemical residue by the rubber. Without the use of disposable AV liners or thorough cleansing of theAV and its liners, the AV may become contaminatedby *Pseudomonas* 1148
spp., *Klebsiella* spp., *Escherichia coli*, *Taylorella equigenitalia*, or other harmful bacteria and therefore 1149
contaminate subsequent semen samples and inoculate the penile surface of the stallion. For these reasons, many farms maintain an individual AV for each stallion at the breeding farm.

16.10.2.2.3

Selection of a Breeding Mount

One can collect semen from the stallion while the stallion is mounted on a behaviorally estrous mare, phantom mare, or breeding mount or while the stallion is standing on the ground.

16.10.2.2.3.1

Live Mare

Selection of a suitable mount mare frequently depends on the experience and breeding mannerismsof the stallion. For example, the novice, inexperienced stallion may need to be taught to mount the mare

from the rear quarters. This training requires a disciplined, cooperative mount that will tolerate being mounted from the side. Some stallions vocalize loudly in the breeding shed and may frighten maiden or timid mares. The mount mare needs to tolerate a certain amount of nipping and biting of the neck, shoulders, flank region, and hocks to be suitable for some stallions. Mares with foals at their sides are frequently protective of their foals and less cooperative than barren mares. The mount mare also should be an appropriate size match for the stallion. For routine breeding farm activities, the reliance on an estrous mare as a mount has significant shortcomings. Additionally, in a cooled, shipped semen program, the breeding farm may not have access to nonpregnant mares, particularly at the end of the breeding season. Therefore some breeding farms maintain one or more ovariectomized mares as mount mares. One should select from these ovariectomized, mount mare candidates based on their size, tolerant attitude toward handling, and their strong estrous behavioral signs as intact mares. A mare with gonadal dysgenesis (XO) may be a good mount mare candidate without having to perform an ovariectomy. Most ovariectomized mares perform well as mount mares while being restrained with a twitch or lip chain placed on the upper gum. In some cases, one may need to administer a low dose of estradiol cypionate (0.5 to2 mg) at intervals of 3 days to 3 weeks to maintain receptivity by the mare.

During the semen collection process, one usually restrains the mount mare using a twitch. One also may apply hobbles to rear pasterns or hocks, but the novice, untrained stallion may become entangled in the hobbles if the collection procedure does not go as planned. One should wrap the long tail hairs at the base of the mount mare to prevent the tail from interfering with deflection and entry of the penis into the AV.

16.10.2.2.3.2

Phantom Mare or Dummy

Because of the lack of readily available mount animals, increased expertise required of an additional horse handler, and increased safety risks encountered while using a mount mare, many farms prefer to train the breeding stallions to mount a phantom or dummy mare for semen collection. Most stallions, including novice stallions, readily accept the phantomas a mount during semen collection. The working area around the phantom should be dust free and allow good footing by the stallion. Adequate space should surround the phantom for the safety of the handlers and to allow a teaser mare to be positioned alongside or in front of the phantom. Many stallions are trained to mount the phantom even when the teaser mare is not close to the phantom.

When one collects semen from a stallion using the phantom, the stallion should approach the mount in a controlled fashion, mount the rear of the phantom, and use his forelimbs to stabilize himself by grasping the padded barrel of the mount. The operator should quickly deflect the penis to the side of the phantom. While on the left side of the stallion, the operator deflects and stabilizes the base of the penis with the right hand. This practice minimizes potential injury to the penis and prepuce during thrusting by the stallion. Some phantom mounts are fitted with a Colorado type AV on the posterior end, which works well for some stallions and requires only one person for the collection procedure. However, some stallions need manual stimulation that is easierto provide when the operator has control of the AV. Stallions regularly used for live cover breedings can be difficult to train to accept the phantom as a suitable mount. For this reason, certain circumstances may require access to an estrous mare.

The breeding phantom usually is made of a hollow cylinder with closed ends. The barrel is covered with 1to 2 inches of firm padding. The padded cylinder thenis covered by a tough, nonabrasive cover that is free of wrinkles. Stallions that repeatedly mount and dismounta phantom abrade the medial aspects of the forearmsand knees. The stallion should be taught to dismount the phantom in a controlled manner by

backing offof the mount rather than making a side dismount. The diameter of the body of the phantom should be 20 to24 inches total. One should keep the legs of the phantom away from the mounting end of the phantom to avoid injury to the hindlegs of the stallion during breeding and dismounting. The mount should be adjustable for height, and the angle of the phantom should be adjustable to accommodate older stallions, stallions with hock problems, and stallions of varying stature.[54,62]

16.10.2.2.3.3 Semen Collection Procedure

Preparation and planning are the keys to the efficient collection of semen from stallions and to assure proper handling of the semen immediately after collection. The laboratory should be prepared so that the 1149
equipment and any extenders used in semen handling after collection are clean and at the desired 1150
temperature (35° to 37° C). One then assembles the AV and fills it with warm water (usually at 48° to52° C) because the AV equipment quickly drops the temperature during equilibration. One adjusts the final temperature of the AV, if necessary, to 45° to 48° C for most stallions; lightly lubricates the inner liner using a nonspermicidal lubricating gel; and adjusts the AV pressure at this time.

One selects a suitable area for semen collection. If an estrous mare is to be used as a mount mare, one should wrap the tail of the mare and wash her perineal areato prevent undue contamination of the stallion's penis during mounting. With the mare adequately restrained, one brings the stallion in to the collection area. Oncethe stallion has achieved full erection, the operator cleans the penis with clear, warm water, wipes the urethral diverticulae clean to reduce bacterial contamination of the semen further, and wipes the penis dry, if necessary, using a clean, soft towel.

The operator presents the stallion to the side of the mare and encourages him to mount after achieving a full erection and after the mare has demonstrated her receptivity. For safety reasons, the mare and stallion handlers should be on the same side of the mount mare as the individual collecting semen from the stallion. After the stallion has mounted, the operator directs the erect penis into the AV using the hand placed on the ventral surface of the penis. This hand continues to stabilize and deflect the base of the penis during thrusting and ejaculation. The operator should hold the AV to accommodate the stallion, which usually involves holding the AV parallel to the ventral abdominal wall of the stallion. Just before ejaculation, the operator can feel strong urethral pulsations with the right hand. Once ejaculation begins, the operator should tilt the AV downward to allow rapid entry of semen into the collection vessel to avoid heat shock to the sperm.

One conducts semen collection from the stallion mounted on a phantom in this same manner. As soon as the stallion dismounts, one takes the semen to the laboratory for processing and evaluation.

16.10.2.3 SEMEN EVALUATION

Depending on the reason for semen collection andits ultimate purpose, the ejaculate must be handled, processed, and preserved in different ways. Evaluation of raw semen for a routine breeding soundness examination or a prepuchase examination might need more detailed analysis than that done for semen collected regularly for an artificial insemination program at the farm. However, semen that will be processed for an artificial insemination program away from the site of collection requires different processing.

16.10.2.3.1 Evaluation of Raw Semen

The goal of most semen evaluations is to try to predict the fertilizing ability of a given ejaculate or the potential fertility of the animal undergoing the evaluation. However, the low predictive value and frequent lackof objectivity of traditional tests such as motility and morphology has led to the refinement of old techniques and the development of new methodologies for semen evaluation.[63] The standard evaluation of a given ejaculate involves the following:

1. Volume and color. One should record the color and volume (in milliliters) of the ejaculate. In general, color of the ejaculate ranges from watery to creamy and depends on the sperm concentration per milliliter. Abnormal colors or volumes can indicate contamination of the ejaculate with blood, urine, or pus. Normal volumes of ejaculates range from 20 to 250 ml, with an average of 50 to 60 ml.[64] Factors that influence the volume are degree of sexual stimulation before collection, breeding conditions, and foreign material in the ejaculate. Low volumes of ejaculate with low sperm concentration in an otherwise normal stallion suggest an incomplete ejaculation, and one should collect another sample.

2. Osmolarity and pH. Osmolarity of stallion semen ranges from 290 to 310 mOsm. Values greater than 350 mOsm can indicate urospermia, and one should measure the level of creatinine. Values less than250 mOsm suggest water contamination. Seminal pH ranges from 6.9 to 7.5, and values higher than those should warn the clinician regarding the possibility of extraneous material in the ejaculate or an infectious process in the reproductive tract of the stallion.

3. Spermatozoal motility. Sperm motility is a rough estimate of the percentage of viable sperm in the ejaculate. Several methods have been used to evaluate motility. First, *visual motility* is the most widely used assay for evaluating semen because of its simplicity and low cost. However, many factors greatly influence the evaluation: individual judgement, thickness of the sample, concentration of sperm in the ejaculate, degree of contamination, degree of agglutination, and temperature. For this reason, one should estimate motility by evaluating a number of fields in a 10 μl drop of well-mixed semen in a microscope with a heated stage.[43] Conversely, one can estimate motility by diluting a portion of the ejaculate with extender to a concentration of 25 to 50 × 10^6 sperm per milliliter.[65] Even under the most tightly controlled conditions, repeatability of visual motility is poor between technicians and laboratories. Visual motility estimates of freshly ejaculated stallion sperm have been reported to account for only 50% to 70% of the variation of fertility in that 1150
sample. The correlation is even worse (r = 0.3) when trying to predict fertility of a frozen-thawed 1151
sample of semen based on postthaw motility.[66] Second, *computer-assisted semen analysis* provides data on characteristics of sperm such as linear velocity, linearity, path velocity, and lateral head displacement that otherwise would be difficult to obtain. In addition, computerized analysis provides information on sperm concentration and percentage of motile cells. Although analysis of sperm motion with a computer is more objective and provides a highly consistent way of evaluating spermatozoa, the fertility of stallion sperm is not well correlated with any of the sperm characteristics measured with these analyzers.[67]

4. Longevity of sperm motility. One can determine duration of motility on raw, undiluted semen or on extended semen. Dilution factor of semen to extender affects the longevity of sperm, so ratios of 1:3

to 1:4 are recommended. To evaluate longevity of motility, one should evaluate semen samples immediately after extension and at regular intervals thereafter for up to 96 hours.[65]

5. Sperm morphology. Several attempts have been made to try to correlate the percentage of morphologically normal sperm present in a given ejaculate with fertility.[68,69] However, because of the lack of consistency among clinicians in reporting sperm morphology, results have been inconclusive. Among the problems that clinicians encounter are the definition of normal and abnormal in light of the tremendous range of normality and the fact that little knowledge exists regarding specific sperm defects that interfere with fertility. This problem is even more notorious when a clinician is trying to interpret the results from a referring veterinarian or a veterinary technician. One can avoid some of these inconsistencies by recording specific morphologic defects rather than grouping defects into primary and secondary because this last method erroneously assumes origin of sperm defects (i.e., testis and posttesticular, respectively). For any sperm morphology evaluation, one should count a minimum of 200 cells. One should record normal sperm cells as well as those with acrosomal, head, midpiece, droplets, and tail defects, noting the specific type of defect for each part. Although only 200 cells are counted routinely, one should record sperm cells with more than one defect as such to help the clinician evaluate the incidence of defects in a particular semen sample. One can evaluate cells as wet mounts under phase-contrast microscopy or differential interference contrast microscopy after fixation in buffered formal saline or 4% glutaraldehyde. If samples are to be preserved for longer periods of time, one should add an antibiotic to the fixative. Alternatively, one can evaluate cells after staining. One should mix one drop of semen well with the stain and then smear it on a glass slide. Common stains currently used include India ink, eosin-nigrosin, eosin–aniline blue, Giemsa, Wright's, and several others. The clinician should be aware that severe changes in osmolarity of the stain as well as mechanical damage to the sperm could alter the normal morphology of the cells.

One can gain additional morphologic information by performing scanning or transmission electron microscopy. Although these procedures are not recommended as a routine, they can prove valuable in cases of stallions with unexplained infertility.

16.10.2.3.2

Alternative Assays for Sperm Evaluation

Other assays for evaluating sperm in stallion semen include the following:

1. Hypoosmotic stress test. As with many other cells, when sperm with intact membranes are exposed to hypotonic solutions, the influx of water across the intact membrane causes swelling of the cell and is evident by a characteristic coiling of the tail.[70]

2. Flow cytometry. Flow cytometry now is used widely to evaluate stallion spermatozoa. This technique, based on labeling of sperm acrosomes or DNA with fluorescent dyes, has the advantage of analyzing a large population of cells, which in turn provides information on the distribution of acrosomal or chromatin integrity.[71–73]

3. Biochemical evaluation. Activity of enzymes such as aspartate aminotransferase, glutamine-oxaloacetic transaminase, lactate dehydrogenase, adenosine triphosphate, hyaluronidase, and acid and alkaline phosphatase are correlated positively with the numberof sperm in the ejaculate. Because these enzymesare located mostly in the acrosome or midpiece, their activities—particularly those of glutamine-oxaloacetic transaminase, lactate dehydrogenase, and hyaluronidase—increase

proportionally with the level of damage inflicted on the sperm. Therefore one can use enzyme activities in the seminal plasma as indicators of the degree of acrosomal or membrane damage.[74–76] Further research is needed in this area to establish normal enzyme activities in stallion seminal plasma.

16.10.2.3.3 Preservation of Semen

The specific processing of the ejaculate is determinedby how long the semen needs to be stored before insemination. Semen can be used fresh, cooled, or frozen.

16.10.2.3.3.1 Fresh Semen

Semen that is collected and used immediately or up to 12 hours later need not be refrigerated and in most cases can be diluted with appropriate prewarmed extender at ratios of 1:1 to 1:4, depending on raw semen concentration and ejaculate volume. Immediately after extension, one should remove semen from 1151
the incubator and cool it to room temperature (15° to 20° C) without loss of its fertilizing potential. One 1152
may use raw semen for artificial insemination within 30 minutes to 1 hour after collection, provided that it is kept at 37° C. Extension of all collected semen before insemination is highly recommended.[77]

16.10.2.3.3.2 Chilled Semen

To retain its fertilization potential, semen that is going to be used 12 and up to 72 hours after collection should be cooled to 5° to 8° C. Besides storage temperature, the most important factors affecting the longevity of extended semen are semen quality, sperm concentration immediately after ejaculation, type of extender and antibiotic used, and dilution rate and cooling rate. Several systems of passive cooling for stallion semen are available; however, the Hamilton-Thorn Equitainer System has proved to provide excellent cooling rates and is considered the most reliable method for shipping cooled semen. In addition to cooling the semen at an appropriate rate, the Equitainer maintains the semen at the desired temperature for up to 72 hours. Furthermore, the Equitainer II has been shown to be the most appropriate container to use if the container is likely to be subjected to freezing conditions for an extended time.[77]

One should dilute semen at a ratio of 1:3 to 1:10 depending on the initial volume and concentration of the ejaculate. In general, longevity of fresh cooled sperm is directly proportional to the dilution ratio, ensuring that the total dose has 1 billion sperm cells and concentrations between 25 million and 50 million sperm per milliliter.[77]

Idiosyncratic differences in individual stallion semens in tolerance of the cooling process or particular extenders are not uncommon. Although the factors that determine why some semen samples from some stallions do not preserve well are unknown, particular components in the seminal plasma, such as oxygen free radicals, are suspected of being involved. In fact in some instances, one can improve longevity and fertility of some stallion semen substantially by removing the seminal plasma and resuspending the semen in the appropriate extender.[78]

When processing or evaluating shipped semen, one must remember the following:

1. Extenders should have an antibiotic. The combination of amikacin and potassium penicillin is popular and does not appear to interfere with fertility.
2. The modification of the traditional nonfat, dried milk solids–glucose extender may improve semen quality of selected stallions, especially if all seminal plasma is removed.
3. Semen-to-extender dilution ratio may need to be altered for each individual ejaculate with dilutions of 1:5 or greater not being uncommon.
4. One should select a commercial storage container in light of the transport time, method of shipment, and ambient temperatures to which the container will be exposed.
5. The use of nontraditional laboratory probes, such as the Sperm Chromatin Structure Assay, can provide meaningful information regarding the effects of semen storage on spermatozoal function.

A common practice for some breeding farms is to ship semen for two inseminations 12 hours apart. In recent years it has become evident that mammalian sperm, including stallion sperm, reaches the oviduct within a few hours after insemination and subsequently attaches to the oviductal epithelium and remains motile for at least 72 to 96 hours. This has led researchers to suggest that the oviduct of the mare is a better storage place than any of the transport systems available. Therefore using all available semen for insemination of the mare as soon as it arrives is highly recommended. However, one must take into account sperm quality and reproductive history of the mare to decide if the uterus of the mare can tolerate two inseminations.

16.10.2.3.3.3

Freezing Semen

When the semen is intended to be inseminated more than 72 hours after collection, it must be frozen to retain some of its fertilizing potential. The only successful way to preserve sperm for long periods of time, that is, for months or years, is cryopreservation or freezing. Different stallion semens tolerate differently the freezing and thawing process, and unfortunately, the number of motile sperm after thawing is a notoriously poor indicator of fertility of frozen-thawed semen.[78]

In general, the freezing process involves the collection of semen from the stallion, evaluation of the semen, dilution and centrifugation of the semen, and resuspension of the sperm in freezing extender. Unfortunately, frozen-thawed sperm appears to have a shorter life span in the reproductive tract of the mare than raw semen or fresh sperm. The reduced life span appears to be related partially to differences in calcium metabolism between fresh and frozen sperm.[79] Because of the apparent short life span of frozen semen, timing of insemination appears to be critical when using frozen semen.

16.10.2.3.3.4

Semen Processing for Freezing.

Unfortunately, the equine frozen semen industry is still in its infancy. The lack of standardization of the procedure, the lack of laboratory test(s) that relate to fertility, and the idiosyncrasy of some stallion semens to tolerate the freezingand thawing process have been major obstacles in the development of standard freezing techniques.

Extenders for freezing stallion sperm need to have energy and protein sources similar to that needed by fresh or cooled semen. However, in addition, freezing extenders must contain a cryoprotectant such as glycerol.[80,81]

Semen that is intended for freezing should be processed as soon as possible. Although some stallion 1152
semens tolerate transportation well, processing the semen more than 1 hour after collection is not 1153
advisable.

The raw semen from stallions that is classified as a "good freezer" in general has a lower volume, higher motility, and higher number of total motile sperm than semen classified as a "poor freezers."[82] However, classification of stallion semen as a good or poor freezer based solely on the percent of motile cells after thawing is risky. One should perform longevity of motility, detailed morphology, and in some cases acrosomal and flow cytometric evaluations to determine the quality of the semen before its commercial use.[83]

Processing an ejaculate for cryopreservation involves the following steps:

1. Collection and evaluation of the raw semen.
2. Dilution of raw semen in a sugar- and protein-based extender.
3. Centrifugation of extended semen and removal of the supernatant.
4. Reconstitution of the pellet with freezing extender.
5. Packaging of the sperm in an appropriate packaging system after adjusting suspension to the desired number of sperm per dose.
6. Placement of straws in liquid nitrogen vapors or in a programmable freezer. One should identify each frozen unit with at least the name and breed of the stallion, registration number for that breed, date of the freeze, and identification of the laboratory processing the semen.

16.10.2.3.3.5 Thawing and Evaluation.

The handling of frozen semen greatly depends on the recommendations given by the laboratory processing the semen and the type of package in which the semen is presented. In general, single 0.5-ml straws are thawed at 75° C for 7 seconds or at 38° C for at least 30 seconds. When an insemination dose consists of multiple 0.5-ml straws, the most common thawing protocol is 37° C for at least 30 seconds, making sure that the straws do not stick together during the thawing period. Frozen semen packed in 2.5-, 4-, or 5-ml straws is recommended to be thawed at 50° C for 40 to 45 seconds. Regardless of the thawing protocol or packaging system, the well-accepted fact is that once semen has been thawed, it should be inseminated almost immediately.[43]

Although the freezing procedure is simple, the evaluation after thawing and the prediction of the potential fertility of a given ejaculate after thawing is not so simple. Sperm quality has a profound effect on the pregnancy rates achieved with frozen-thawed semen. Motility after thawing, concentration per dose, morphology, and acrosome integrity are parameters one should evaluate to determine the quality of frozen semen. Unfortunately, a battery of tests, let alone a single test, cannot predict fertility of

frozen-thawed sperm so as to determine what is good and what is poor semen. In general, semen with higher motility is considered to be of better quality; unfortunately, this is not always the case. The motility of most stallion semen after thawing, regardless of the motility before freezing, is 30% to 45% less than for fresh sperm. But some stallion semens with motilities of less than 30% after thawing have acceptable pregnancy rates per cycle, whereas others with motilities greater than40% have low pregnancy rates.[83,84]

16.10.3 Management of the Breeding Stallion

One should consider many things to optimize management of the breeding stallion, including feeding, exercise, and vaccination programs. One determines many management decisions by the number of mares that a stallion will breed in a season, breed, type of housing, and the method of breeding.

16.10.3.1 GENERAL MANAGEMENT

16.10.3.1.1 Feeding Program

Successful breeding programs require a balanced feeding program. Overfeeding and oversupplementing of stallions is probably the most common form of malnutrition in stallions. Obesity may affect libido and mating ability adversely. In general, the nutritional needs of a stallion during the breeding season do not appear to be different from those of maintenance. A maintenance ration consists of enough balanced nutrients to support normal, basic bodily functions. Adequate pasture or good-quality hay usually can meet these requirements. Free access to trace mineralized salt and fresh water ad lib are also necessary. Grain as an energy supplementin cold weather or under certain stressful conditionsalso may be warranted. The size, condition, activity, and temperament of the stallion play a role in his nutritional needs.

The healthy stallion consumes 2% to 3% of his body weight daily. At least 50% of this should be in the form of roughage.[85] Stallions generally require 10% proteinin their feed, with younger stallions requiring 12% to 14%.[86] Although micronutrients such as vitamins A and E, selenium, copper, and zinc play an important part in the successful completion of spermatogenesis, to date no evidence exists that any of these nutrients fed in excess increase sperm numbers or quality.

16.10.3.1.2 Exercise

Horses naturally are roaming and grazing animals. Exercise for stallions is an integral part of their management and affects their mental and reproductive well-being. The goal is to keep a stallion fit for the breeding shed, not the racetrack, so that he has a good attitude toward his daily duty, that is, covering 1153
mares. The amount of exercise time must be tailored to the temperament and needs of the stallion. Owner 1154
should provide regular exercise to maximize the fertility and longevity, as well as physical and mental fitness, of the stallion. In addition, lack of exercise may lead to vices such as weaving, stall walking, or cribbing.[87]

16.10.3.2 ESTIMATION OF STALLION BOOKAND FREQUENCY OF SERVICE

A stallion usually is chosen as a breeding animal basedon pedigree, performance, and conformation. Little or no emphasis is placed on reproductive potential, and a significant number of stallions enter the breeding pool

with poor or marginal fertility. A complete breeding soundness examination on maiden stallions or a complete review of past reproductive performance for new stallions is an integral part in the management process. Information concerning the number of mares bred, number of covers made, pregnancy rate per cycle, and covers per pregnancy is helpful in evaluating reproductive efficiency. If a stallion has stood at stud in previous breeding seasons, collection of one or two ejaculates is sufficient to determine that his semen quality has not declined during his off time because of illness, trauma, or age.

Libido of the stallion certainly can play a large role in determining the number of mares a stallion can service during a breeding season. Libido, which is thought to be a genetically acquired trait, can be modified by handling and environmental conditions such as housing. Therefore testosterone levels and libido can be altered by interaction between stallions and mares and with exercise. Many times, poor libido is a limiting factor in the number of mares a stallion can cover. The number of covers a stallion can make in a day varies with the individual stallion. Factors such as age, physical abnormalities, and testosterone levels play an important role. Some stallions can breed 2 to 3 times a day, 7 days a week, whereas some can cover only one mare per day.

The length of the breeding season also plays a rolein the number of mares the stallion can mate. The Thoroughbred season is generally from February 15 to July 15. Therefore the number of mares that can be presented during this time is limited in a natural breeding program. In Warmblood stallions, an excess of 350 mares can be bred with every other day collection of semen of average quality.

One must also consider the age and physical condition of the stallion. Stallions typically retire to stud at 3 to 5 years of age. Age influences reproductive capacity. Stallions reach puberty at 12 to 24 months of age but continue to mature and increase reproductive performance until at least 5 years of age or older. During the breeding season, seasonal fluctuation of sperm production occurs.[88] In addition, physical problems, especially of the hindlegs may limit the number of mares that the stallion can mount and service. Furthermore, the quality of the book of mares that the stallion has for a particular year can have a significant effect on the number of mares that he can cover. For example, a stallion that has a book in which most of his mares are maiden or young foaling mares will be able to cover significantly more than one who is booked to a majority of old barren mares.

The effect that the reproductive potential of a stallion has on the overall breeding program is significantly more important than that of a single mare. However, for veterinarians and managers to understand the complex interactions between management, endocrinology, and the quality of the mares that a stallion breeds is imperative so that they can diagnose or treat infertility problems in the stallion.[89] One can estimate potential sperm production by using the combined testicular volume as described in a previous section of this chapter.

16.10.3.3 STALLION AS A SOURCE OFDISEASE TRANSMISSION

With increasing breeding management—that is, more mares bred to a stallion on a given year—the significance of venereally transmitted diseases in horses has gained importance. Thoroughbred stallions commonly may breed more than 100 mares by natural cover and Standardbred or Warmblood stallions may breed twice that number, or more, in a single 5- to 6-month breeding season through artificial insemination. With the implementation of frozen semen technology, semen from virtually any country can spread disease in a country or continent far removed from the direct area of influence of the stallion.

Veterinarians must be aware of the risk factors for disease transmission, diagnostic methods, and some management measures that can reduce the incidence of disease with its potentially devastating effects on fertility.[90]

16.10.3.3.1

Risk Factors

Several risk factors may increase the chances of disease transmission through semen:

1. Natural mating: Direct sexual contact perhaps poses the biggest risk for venereally transmitted disease. One should culture all mares, but particularly those with poor fertility histories, before breeding. One should monitor stallions breeding by natural cover regularly.
2. "Backyard stallions": Stallions that are not standing at a breeding farm tend to have lower numbers of mares per season and poorer reproductive management. Hygienic procedures in these cases often are neglected, and these stallions or mares can be carriers of infectious disease. Often these horses are poorly housed, which can contribute to colonization of the penis by pathogenic bacteria.
3. Inconsistent breeding method: In breeds for which artificial insemination is allowed, a stallion 1154
   commonly may breed several mares by natural cover at the farm, under no veterinary supervision. 1155
   The owner also may request that semen from the stallion be collectedto be shipped to other mares. These inconsistent practices can increase the risk of a stallion becoming contaminated or of spreading microorganisms to several mares.
4. Artificial insemination: Artificial insemination has been advocated as a technique that greatly reduces the risk of disease transmission. Stallions breeding artificially could breed more than 200 mares during the year. These horses usually are scrutinized carefully for venereal diseases and are housed with other animals of similar health status. Other factors—such as the hygiene of the artificial vaginal, lubricants, collection bottles, dummy mount, or teaser mare and semen packing material—could serve as sources of contamination for venereal disease transmission. If cleanliness and hygiene factors are overlooked, the process of artificial insemination may serve as a multiplier of disease.[91]

16.10.3.3.2

Types of Disease

The types of diseases that can be transmitted through semen include bacterial, viral, protozoal, and genetic.[90]

16.10.3.3.2.1

Bacterial Diseases.

The source of bacterial infections can be from the external or the internal genitalia.

16.10.3.3.2.2

External Bacterial Infections.

Virtually every stallion and all ejaculates have contaminants that could be potential pathogens in the mare because a variety of commensal bacteria inhabit the surface of the penis and prepuce. These bacteria constitute the normal flora of the penis and rarely produce genital infections in reproductively

sound mares. One commonly can culture an unwashed stallion penis or fossa glandis and harvest a milieu of bacteria including *Escherichia coli, Streptococcus zooepidemicus, S. equisimilis, Staphylococcus aureus, Bacillus* spp., *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.[92] However, when the normal bacterial flora is disrupted, potentially pathogenic bacteria, particularly *P. aeruginosa* and *K. pneumoniae*, can colonize the penis and prepuce. These organisms rarely produce clinical disease in stallions but can be transmitted to the genital tract of the mare at the time of breeding, resulting in infectious endometritis and associated subfertility. The factors that contribute to the colonization of the penis by these bacteria have not been determined clearly. Normal bacterial microflora of the external genitalia of the stallion may combat proliferation of pathogens, and frequent washing of the penis, especially with soaps, may remove these nonpathogenic resident bacteria, increasing the susceptibility of the penis and prepuce to colonization by pathogenic organisms.[93] Others dispute this concept, asserting that repeated washing of the external genitalia alone does not contribute to overgrowth of pathogenic microorganisms. The environment in which a stallion is housed may influence the type of organisms harbored on the external genitalia. The stallion also can acquire these organisms at the time of coitus with a mare that has a genital infection.[94]

One diagnoses pathogenic colonization of the stallion's penis first of all by careful evaluation of breeding records and early pregnancy diagnosis. A sudden and unexplained drop in pregnancy rates should warn the stallion manager about a possible problem. Definitive diagnosis is by isolation of the microorganism in culture. In addition, isolation of the same microorganism with a similar sensitivity pattern from the nonpregnant mares helps confirm the diagnosis.

Treatment of penile colonization depends on the type of bacteria and method of breeding. For stallions breeding by artificial insemination, a thorough penile wash before semen collection is recommended. One then dilutesthe filtered semen with extender containing antibiotic for which the bacteria is sensitive. Incubation should be for at least 30 minutes before insemination. Operators thoroughly should wash and scrub each stallion breeding by natural cover and dry the penis after washing. After the stallion covers the mare, the operator lavages the uterus, infusing the mare with appropriate antibiotics between 4 and 6 hours after breeding.

One can wash stallions with penile colonization by *Klebsiella* or *Pseudomonas* spp. with a weak solution of HCl or sodium hypochlorite. One should avoid systemic treatment because it has proved unrewarding in most cases.[95]

16.10.3.3.2.3

Contagious Equine Metritis.

Contagious equine metritis, caused by the coccobacillus *Taylorella equigenitalis*, is perhaps the only true venereal sexually transmissible disease in horses. Contagious equine metritis, although not present in North America, could be imported, particularly through frozen semen from untested stallions.

Stallions infected with contagious equine metritis are asymptomatic carriers and harbor the organism in the urethral fossa, the urethra, or the sheath. Mares bred to infected stallions develop a severe purulent vaginitis, cervicitis, and endometritis. Infection in these mares appears to resolve, but they remain infected and the organism can be cultured from the clitoral fossa. Diagnosis of contagious equine metritis is by culture of the organism. Aimes medium supplemented with charcoal is recommended for transport of culture specimens. Swabs are plated on Columbia blood-chocolate agar at 37° C and 7%

carbon dioxide. Because of the slow growth of *T. equigenitalis*, the possibility of false-negative results is high.[96]

16.10.3.3.2.4

Internal Bacterial Infections.

Although infections of the accessory sex glands are uncommon, unilateral or bilateral seminal vesiculitis 1155
can occur. Bacterial infection of the seminal vesicles is not accompanied by clinical signs except for 1156
white blood cells in the ejaculate.[7] In some stallions with seminal vesiculitis, the glands may be enlarged, firm, and painful on palpation if the condition is acute. One cannot consider gland size alone and character of seminal vesicle fluid on ultrasound examination as accurate indicators of infection, because the glands vary in size and appearance within individual stallions and across breeds. Diagnosis of seminal vesiculitis is made best by rectal palpation, observation of large numbers of neutrophils in the semen, bacterial culture of semen, and endoscopy of the urethra and seminal vesicles. Direct culture of the seminal vesicles during endoscopy increases the clinician's confidence in the significance of organisms cultured. Treatment is difficult and the prognosis is guarded. Reported treatments include systemic treatment with antibiotics, or alternatively, addition of extenders containing appropriate antibiotics to the semen of the affected stallion. Recently, endoscope-aided direct lavage followed by antibiotic instillation into the vesicular gland lumen has been advocated.

Bacterial infections of the accessory genital glands, epididymides, or testes are uncommon in stallions but are clinically important because of their persistent nature, tendency for venereal transmission, and detrimental effect on fertility of stallions. One usually identifies these infected stallions by the presence of numerous neutrophils in ejaculates, whereas one uses other diagnostic procedures such as ultrasound and endoscopy to localize the site of infection. Treatment generally consists of combining local and systemic therapy.[97]

16.10.3.3.2.5

Viral Diseases.

Although many viruses can affect the reproductive performance of a stallion, only two are considered to be venereally transmissible. Equine herpesvirus 3, the causative agent for equine coital exanthema, and equine arteritis virus (EAV), which is responsible for equine viral arteritis (EVA).

16.10.3.3.2.6

Equine Coital Exanthema.

Equine coital exanthema can be transmitted by the stallion to the mare or from the mare to the stallion. The disease is characterized by the formation of small (0.5- to 1-cm) blisterlike lesions on the penis and prepuce or on the perineal area of the mare. These lesions eventually break to form skin ulcers that usually resolve completely in 3 to 4 weeks, leaving some round white scars in the area that was affected. Sometimes one can observe mild fever and slight depression. The effect on fertility is not known, but stallions and mares during the acute phase of the disease should be rested sexually to avoid further spread of the disease.[91]

16.10.3.3.2.7

Equine Viral Arteritis.

Equine arteritis virus is a small RNA virus that can infect horses and donkeys and is thought to be present in most countries except Iceland and Japan. Presently, EAV is responsible for major restrictions

in the international movement of horses and semen. Although most EAV infections are asymptomatic, acutely infected animals may develop a wide range of clinical signs, including fever, limb and ventral edema, depression, rhinitis, and conjunctivitis. The virus may cause abortion and has caused mortality in neonates. After natural EAV infection, most horses develop a solid, long-term immunity to disease. Mares and geldings eliminate the virus within 60 days, but 30% to 60% of acutely infected stallions become persistently infected and maintain EAV within the reproductive tract, permanently shedding the virus in the semen and efficiently transmitting the virus through the semen.[98] Mares infected venereally may not have clinical signs, but they shed large amounts of virus in nasopharyngeal secretions and in urine, which may result in the lateral spread of infection by an aerosol route.

The consequences of venereally acquired infection are minimal, with no known effects on conception rate, but mares infected at later stages of gestation may abort. Identification of carrier stallions is crucial to control the dissemination of EAV. One can identify these animals by serologic screening using a virus neutralization test. If the test is positive at a titer of 1:4 or higher, one should test the stallion for persistent infection by virus isolation from the sperm-rich fraction of the ejaculate or by test mating. Shedding stallions should not be used for breeding or should be bred only to mares seropositive through natural infection or vaccination that are isolated subsequently from seronegative horses for 3 weeks after natural or artificial insemination.

One of the greatest risks of EAV infection is abortion, which may occur even if the mare had no clinical signs. In cases of natural exposure the abortion rate has varied from less than 10% to more than 60% and can occur between 3 and 10 months of gestation. The abortions appear to result from the direct impairment of maternal fetal support and not from fetal infection.[98]

Although mares and geldings are able to eliminate virus from all body tissues by 60 days after infection, 30% to 60% of stallions become infected persistently. In these animals, virus is maintained in the accessory organs of the reproductive tract, principally the ampullae of the vas deferens and is shed constantly in the semen.[99] Three carrier states exist in the stallion: (1) *a short-term state during convalescence* (duration of several weeks), (2) *a medium-term carrier state* (lasting for 3 to 9 months), and (3) *a long-term chronic condition* that may persist for years after the initial infection. The development and maintenance of virus persistence depends in large part on the presence of testosterone. Persistently infected stallions that were castrated but given testosterone continued to shed virus, whereas those administered a placebo ceased virus shedding. In addition, virus could not be detected in geldings 1156
after 60 days after infection.[16] The ability of a large percentage of stallions to eliminate the virus 1157
effectively in time suggests that differences in the immune response of the host may be involved, or alternatively, virus strains may have biologic differences that influence their ability to persist in the reproductive tract. Establishment of persistence may involve a multifactorial process, with dependence on host and viral factors.[98]

After clinical recovery from initial infection, no significant decrease in the fertility of shedding stallions occurs. Mares infected after service by a carrier stallion do not appear to have any related fertility problems during the same or subsequent years, and no reports indicate mares becoming EAV carriers or chronic shedders or virus passage by the venereal route from a seropositive mare, causing clinical disease or seroconversion in a stallion.[98]

The two major routes by which EAV is spread are *aerosols* generated from secretions (respiratory or urine) from acutely infected animals or from secretions from recent abortions, and *venereal* from semen

from a shedding stallion. Close contact between animals generally is required for efficient virus spread in aerosol transmission. Personnel and fomites may play a minor role in virus dissemination. Virus is viable in fresh, chilled, and frozen semen, and venereal transmission is efficient, with 85% to 100% of seronegative mares seroconverting after being bred to stallions shedding virus. In several cases, outbreaks of clinical disease have been traced to a persistently infected stallion.[99]

Clinically, EVA resembles several other viral infections of horses, and a definitive diagnosis requires laboratory confirmation. One can diagnose acute infections by virus isolation or by serologically identifying a fourfold or greater rise in neutralizing titer between acute and convalescent serum samples. In the case of abortion, one can attempt virus isolation from fetal and placental tissuesor can demonstrate seroconversion in the mare. One can diagnose persistent infection in stallions by first screening serum for antibody in a serum neutralization test. If the serum is seropositive at a titer of 1:4, one should perform virus isolation on the untreated, sperm-rich fraction of the ejaculate or should test-mate the stallion to seronegative mares and monitor them for seroconversion.

The method of choice for antibody detection is the serum neutralization test. Antibody titers develop 2 to4 weeks following infection, are maximal at 2 to 4 months, and remain stable for several years. A titer of 1:4 or greater in duplicate sera is considered EAV seropositive. One must test semen from a seropositive stallion to determine the EVA status of the stallion as a carrier.[100] The current test for identifying virus in tissues and semen is virus isolation in cell culture. In EAV related-abortion, the fetus and placenta contain large amounts of virus. One should collect samples of placenta, spleen, lung, and kidney along with fetal and placental fluids in a sterile manner as soon as possible after the abortion occurred, chill the samples on ice, and submit them for virus isolation. One should obtain blood from the mare at the time of the abortion and 3 weeks later for testing by serum neutralization.[98]

After having determined that a stallion is seropositive at a titer of 1:4 in a serum neutralization test, one should collect a semen sample using an AV or a condom and a phantom or a teaser mare. If this is not possible, one can collect a dismount sample at the time of breeding; however, this is less satisfactory. The sample should be from the sperm-rich fraction of the full ejaculate and should be chilled immediately and shipped at 4° C to arrive at the diagnostic facility within 24 hours. If this is not possible, one should freeze the sample at below –20° C and ship it to the diagnostic facility under these conditions. Submission of two samples, collected the same day or on consecutive days is recommended. One should avoid washing the penis with antiseptics or disinfectants before collection of the samples. Samples of commercial frozen semen also may be tested, but the sample must have at least 2 billion sperm cells to be representative. False negatives have been reported because of the lack of seminal plasma in cryopreserved semen.[99]

16.10.3.3.2.8

Prevention and Control of Equine Viral Arteritis.

A modified live (ARVAC, Fort Dodge, Iowa) and a formalin-inactivated (ARTERVAC, Fort Dodge, Iowa) vaccine are available. Both vaccines induce virus-neutralizing antibodies, the presence of which correlates with protection from disease, abortion, and the development of persistent infection. EAV has a worldwide distribution and its prevalence is increasing. As a consequence, an increasing number of EVA outbreaks are being reported. The trend is likely to continue unless action is taken to slow or halt the transmission of this agent through semen. The modified live virus vaccine (ARVAC) does not produce any adverse effects in stallions apart from a possible short-term abnormality of sperm morphology and a mild fever with no overt clinical signs. However, one can isolate live virus

sporadically from the nasopharynx and blood after modified live virus vaccination. Serum neutralization antibody titers are induced within 5 to 8 days and persist for at least 2 years. The modified live virus protects against clinical disease and reduces the amount of virus shed from the respiratory tract in experimental infection. Horses in contact with and mares served by vaccinated stallions are not infected by EAV, and vaccinated mares experimentally challenged by artificial insemination are protected from clinical disease, but not infection. In the field the modified live virus vaccine has been used to control EAV outbreaks in some states of the United State since 1984, but the vaccine is not licensed worldwide. [96]

EVA is entirely preventable if horse owners, breeders, and barn managers follow simple 1157 serosurveillance and hygiene procedures. Controlling the dissemination of EAV requires a concerted 1158 effort on the part of all those involved in the equine industry. The presence of neutralizing antibody that correlates well with protection from disease, abortion, and the development of persistent infection in stallions is evidence that control programs, once instituted, have been successful.

16.10.3.3.2.9 Protozoal Diseases

Trypanosoma equiperdum is the causative agent for dourine, or mal du coit. The organism is not present in the United States or Europe but is still endemic in Africa and some areas in Asia and South America. *T. equiperdum* is perhaps the only protozoal organism that can be transmitted venereally. Tentative diagnosis is by the clinical manifestation of the disease, which includes intermittent fever, depression, progressive loss of body condition and severe purulent discharge from the urethra. Definitive diagnosis is by complement fixation and culture.[96]

Other microorganisms with the potential to be transmitted venereally include *Chlamydia* spp. and *Mycoplasma* spp. Although both of these organisms have been isolated from the urethra of stallions, their effect on equine fertility is not well known. One must be aware of the possibility of these agents causing infertility in mares and stallions. *Candida* spp and *Aspergillus* spp., although not commonly present in semen or the genital tract of the stallion, can be potential pathogens, particularly in artificial insemination programs in which the hygiene of the collection and processing equipment is not well monitored.

16.10.3.3.2.10 Genetic Diseases

One of the main reasons for stallions standing at stud is to pass on to their offspring their genetic attributes. However, sometimes stallions can be carriers of a hereditary condition that may be expressed in his foals when he is mated to certain mares. Perhaps the clearest example of the potential effect of the genetic effect is the “Impressive Syndrome” of American Quarter Horses, in which thousands of mares were bred by one stallion transmitting the gene for hyperkalemic periodic paralysis.

Conditions such as genetic mosaics (63 XO/64 XY or 65 XYY), certain sperm defects such as detached heads, or midpiece defects and testicular characteristics such as small testicular size or premature testicular degeneration are or could be transmitted genetically.

Other conditions that have been proved to have or are suspected of having a hereditary basis include combined immune deficiency in Arabians, umbilical and scrotal herniae, parrot mouth, cryptorchidism, and testicular rotation. The expression of any of these genetic traits can have profound and devastating

effects on a breeding program. When one suspects a condition, one must use cytogenetic or molecular diagnostic procedures to identify undesirable traits that will be expressed in the adult animal.

The pivotal point in prevention and control of infectious disease is the identification of infected stallions and mares and the institution of management procedures to prevent the further spread of any disease to susceptible populations through the breeding of mares by natural service or artificial insemination. If a stallion proves tobe a carrier, he should not be used for breeding through natural service or his semen should be treated with proper antibiotics in cases of bacterial disease. In the case of EVA, stallions might still be used, provided that the mare owners are informed that the stallion is a shedder so that they can take the preventive measurements such as vaccinating the mares.

The option to use a particular stallion in a breeding facility may depend on the value of the stallion as a breeding animal and individual regional regulations. Whatever the case may be, all stallions should have a diagnosed status before each breeding season. Breeding managers and stallion owners must be aware that poor genital hygiene of a breeding stallion and of the maresat the time of breeding greatly increases the chances of spreading disease from a stallion to a group of maresor from a mare to a stallion. Poor management with poor breeding records and poor hygiene at the time of breeding are perhaps the most common reasonsfor venereal diseases causing severe and irreversible problems in a breeding operation.

16.10.4 Pathologic Conditions of the Reproductive Tract

Clinicians must be able to recognize pathologic conditions accurately to make sound decisions respecting the therapy and management of stallions. Acquired conditions may be reversible by surgical or other therapeutical means, but diagnosis of irreversible or terminal conditions must be well substantiated because the conditions could have a significant effect on the economics of a breeding operation. One also must take into account ethical considerations because some genetic diseases could have a significant effect on the breed as a whole.

16.10.4.1 CONGENITAL DEFECTS

For an individual to develop into a normal male the Y chromosome must be expressed. Horses with genetic abnormalities can vary in their genotype, anatomic features, and behavior.[101] The most common causes of congenital defects are hermaphroditism, XY sex reversal, and testicular feminization or androgen insensitivity.

Hermaphrodites are classified based on the type of gonadal tissue present. *True hermaphrodites* have testicular and ovarian tissue,[101] whereas *pseudohermaphrodites* have testes (male pseudohermaphrodites) or 1158
ovaries (female pseudohermaphrodites), with various combinations of male and female internal reproductive 1159
organs.[102–105] Perhaps the most common of the intersexes is the male pseudohermaphrodite. For the veterinarian to examine a “mare” with a small vulva and an extremely large clitoris or what appears to be a glans penis is not uncommon. Behavior in these animals most likely depends on the gonadal tissue present.

Horses with XY sex reversal syndrome are characterized by female external genitalia but a normal 64XY karyotype.

Androgen insensitivity or testicular feminization syndrome is a well-characterized genetic disease in human beings and in some domestic species. Animals have a normal XY karyotype and male behavior but female genotype. The syndrome has two possible causes: a mutation at the level of the gene that codes for the androgen receptor or a deficiency in 5α-reductase, the enzyme responsible for conversion of testosterone

tothe active androgen dihydrotestosterone.[104] In either situation the reproductive tract is underdeveloped and only external female genitalia are present.

One can diagnose genetic defects tentatively by visual inspection of the external genitalia, rectal palpation, and ultrasonography. However, one can reach a definitive diagnosis only by cytogenetic evaluation. One must submit blood samples in tubes with EDTA anticoagulant to the appropriate laboratory.

16.10.4.2 DISEASES OF THE SCROTUM

The scrotum in the horse is a pliable and thin-skinned pouch with a fine layer of short hair, numerous sweat glands, and the thick muscle layer (dartos). The scrotum is usually darkly pigmented. Functional integrity of the scrotum is vital because it is perhaps the most important structure regulating testicular temperature.[106] Abnormal conformation, absence, or increase in thickness can have a dramatic effect on spermatogenesis. The scrotum is easy to access for examination by visual inspection and palpation. The absence of the scrotum or an extremely small scrotum in most instances indicates genetic abnormalities such as bilateral cryptorchidism or intersex conditions. One should record increases in the thickness of the scrotal wall, surface irregularities, or changes in color of the skin. Lesions of the scrotum can be physical or infectious in origin.

16.10.4.2.1 Physical Problems

Traumatic insults to the scrotum or the scrotal area and other inflammatory processes in the genital area often result in scrotal edema. The clinician must identify the cause or possible causes of the swelling to initiate adequate therapy. Chronic edema of the scrotum can result in abrasions or secondary lacerations and can be complicated further by cutaneous bacterial contamination. Differential diagnoses for scrotal swelling include systemic infectious processes, scrotal herniae, spermatic cord torsion, or hemorrhagic processes associated with the testicles or spermatic cords. One must be sure not to confuse primary edema of the scrotum with peritoneal fluid accumulation between the vaginal and parietal tunics of the testicle.[107] One can use ultrasonographic examination or fine needle aspiration for the differential diagnosis.

One should direct therapy of scrotal edema toward removing the primary cause and controlling the inflammation with supportive therapy. Antiinflammatory drugs, diuretics, and frequent cold-water therapy are measures aimed at reestablishing the circulation in the area. If the skin is broken, one should institute broad-spectrum antibiotic therapy. Gentle and continuous lubrication of the scrotum with emollients and a tetanus toxoid booster are recommended. Depending on the degree of involvement, unilateral or bilateral orchiectomy also may be indicated.[107]

16.10.4.2.2 Infectious Causes

16.10.4.2.2.1 Viral Causes

Equine infectious anemia or diseases causing severe hypoproteinemia can cause scrotal edema. Hypoproteinemia is confirmed by analyzing levels of total protein in serum. One can demonstrate the presence of the equine infectious anemia virus by agar gel immunodiffusion or Coggins test.

During the acute phase of EVA, males often display different degrees of lower abdominal edema that may involve the scrotum.

Neoplastic conditions of the scrotum that have been reported include melanomas and sarcoids. Melanomas have the characteristics of benign tumors of the dermis and epidermis but may become metastatic in somehorses. Sarcoids are common nonmetastatic skin tumors. One diagnoses both conditions by their general appearance and makes definitive diagnosis by histologic sections.[107]

16.10.4.2.2.2 Parasitic Causes

Although *Trypanosoma equiperdum*, the causative agent of *dourine*, is not present in North America, scrotal edema is a typical sign of the initial phases of this venereally transmitted disease.

Onchocerca cervicalis, a microfilaria, and summer sores caused by *Habronema* sp. are seldom a cause of scrotal dermatitis. These infections are transmitted by several species of flies and mosquitoes. Ivermectin therapy at regular intervals is recommended for treatment of these conditions.[108]

16.10.4.2.2.3 Bacterial Causes

Scrotal infections most often result from secondary breaks in the skin caused by castrations, traumatic lacerations, or puncture wounds. Less often, infections of the scrotum can result from septic processes in the testis or peritoneum.[107] In addition to appropriate antibiotic therapy, one should implement 1159
continuous washing of the infected area, antiinflammatory drugs, and supportive therapy. 1160

16.10.4.3 DISEASES OF THE TESTES

The four major cell types of the testis are Sertoli's, Leydig's, myoid, and germ cells. During the last month of gestation or first month of life in the normal horse, the gonads migrate through the inguinal canal into the scrotum. Because of this migration the testis is surrounded by the *tunica vaginalis* or peritoneum and the *tunica albuginea* or the capsule proper.

Sexual developmental abnormalities often are expressed as testicular problems. Among the congenital testicular defects that have been reported in stallions are *monorchidism, anorchidism*, and *polyorchidism*, which are the absence of one or both testicles or the presence of more than two testes, respectively.[109]

16.10.4.3.1 Ectopic Testis

A gonad that fails to reach the scrotum and deviates from the normal path of descent is termed an *ectopic testis*. These testes may be subcutaneous in the inner thigh or abdominal or perineal region. Some authors report splenic-testicular fusion on the left side.

16.10.4.3.2 Cryptorchidism

In a cryptorchid animal, one or both testicles are in an ectopic location for an adult animal, although the gonads remain in the normal path of testicular descent. Cryptorchid testes may be abdominal or inguinal.

Because of the complexity of the process of testicular descent, the exact mechanism for the failure of normal gonadal migration is not well understood. Although cryptorchidism has been speculated to be a hereditary condition, none of 56 colts sired by a cryptorchid Quarter Horse had abnormalities in their testicular descent. If the condition is heritable, perhaps a gene with low penetrance controls the process or is associated with several autosomal genes, as has been shown in men. The incidence of the condition according to Stickle and Fessler[110] and Hayes[111] is believed to be between 15% and 20%. Prevalence is higher in Percherons, followed by Palominos and Quarter Horses. Prevalence in Thoroughbreds is lower than in other breeds.

One can diagnose the unilateral cryptorchid horse easily by palpation of the scrotal contents and the inguinal region. When the testis is closer to the internal inguinal ring, in an abdominal or ectopic location, diagnosis can be difficult. Although frequently unrewarding, rectal palpation and ultrasonography are used as diagnostic aids. The experienced clinician can locate the testis and epididymis in or around the internal inguinal ring just cranial to the pelvic brim slightly off the midline. When palpation and ultrasonography have failed to identify testicular tissue, one should perform hormonal assays. Circulating testosterone and estrone sulfate levels are of great diagnostic value. Because of the variation in baseline testosterone levels, one should measure the hormone after stimulation with 5000 to 10,000 IU of human chorionic gonadotropin.

Collection of serum samples immediately before stimulation and 30 and 60 minutes or 60 minutes and4 hours after the injection is recommended. A twofold to threefold increase in the level of testosterone is diagnostic for testicular tissue. Although false-positive results are rare, false-negative results can occur in 5% to 10% of horses. A single measurement of estrone sulfate has been reported to be highly accurate for diagnosis of the condition in adult cryptorchids. In very young equids, one should interpret low hormonal levels carefully because false-negative results may be more common than in adult horses. The treatment for the condition is invariably castration.

16.10.4.3.3

Gonadal Hypoplasia

Small testes may result from a number of underlying complex processes such as spermatogenic arrest and germ cell deficiencies. Testicular hypoplasia is the failure of the gonads to reach their full adult size and must be differentiated from gonadal atrophy or testicular degeneration, which is the reduction in testicular size after the gonad has reached full adult size. The cause of the hypoplastic gonad is complex and is thought to be congenital or acquired. Although not clear for horses, a genetic component has been identified in other species.[112] In general, testicular hyperthermia, malnutrition and endocrine imbalances particularly in steroid-treated young stallions can affect testicular size negatively. One must remember that the testicles do not start developing before 15 to 18 months and continue to increase in size until the age of 4 to 5 years. One should not confirm testicular hypoplasia before the stallion is 2 to 3 years old. One should discourage the breeding of mares to stallions with hypoplastic gonads; however often because of the value or the performance record, animals with small testicular size are used at stud. In these cases, implementation of managerial practices to maximize the reproductive performance of the animal is important and should include reducing the number of mares in the book and breeding mares only once close to ovulation.

16.10.4.3.4

Gonadal Atrophy

Also known as testicular degeneration, gonadal atrophy is found most commonly in the mature stallion and isa consequence of the disruption of the process of spermatogenesis. One must differentiate atrophy and hypoplasia. A thorough reproductive examination and an accurate history are fundamental in 1160
differentiating the two conditions. One should note inconsistencies between sperm output and testicular 1161
size and patterns of agglutination of the sperm cells. Small testicular size in relation to the epididymis indicates atrophy. However, developmental abnormalities such as a small penis and enlarged inguinal rings associated with small testicular size often indicate hypoplasia. The veterinarian should know that atrophy or degeneration is an acquired condition thatin some cases is reversible.

Testicular atrophy is caused most commonly by administration of anabolic steroids or other products such as altrenogest to colts or stallions during their racing or show careers. Although the negative effects of steroids on testicular function are considered to be reversible, a between length of exposure and age of administration is suspected. Colts injected with anabolic steroids during their first year of life are at greater risk of having permanent damage of the testicular parenchyma. The detrimental effects on spermatogenesis are caused by an increase in the circulating levels of androgens, which in turn havea negative feedback on LH secretion by the pituitary with a consequent decline in endogenous testosterone. The reduction of endogenous testosterone reduces testicular function with a significant reduction in sperm production.

Hyperthermia caused by prolonged recumbency; trauma; torsions; infections with disruption of the blood-testis barrier and the consequent production of antisperm antibodies; inappropriate or prolonged steroid therapy especially with testosterone or anabolic steroids; accumulations of fluids such as in hydroceles; and advanced age have been implicated as possible causes of testicular degeneration. Scrotal lesions that impair the normal testicular thermoregulation may be a significant factor causing gonadal atrophy. Other causes of testicular degeneration include radiation exposure; nutritional disorders, particularly those of vitamin A and zinc; and toxicity with heavy metals or nitrogen, phosphorus, and halogenated compounds.

In the initial phases of testicular degeneration, the testis feels softer. As the process becomes more chronic, the testicular tissue is replaced by connective tissue, making the testis feel firmer on palpation.

Leydig's and Sertoli's cells and spermatogonia and spermatozoa are more resistant to degeneration than cells of the intermediate stages of spermatogenesis; therefore semen analysis varies depending on the extent of damage.[113] In most cases, gonadal atrophy does not affect libido.

Although not easy to do, one can diagnose the condition by evaluating circulating levels of LH, FSH, testosterone, and total estrogens and inhibin. Elevated serum concentrations of FSH often indicate seminiferous epithelial damage,[114] whereas low levels of LH could indicate a pituitary problem. Evaluation of efficiency of sperm production also can aid in the diagnosis of gonadal degeneration.

A degenerative process in the testicle often is reversible, provided one removes the causal factor. The condition of hypogonadotropic hypogonadism in men is treated routinely with Gn-RH administered in a pulsatile manner. Anecdotal evidence suggests that similar conditions in stallions respond to Gn-RH therapy administered as subcutaneous osmotic minipumps. To date, no study indicates the benefit of Gn-RH therapy in stallions with poor semen quality.[114]

16.10.4.3.5

Testicular Hypertrophy/Hyperplasia

Hypertrophy refers to a condition in which the individual cells of the testes increase in size. The most common cause of testicular hypertrophy is removal of one testis, triggering a compensatory growth of the contralateral gonad.

Hyperplasia refers to an increase in the number of cells and can be focal or generalized. Testicular hyperplasiais rare in stallions.

16.10.4.3.6

Testicular Neoplasia

Among domestic species the stallion has a low incidence (4%) of testicular neoplasia.[115,116] Of 30 equine testicular neoplasias, McEntee reported that *teratomas* were the most common (37%), followed by interstitial cell tumors (30%), seminomas (23%), lipomata (7%), and mast cell tumors (3%).[115]

The cause of testicular tumors is not clear. Environmental and genetic factors may be important. Becauseof the alteration in temperature and hormone supply, cryptorchid stallions appear to have a higher incidence of germ cell tumors, particularly Leydig's cell tumors or seminomas, of the retained testis.[117] In general, equine testicular tumors have a low degree of metastatic activity; however, because of the potential of spreading to somatic organs, they are considered malignant. The incidence of regional lymph node involvement appears to be low compared with other domestic species.[118]

Diagnosis of testicular neoplasia if both testiclesare in the scrotum is based on a careful examination of the suspect and the contralateral testis. One should palpate the scrotal contents and note soft spots, nodules, or asymmetry. Ultrasonographic examination is crucial for the identification of fluid-filled or solid nonpalpable lesions embedded deep in the testicular stroma. Spermiogram can be useful if the semen collected has a high incidence of abnormal spermatozoa combined with round spermatids and other testicular cells. One commonly finds low sperm numbers but otherwise normal seminal parameters in horses with testicular neoplasia.[119] 1161

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One can diagnose a testicular tumor in an abdominal testicle by rectal palpation and ultrasonography, combined with endocrinologic tests. Depending on the size and location of the testicle, an inguinal, flank, or ventral midline incision is recommended. Often abdominal tumors are only an incidental finding during routine postmortem examinations.

Gross appearance of some tumors can help the clinician make a presumptive diagnosis. Leydig's (interstitial) cell tumors are usually soft, orange, and nodular withno clear demarcation with the adjacent testicular tissue. Seminomas can vary in color from white to dark gray with a glistening appearance; the neoplastic area frequently bulges above the adjacent testicular tissue. Fluid-filled cysts are often present. Sertoli's cell tumors are usually firm and nodular and pale gray. Teratomas are easily identifiable by the presence of tissue of different origins (bone, hair, etc.). Ultimately, one confirms diagnosis by histopathologic examination. One can obtain samples for histopathologic examination by fine needle aspiration or by testicular biopsy. Unilateral or bilateral orchiectomy is the treatment of choice regardless of the type of tumor. Ligation and removal of as much of the cord as possible is strongly recommended. If one suspects metastasis, excision of the adjacent lymph nodes is recommended.

16.10.4.3.7

Orchitis

An inflammatory process of the testicles is referred to as *orchitis*. The cause of orchitis in stallions may be bacterial, viral, parasitic, or aseptic following trauma. Orchitis can be primary or secondary as a postoperative complication of abdominal surgery. One must differentiate the condition from the more common periorchitis or scrotal edema, although the conditions may be present simultaneously. Bacterial orchitis in horses may be caused by *Brucella abortus, Actinobacillus equuli, Pseudomonas pseudomallei, Streptococcus zooepidemicus* and *S. equisimilis, Salmonella* spp., *Escherichia coli, and Staphylococcus* spp.[115]

Parasitic orchitis is usually a sequela of migratory larvae of the parasite *Strongylus* spp.[120] The condition can affect descended or undescended testicles and the tunics and spermatic cords. A possible secondary lesion associated with the larvae is the condition known as *focal lymphocytic orchitis*, which occurs around the seminiferous tubules.[121] Focal lymphocytic orchitis is different from the condition of autoimmune orchitis reported for the mouse in which foci of lymphocytes are localized exclusively at the rete testis and efferent ductules. Initial diagnosis of the granulomatous-type lesions in the testicle caused by strongylosis sometimes can be done by ultrasonography; however, histopathologic identification is necessary for a definitive diagnosis. Regular deworming programs with ivermectin can help control this condition.

Equine viral arteritis and equine infectious anemia are the primary viral diseases that potentially may affect the testis. The viral agents of equine infectious anemia and EVA are shed in the semen of affected stallions. Onemay observe focal lymphocytic infiltrations in affected stallions.

As soon as spermatogonia enter leptotene stage during the meiotic phase, they become isolated from the general immune system by tight junctions between adjacent Sertoli's cells. These gap junctions are known as the blood-testis barrier. A fine balance is maintained so that maturing spermatids can migrate toward the adluminal compartment without eliciting an immunologic response. In addition to the blood-testis barrier, local immunosuppresors are present in the testicular interstitium. Factors that disrupt the blood-testis barrier with the consequent formation of antisperm antibodies include tumors, trauma, biopsies, and testicular torsions of more than 360 degrees. The association between antisperm antibodies and infertility, although reported for the stallion, warrants further investigation.[122]

16.10.4.4

DISEASES OF THE EPIDIDYMIS

Problems that affect the epididymis can be grouped into congenital abnormalities and into infectious or physical causes. In the stallion a condition known as blind-ending ductules has been observed. If sufficient numbers of tubules are blocked, the condition may lead to spermiostasis with development of cystic dilations, formation of sperm granulomas, and reduction of fertility. One may diagnose these cystic dilations by palpation and ultrasonography.

Because the epididymis in the stallion is not fused completely with the testicle as in other species, a diagnosis of epididymal aplasia or agenesis is not uncommon. In some cases an abdominal testicle may have a caput attached with the corpus and cauda epididymis present in the scrotum. The inexperienced clinician sometimes mistakenly removes an epididymis and leaves the testicle in the inguinal canal or in the abdomen when performing a castration. Epididymal aplasia is rare in the stallion and if present is related to other

anomalies of the wolffian duct system. One commonly detects spermatoceles and cystic dilations by palpation or ultrasonography. Other less common conditions include adenomyosis and tumors.[123]

Bacteria or trauma to the scrotal area may cause inflammation of the epididymis, or epididymitis. Infectious epididymitis as a primary disease is rare in stallions and is considered a sequela to orchitis or to deep lacerations of the scrotal area. However, some authors report the presense of *Streptococcus zooepidemicus* in association with epididymal infection. Migration of *Strongylus edentatus* larvae also may cause epididymitis, with the consequent formation of granulomas.[124] 1162

1163 One confirms a diagnosis of epididymitis by palpation, ultrasonography, the presence of inflammatory cells in the ejaculate, or bacterial growth on culture.[125]

Other causes of epididymal dysfunction can be attributed to abnormal accumulation of sperm in the cauda epididymides or generalized dysfunction of the epididymal epithelium associated with deficiencies in electrolyte, protein, or steroid secretion or resorption. Such dysfunction would cause changes in pH and osmolarity that might affect adversely the ability of sperm cells to fertilize. One should diagnose this condition by frequent semen collections (twice daily for 7 to 10 days) and evaluation of sperm morphology and motility.

16.10.4.5 DISEASES OF THE SPERMATIC CORD

Problems associated with the spermatic cord are limited to infections or vascular problems. Infectious processes of the cord result from larvae migration or secondary contamination with *Streptococcus* spp. after castration. Failure of the castration site to heal and continuous draining of purulent material with intermittent febrile periods often indicates an infection of the spermatic cord known also as scirrous cord or *champignon*. Therapy includes opening the area and aggressive therapy with penicillin.

Vascular problems associated with the cord include torsion, varicoceles, and thrombosis.[126,127] Torsion of the cord is significant when the cord has rotated more than 180 degrees. Torsions of less than 180 degrees are an incidental finding and can be permanent or transient and are of little clinical significance. Torsions greater than 270 degrees are associated with scrotal swelling, severe pain, abnormal gaits, and colic symptoms and are considered an emergency. Diagnosis is by palpation and history, and one must differentiate torsion from scrotal herniae. If the tail of the epididymis is palpable, in torsions of 360 degrees the tail will be located in a dorsal position with respect to the normal stallion. The treatment of choice is hemiorchiectomy because the affected testicle will be nonfunctional. In addition, one should consider the possibility of developing immune-mediated infertility because of antisperm antibody production.[121]

Varicoceles and thrombosis of the spermatic cord are rare in stallions; however, they might interfere with testicular thermoregulation.[126] The presence of varicoceles is of questionable significance in the stallion and is diagnosed easily by ultrasonography. One must ensure that no adhesions of the spermatic cord occur because adhesions may result in fluid accumulation betweenthe testicular tunics.[128] Thrombosis of the cord is a more serious condition. The clinical signs resemble thoseof higher-degree torsions, and unilateral castration is recommended.

16.10.4.6

SEMEN

Problems associated with semen may be divided into two distinct groups: (1) volume, color, and pH/osmolarity of seminal fluids and (2) spermatozoa. Abnormal volumes may be too small or too large. Extremely low volumes are associated with incomplete ejaculation or nonejaculation. Large volumes may be associated with extraneous fluids such as water, urine (urospermia), blood (hemospermia), or pus (pyospermia).[128]

16.10.4.6.1

Urospermia

Urine-contaminated semen is readily detectable. Color, odor, and increase in volume are obvious on gross examination. One may evaluate small amounts of urine for urea nitrogen and creatinine concentration. The effect of urine on the sperm cells is unknown. However, the reduction in motility and perhaps infertility is significant because of the effects of hyperosmotic medium and water removal from the sperm cells. Neurologic or behavioral dysfunctionshave been associated with urospermia.[129] The author has observed several cases associated with self-mutilating stallions. Pharmacologic therapy of the problem is purely empirical and limited to drugs that act to close the neck of the bladder such as α-blockers. A more common approach is to use managerial procedures such as collection of the semen directly into extender and immediate centrifuging. The ideal procedure is to be able to fractionate the ejaculate during collection with an open-ended AV. For breeds for which artificial insemination is not allowed, one may infuse extender into the uterus and flush it out a few hours later.

16.10.4.6.2

Hemospermia

Lacerations of the penis and urethra can result in the presence of blood cells in the semen. Diagnosis is often obvious because of the pinkish or red color of the ejaculate. The presence of blood is believed to interfere with fertility, but no study has proved this. Stallions infected with *Pseudomonas aeuroginosa* may be at a higher risk for hemospermia.[130] Diagnosis is based on identification of the site of bleeding. If one suspects urethral bleeding, one may use a pediatric endoscope to examine the penile and pelvic urethra. If one cannot find the bleeding site externally, a common bleeding site is where the urethra folds over the ischium. A common approach to therapy is to rest the stallion so that cauterization takes place. A complete urethrostomy of the area is indicated sometimes until the urethra heals. One also may consider some of the management procedures described for urospermia.

16.10.4.6.3

Bacteriospermia/Pyospermia

Every ejaculate contains a small amount of mixed bacteria, with no signs of inflammation. These bacteria 1163
are of little significance and should be interpreted in light of the fertility of the stallion. The presence of 1164
large numbers of white blood cells in the ejaculate usually indicates accessory sex gland infection. One should culture quantitative pre- and postejaculatory swabs and semen samples in an attempt to isolate the causative bacteria. Giemsa, Wright's, or Dif-quick stains of the semen aid in identifying the type of white blood cell present.

16.10.4.6.4

Spermatozoal Problems

Spermatozoal defects may be in number, motility, and morphology. Reduction in number, also known as oligospermia, can have several levels of severity culminating in azoospermia, or the lack of sperm in the ejaculate. Poor motility is referred as asthenospermia, whereas poor morphology is known as teratospermia. If the defectsare in number, motility, and morphology simultaneously, the condition is described as oligoteratoasthnospermia. Testicular problems such as degeneration or hypoplasia; sperm stasis in the efferent ducts, epididymis, vas deferens, or ampulla; inadequate collection procedures or ejaculation failure; and increased frequency of ejaculation have a dramatic effect on the total number of sperm present in the ejaculate. Accurate assessment of the sperm production of the stallion and a good history are essential for diagnosis and prognosis of the condition.

Abnormal spermatozoal motility and morphology can result during the process of sperm formation, during posttesticular sperm transport, and epididymal storage and during collection and handling. Improper handling procedures also may adversely affect the quality of a semen sample.

Stallion spermatozoa do not always display the same type of forward progressive motility as observed in human or ruminant sperm. Equine sperm normally may tendto swim in a wide circular pattern.[130] Backward motility, tight circular movement, or static motion (not boundto the glass) is considered abnormal. Midpiece and tail abnormalities—whether induced by cold shock, by other mechanical means, or as a direct result of spermatogenesis, spermiogenesis, and epididymal transport—are the most common causes of motility abnormalities.[128]

Every ejaculate contains some degree of sperm abnormalities, but in general normal stallions are expected to have at least 60% morphologically normal sperm. One can evaluate spermatozoal morphology by diluting raw semen in buffered formal saline under phase-contrast microscopy or under light microscopy using eosin-nigrosin or eosin–aniline blue stains (see the previous section).

16.10.4.7

DISEASES OF THE PREPUCE AND PENIS

16.10.4.7.1

Prepuce

Preputial inflammation or posthitis may be traumatic or infectious. Regardless of the origin, inflammation of the prepuce invariably results in phimosis or paraphimosis, which is the ability to exteriorize or retract the penis, respectively. One should differentiate inflammation caused by trauma, infection, or parasitic diseases from edema following priapism or penile paralysis. Traumatic injuries to the prepuce most commonly are associated with breeding accidents that can be avoided with proper breeding management practices.

Infectious, parasitic, and neoplastic preputial problems are similar to those affecting the scrotum and were described previously. Infections caused by equine herpesvirus 3 or coital exanthema are a common occurrence, as is colonization of the penis by gram-negative bacteria. Congenital problems affecting the prepuce, penis, and scrotum often are linked to developmental abnormalities and are diagnosed easily.

16.10.4.7.2

Penis

16.10.4.7.2.1

Penile Tumors

The most common neoplasia affecting the penis is squamous cell carcinoma. Smegma accumulation on the penis may be a predisposing factor for this type of neoplasia. The tumors start as small keratinized plaques that slowly progress into necrotic foci with foul-smelling material caused by secondary bacterial contamination. Conclusive diagnosis is based on histopathologic examination. Treatment of the condition is with cryosurgery, reefing, or phallectomy.

Penile warts or squamous papillomas, sarcoids, melanomas, fibromas, and lipomas may occur on the penis. Cryotherapy or autogenous vaccines have been used with varying degrees of success to treat these conditions.

16.10.4.7.2.2

Penile Paralysis/Priapism

Malnourished or exhausted horses, animals with neurologic disease, or treatment with phenothiazine derivatives sometimes may induce loss of tone of the retractor penis muscle in horses that culminates in relaxation of the penis, extensive penile edema, and secondary trauma.[131,132] Because venous return is impaired, the condition progresses to the development of ulcers, secondary bacterial contamination, and necrosis. Treatment of the condition aims to restore the venous blood flow. Hydrotherapy, mechanical support of the penis, and application of topical emollients, antiinflammatory drugs, and diuretics are recommended. In some instances, flushing of the corpus cavernosum penis with heparinized saline or intracavernous injection of phenylephrine may aid in blood drainage and penile retraction. Traumatic accidents involving the penis are not uncommon. Cuts with mare tail hair, poorly constructed breeding phantoms, or AVs may result in laceration or abrasion of the penis varying from simple skin cuts to severe hematomas. One treats these wounds with supportive therapy or surgical intervention. Prevention of secondary contamination and adhesion formation is important. 1164

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Other mechanical problems observed in the penis and prepuce of stallions include fibrosis, strictures, or lacerations caused by the misuse of penile rings or brushes to dissuade the stallion from masturbation. The use of such instruments is inhumane and should be discouraged.

16.10.5

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17 # CHAPTER 17 DISORDERS OF THE URINARY SYSTEM

17.1 ## 17.1—Anatomy and Development

Harold C. Schott, II

17.1.1 ### Anatomy

The urinary system of the horse, like that of most mammals, consists of paired kidneys and ureters, the bladder, and the urethra. With the exception of the abdominal portion of the urinary bladder, the entire urinary tract is located in the retroperitoneal space. In a newborn foal, each kidney weighs about 175 g. In an adult horse the left kidney weighs 600 to 700 g, and the right kidney is usually 25 to 50 g heavier, although this is not a consistent finding, and one may observe the reverse relation.[1,2] Thus the kidneys account for approximately 0.65% to 0.75% and 0.27% to 0.37% of the total body mass of the foal and adult horse, respectively.[1,3] The right kidney is located immediately below the dorsal extent of the last two or three ribs and the first lumbar transverse process, is shaped like a horseshoe, and measures about 15 cm long, 15 cm wide, and 5 to 6 cm high (dorsal to ventral). Craniolaterally, the right kidney is embedded into the liver, and its more craniad position compared with the left kidney prevents it from being accessible on rectal palpation. Although not the classically bean-shaped organ found in human beings and small animals, the left kidney is more elongated than the right kidney, with the cranial pole at the level of the hilus of the right kidney and is about 18 cm long, 10 to 12 cm wide, and 5 to 6 cm high. Because of its more caudal location, one routinely can palpate the caudoventral aspect of the left kidney during rectal examination. The blood supply to the kidneys comes from one or more renal arteries branching from the aorta. Accessory renal arteries (which generally enter caudally) may arise from the caudal mesenteric, testicular or ovarian, or deep circumflex iliac arteries.[1,2]

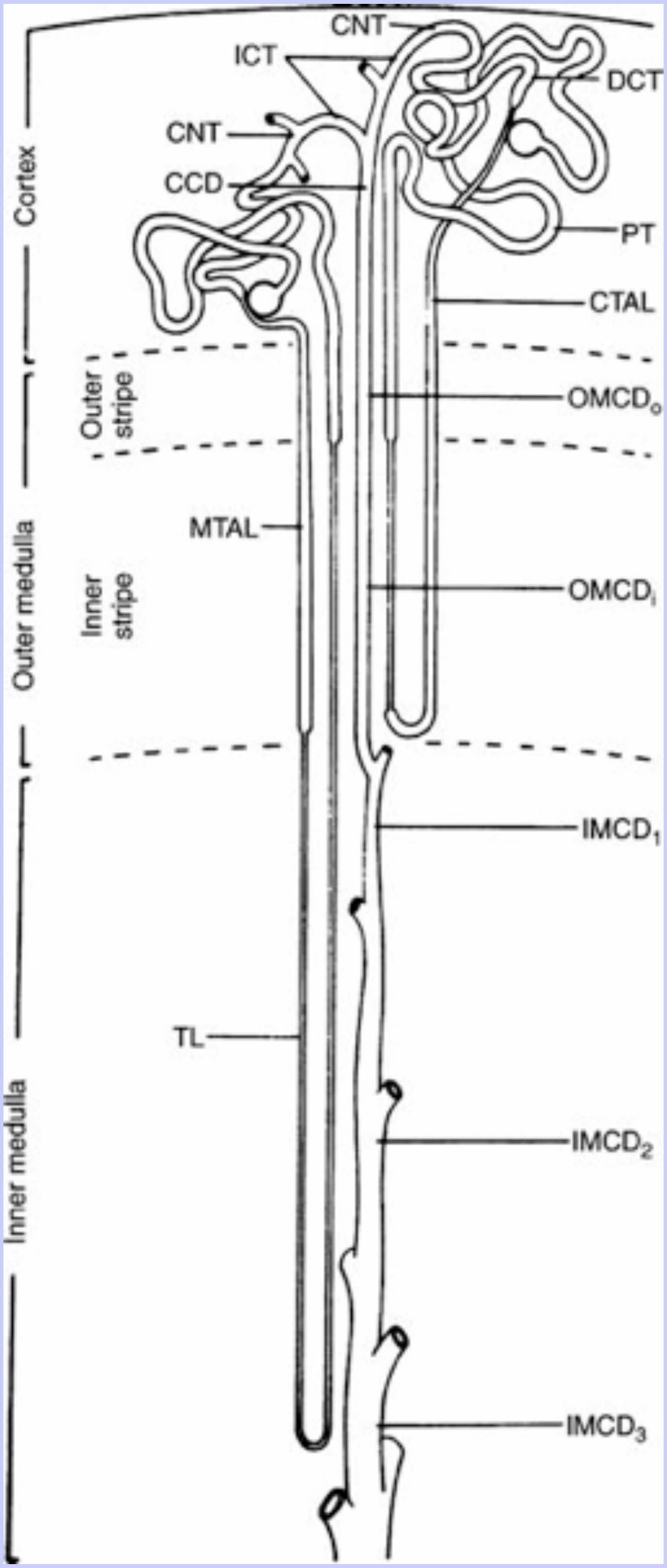
The ureters are 6 to 8 mm in diameter and travel about 70 cm to their insertions in the dorsal bladder neck or trigone, close to the urethra. The distal 5 to7 cm of each ureter courses within the bladder wall. This intramural segment of the ureter functions as aone-way valve to prevent vesicoureteral reflux with progressive bladder distention. The urinary bladder lies on the pelvic floor when empty but can increase in size and drop forward over the pelvic brim when filled with urine. The bladder can accommodate up to 3 to 4 L of urine before stimulation of micturition. In the foal the bladder is attached to the ventral abdominal wall by the urachus and remnants of the umbilical arteries. Consequently, when empty, the bladder is commonly a band-shaped structure in a neonatal foal. During the first few months of life, this ventral attachment loosens as the urachal remnant becomes the middle ligament and the umbilical arterial remnants become the round ligaments of the free border of the paired lateral ligaments of the bladder.[1]

The urethra is about 2 to 3 cm long in a mare and75 to 90 cm long in a male. In the intact male the pelvic urethra, which is 10 to 12 cm long, widens in an elliptic pattern to a diameter of 5 cm across and 2 to 3 cm from dorsal to ventral. A rounded dorsal prominence, the colliculus seminalis, is located immediately caudal tothe urethral orifice and is the site of the common openings of the ductus deferens and ducts of the seminal vesicles. The openings of the prostatic ducts are on two groups of small papillae lateral to the colliculus seminalis. 1169
Between 2 and 3 cm farther caudad, the ducts of the bulbourethral glands open in paired dorsal lines. The 1170
smaller openings of the ducts of the lateral urethral glands open at the same level on the lateral aspect of the urethra.[1]

The surface of each kidney is covered by a fibrous capsule that peels easily from the normal kidney. The equine kidney consists of an outer cortex slightly wider than the inner medulla. The cortex is dotted with dark spots—renal corpuscles or glomeruli within Bowman's capsules. In horses, the corticomedullary junction is less distinct than in other species and is typically a deep red that contrasts well against the paler medulla and red-brown cortex. This region undulates along renal pyramids (cortex) and renal columns (medulla). The pyramidsare subdivisions of the renal parenchyma, which are separated by arcuate arteries at the level of the corticomedullary junction. The equine kidney contains a total of 40 to 60 pyramids arranged in four parallel rows. The renal pelvis is the dilated proximal portion of the ureter. Microscopic examination reveals numerous small openings of the collecting ducts (ducts of Bellini). Additionally, the renal pelvis and proximal ureter are lined with compound tubular mucous glands and goblet cells that secrete thick, viscid mucus usually found in the renal pelvis and urine of normal horses.[1,4]

The functional unit of the kidney is the nephron. Each nephron is composed of a renal corpuscle (glomerulus within Bowman's capsule), a proximal tubule (convoluted and straight components), an intermediate tubule (loop of Henle), a distal convoluted tubule, a connecting tubule, and cortical, outer medullary, and inner medullary collecting ducts (Figure 17.1-1). The two populations of nephrons are (1) the superficial (or cortical) nephrons possessing short loops of Henle and (2) the juxtamedullary nephrons with long loops of Henle. Gradations exist between these two general categories of nephrons, as well as species variationin the ratio of short-looped nephrons to long ones. For example, human beings have 7 times more short- than long-looped nephrons, whereas essentially 100% of nephrons in dogs and cats have long loops.[5] An early anatomic study found approximately 4 million glomeruli (nephrons) in the adult bovine kidney[6]; however, a recent study of kidney organogenesis using unbiased stereologic techniques to examine 45 equine left kidneys indicated that the left kidney of the horse may contain closer to 10 million glomeruli (for a total of 20 million in both kidneys).[7] The latter study also confirmedthat the total number of glomeruli does not increase after birth despite continued growth of the kidney until about 1 year of age. At present, little information is available on the ratio of short- to long-looped nephrons in horses. Histologically, equine nephrons are similarto those of other 1170
mammalian species; however, the diameter and epithelial height of the tubule and collecting duct segments are 1171
comparatively larger. In addition, the equine macula densa (segment of the ascending loop of Henle that lies in close association with the juxtaglomerular apparatus of the afferent arteriole) appears more prominent than that of other mammals.[8] Whether these subtle histologic differences are accompanied by functional differences has not been investigated.

Figure 17.1-1 Diagram of a superficial and juxtamedullary nephron. *TL*, Thin limb of Henle's loop; *MTAL*, medullary thick ascending limb of Henle's loop; *CCD*, cortical collecting duct; *CNT*, connecting segment; *ICT*, initial collecting tubule; *DCT*, distal convoluted tubule; *PT*, proximal tubule; *CTAL*, cortical thick ascending limb of Henle's loop; $OMCD_0$, collecting duct in outer stripe of outer medulla; $OMCD_1$, collecting duct in inner stripe of outer medulla; $IMCD_1$, outer third of inner medullary collecting duct; $IMCD_2$, middle third of inner medullary collecting duct; $IMCD_3$, inner third of inner medullary collecting duct. (From Tisher CC, Madsen KM: Anatomy of the kidney. In Brenner BM, Rector FC, editors: *The kidney*, ed 6, vol 1, Philadelphia, 2001, WB Saunders.)


CNT
ICT
DCT
CNT
CCD
Cortex
PT
CTAL
Outer stripe
OMCD$_o$
MTAL
Outer medulla
Inner stripe
OMCD$_i$
IMCD$_1$
TL
Inner medulla
IMCD$_2$
IMCD$_3$

Relative to its size, the mammalian kidney has aricher innervation than almost any other organ.[9] Although the neuroanatomy of the equine kidney has not been well studied, autonomic nerves course from the aorticorenal and renal ganglia along the major renal vessels into the kidneys.[1] These nerves are predominantly sympathetic, for the kidneys appear to be poorly supplied by cholinergic nerves. Although the best-recognized effect of renal nerves is control of renal vascular resistance (for regulation of renal blood flow over a wide range of perfusion pressures), the nerves also act directly on renal tubules and juxtaglomerular cells. For example, low-frequency stimulation of renal nerves (below the threshold for vasoconstriction) increases proximal tubular sodium reabsorption and renin release by activation of α_1-adrenoceptors.[10] In addition to α- and β-adrenoceptors, renal vasculature is rich in dopaminergic adrenoceptors, and activation of the latter, specifically dopamine type 1 receptors, leads to increased perfusion of the outer renal medulla. Presence of these receptors is the basis for use of dopamine, and more recently the DA-1 receptor agonist fenoldopam, in an attempt to improve renal blood flow in acute renal failure or to decrease the risk of radiocontrast nephropathy.[11–13] The administration of drugs also can activate renal adrenoceptors unintentionally. A common clinical example is the diuresis induced by administration of the α_2-agonists xylazine and detomidine. Although the diuresis has been attributed to a transient hyperglycemia and glucosuria, the latter is often absent.[14,15] An alternative explanation may be drug binding to α_2-adrenoceptors located on collecting duct epithelium. Activation of these receptors can lead to antagonizm of the effects of antidiuretic hormone on cortical collecting ducts, which results in diuresis.[16] More recently, renal afferent nerves have been identified, and these nerves appear to play a role in the pathogenesis of hypertension in species affected by this disorder.[9]

Autonomic innervation of the ureters, bladder, and urethra is important to ureteral peristalsis and micturition. The equine ureteral smooth muscle contains α_1- and β_2-adrenoceptors, which induce contraction and relaxation, respectively, when activated by norepinephrine.[17] Recent studies of the innervation of the equine ureter demonstrated greater densities of adrenergic neurons in the proximal (renal pelvis) and intravesicular (bladder wall) portions of the ureter.[18] Increased densities in these regions are consistent with the suspected pacemaker activity of the renal pelvis, which initiates ureteral peristalsis and the sphincterlike function of the distal segment of the ureter. The sympathetic nerve supply to the urinary bladder is provided via the hypogastric nerve, with preganglionic fibers arriving from spinal segments L1 to L4 to synapse in the caudal mesenteric ganglion. Postganglionic fibers supply the bladder (β_2-adrenergic receptors) and proximal urethra (primarily α_1- and some α_2-adrenergic receptors).[19,20] In addition to adrenergic innervation, the equine bladder also is innervated by cholinergic and peptidergic nerve fibers.[21] Parasympathetic innervation originates in the sacral segments of the spinal cord with neurons joining to form the pelvic nerve.[19,20] Many complex interneuronal connections exist between sympathetic and parasympathetic nerves in the wall of the bladder, along with small adrenergic cells that facilitate interaction between sympathetic and parasympathetic pathways.[22] As a result, complete denervation of the bladder is virtually impossible. Somatic innervation of the lower urinary tract is primarily to the striated muscle of the external urethral sphincter via a branch of the pudendal nerve, which originates from the sacral cord segments (S1 to S2).[1]

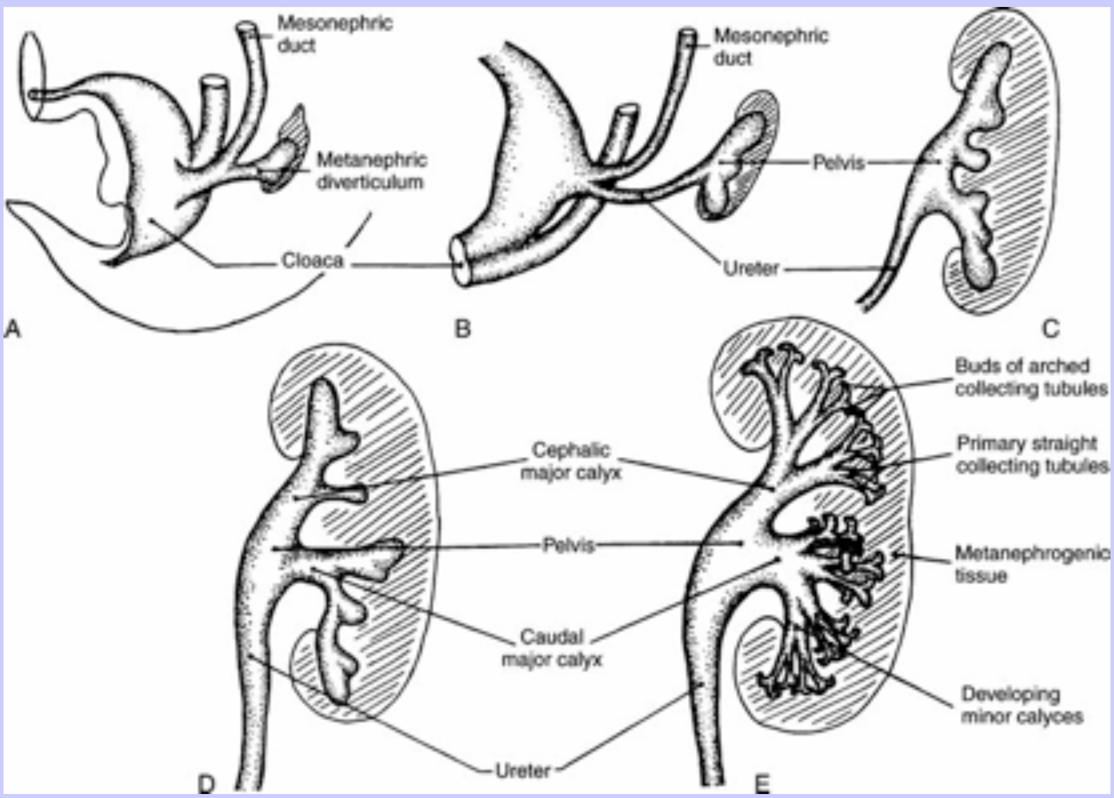
17.1.2 Development

The embryonic upper urinary tract arises from bilateral primordial mesonephric ducts and intermediate mesoderm. The metanephric diverticulum originates from the caudal end of each mesonephric duct and develops craniad to become the ureter and renal pelvis. The advancing metanephric diverticuli collect about

their ends intermediate mesoderm (metanephrogenic tissue), which becomes the collecting system and parenchyma of the mature kidney (Figure 17.1-2). The vascular supply is derived from a branch of the aorta (renal artery) that invades the metanephrogenic tissue. The urinary bladder develops as a dilated proximal portion of the allantois. The bladder is separated from the hindgut by the craniocaudad growth of the urorectal fold, which divides the rectum from the urogenital sinus. The latter structure gives rise to the urethra (Figure 17.1-3). The mesonephric and metanephric ducts initially open into the urogenital sinus, but as development continues, the distal segments of the mesonephric ducts are absorbed into the bladder wall and the openings of the metanephric ducts are pulled craniad to their final site in the dorsal bladder neck.[23]

The fate of the mesonephric tubules (mesonephros) and mesonephric ducts varies with gender. Paired paramesonephric ducts (müllerian ducts) arise parallel to the mesonephric ducts in both sexes. In the female, the 1171
ducts fuse distally to become the vagina and uterine body, whereas proximally they remain separate to give rise 1172
to uterine horns and oviducts. The mesonephric ducts regress into vestigial remnants termed the *epoöphoron* proximally (near the ovaries) and *Gartner's canals* distally (near the vagina and uterus) (Figure 17.1-4). In the male, sexual differentiation of the gonads and production of androgenic steroid hormones leadto regression of the müllerian ducts. The duct system of the male reproductive tract is appropriated from the mesonephros and mesonephric ducts (also termed *wolffian ducts*). Androgenic steroid hormones also stimulate these structures to develop into the seminiferous tubules, epididymis, and ductus deferens. The distal portion of the mesonephric duct becomes the ejaculatory duct, the terminal portion of the ductus deferens.[23]

Figure 17.1-2 Progression of the differentiation and development of the metanephric diverticulum into the collecting system of the mature kidney. **A**, Metanephric diverticulum originates from the caudal end of the mesonephric duct. **B**, Metanephric diverticulum develops craniad, and intermediate mesoderm (metanephrogenic tissue, hatched lines) collects about its cranial end. **C** to **E**, The metanephric diverticulum becomes the ureter and renal pelvis, and the metanephrogenic tissue becomes the collecting system and parenchyma of the mature kidney. (From Patten BM, Carlson BM: *Foundations of embryology*, ed 3, New York, 1974, McGraw-Hill.)



17.1.3 Developmental Malformationsof the Urinary Tract

Anomalies of the urinary tract are uncommon in horses. A survey by Höflinger revealed a similar frequency of unilateral renal agenesis (0.07%) in horses[24] and human beings (0.10%).[5] In contrast, horseshoe kidneys

(attached at the cranial or caudal poles) are the most common anomaly in human beings (0.25%) but rarely have been described in horses.[5,25]

17.1.3.1 RENAL AGENESIS, HYPOPLASIA, AND DYSPLASIA

Renal agenesis, which may be unilateral or bilateral, results from failure of the metanephric duct to fuse with the metanephrogenic mesodermal tissue. Although unilateral anomalies have been described more frequently, this simply may reflect the incompatibility of bilateral agenesis with postnatal life.[24,26–28] Brown, Parks, Mullaney, et al. described a foal with bilateral renal agenesis in which severe azotemia was detected shortly after birth. Bilateral ureteral dysgenesis and cryptorchidism, agenesis of the right adrenal gland, and atresia ani accompanied the renal agenesis in this foal.[28] Unilateral defects may be incidental findings in otherwise healthy horses[29] or may be detectable during examination ofthe reproductive tract, because many horses have associated anomalies of that system. Occasionally, unilateral agenesis may result in clinical renal disease if a problem arises in the contralateral kidney. Johnson, Klingborg, Heitman, et al. described a 4-year-old Quarter Horse with unilateral renal agenesis and a ureterolith causing contralateral hydronephrosis. The gelding was presented for weight loss, pollakiuria, and stranguria. In addition to the renal anomaly, unilateral agenesis of the ipsilateral testicle also was found on necropsy.[27] Renal agenesis may be a familial disorder in several species.[25,30] Although no information is available to suggest a hereditary basis in horses, one probably should discourage repeat matings after detecting such an anomaly.

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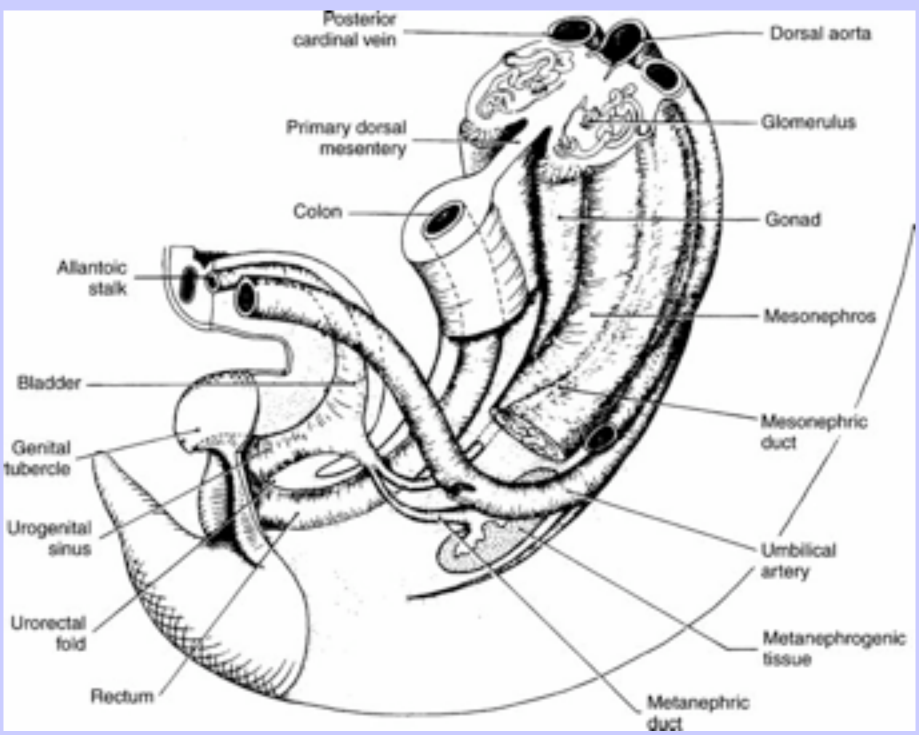
Figure 17.1-3 Developing urogenital tract of the young mammalian embryo. (From Patten PM, Carlson BM: *Foundations of embryology*, ed 3, New York, 1974, McGraw-Hill.)



One diagnoses renal hypoplasia when one kidneyis at least 50% smaller than normal or when the totalrenal mass is decreased by more than one third.[25] Renal hypoplasia is a quantitative defect caused by a reduced mass of metanephrogenic tissue or by incomplete induction of nephron formation by the metanephric duct. The condition may be confused with renal dysplasia. Unilateral renal hypoplasia usually is associated with contralateral hypertrophy and normal renal function, whereas bilateral hypoplasia generally leads to chronic renal failure.[25,30] Andrews, Rosol, Kohn, et al.[31] described bilateral renal hypoplasia in a foal presented after death and in three young horses with chronicrenal failure that had poor growth from birth. Anomalies in these four horses were limited to the upper urinary tract.

Renal dysplasia is disorganized development of renal tissue caused by anomalous differentiation, intrauterine ureteral obstruction, fetal viral infection, or teratogens.[25,32] Bilateral dysplasia usually leads to renal failure. In general, dysplastic kidneys are normal in size unless concurrent hypoplasia exists or the animal lives for 1173
months to years before developing renal failure. Roberts and Kelly reported a case of bilateral renal dysplasia 1174
in a 19-month-old pony gelding.[33] The pony was presented for weight loss over a 3-month period, and clinicopathologic assessment revealed chronic renal failure. A small, firm, and nodular left kidney was palpable per rectum. At necropsy, the kidneys weighed 280 g each (33% smaller than normal for body weight) and were nodular. Renal dysplasia was suspected because glomeruli in the collapsed areas of the kidneys were small, tubules were immature, and inflammatory cells were scant. Six similar cases of bilateral renal dysplasia resulted in chronic renal failure in horses from 2 months to 7 years of age.[34–38] Small kidneys with increased echogenicity and an indistinct corticomedullary junction were typical ultrasonographic findings,[36–38] and these findings were corroborated by computed tomography in one Miniature horse foal.[38] At necropsy, kidneys were typically small and irregular, the cortex and medulla were not well-delineated, and immature glomeruli and primitive tubules were found on histologic examination (Figure 17.1-5). Renal dysplasia also may cause renal failure in neonates. For example, Zicker, Marty, Carlson, et al. reported a case 1174
of renal dysplasia in a 2-day-old Quarter Horse foal presented for diarrhea and depression.[39] 1175
Clinicopathologic assessment revealed azotemia, hyponatremia, hypochloremia, and urinary sodium wastage. At necropsy, the kidneys were normal in size (380 g), but histologic examination revealed immature glomeruli, hypoplastic tubules and vasa recta, and extensive myxomatous connective tissue occupying 90% of the total medullary volume. Finally, renal dysplasia also may be a unilateral problem that does not result in renal failure. Jones, Langer, Sterner-Koch, et al. found ureteropelvic polyps to be the cause of unilateral hydronephrosis and renal dysplasia in a Trakehner colt.[40] Poor growth and hematuria of several weeks' duration were the presenting complaints. Renal function remained normal for 8 months following nephrectomy until the colt developed a severe bout of colic, prompting euthanasia. Ureteral obstruction by the polyps was the suggested cause of renal dysplasia, because urinary tract obstruction has been found in a large percentage of cases of human renal dysplasia.[32]

Figure 17.1-4 Development of the mesonephric tubules (mesonephros) and mesonephric ducts into the female reproductive tract. **A** and **B**, Paired paramesonephric ducts (müllerian ducts) arise parallel to the mesonephric ducts in both sexes. **C** to **E**, In females, the paramesonephric ducts fuse distally to become the vagina and uterine body but remain separate proximally to give rise to uterine horns. **D** and **E**, The mesonephric ducts regress into vestigial remnants termed *Gartner's canals*. (From Patten BM, Carlson BM: *Foundations of embryology*, ed 3, New York, 1974, McGraw-Hill.)

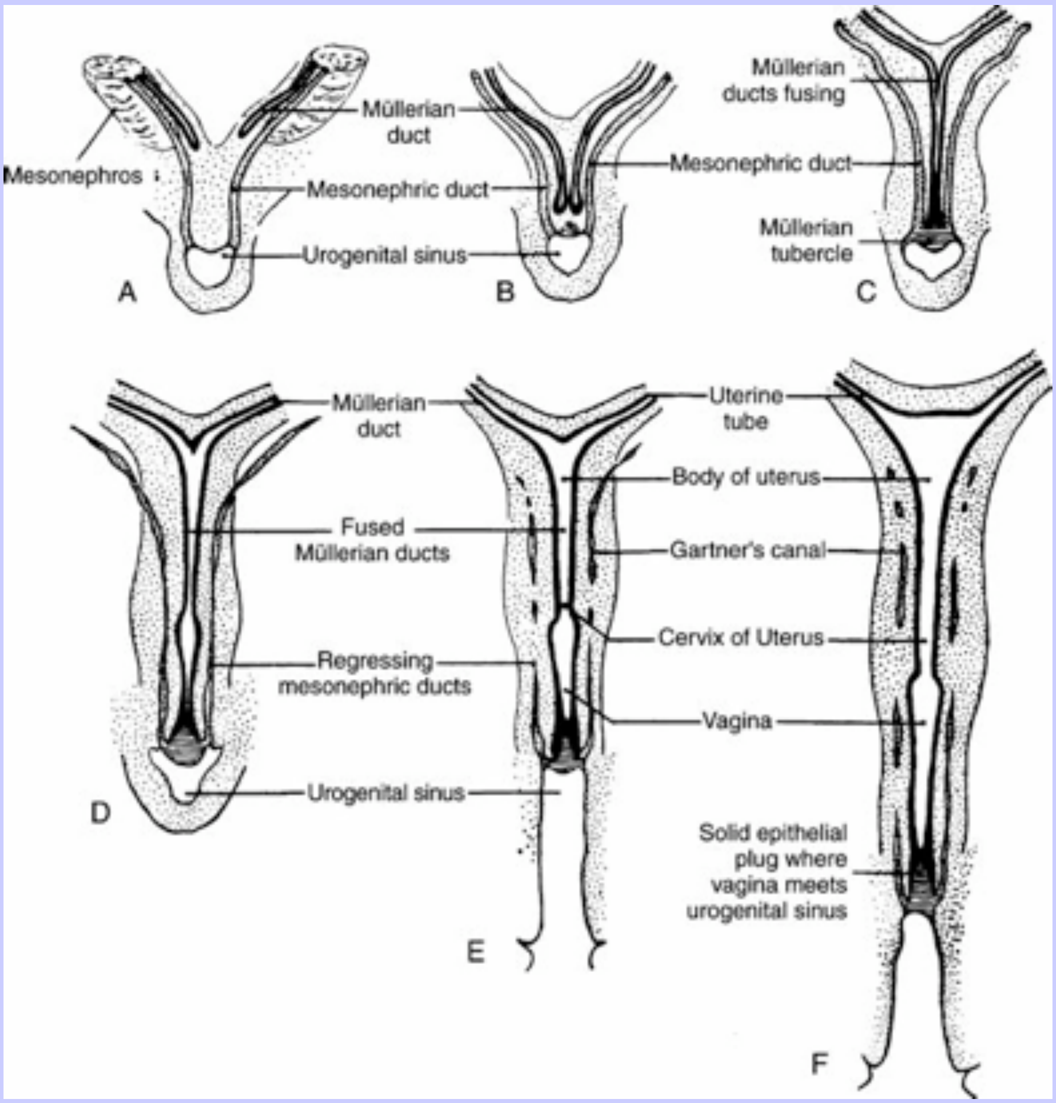
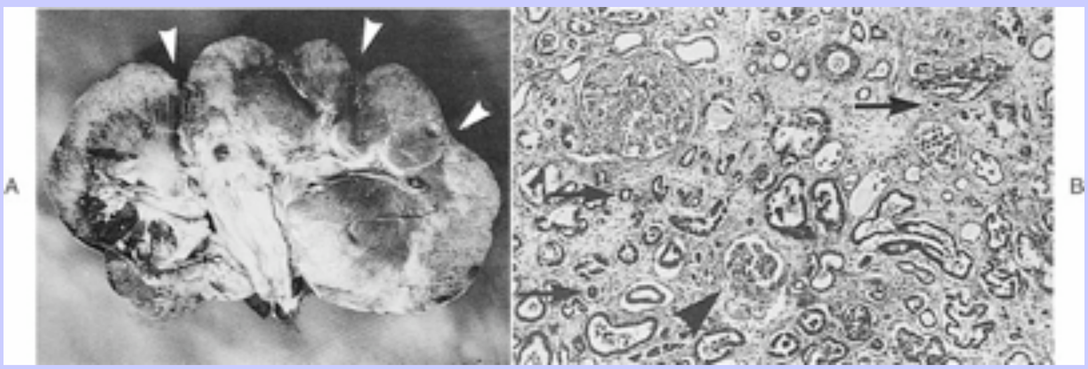


Figure 17.1-5 A, Longitudinal section of the right kidney from a 7-year-old Arabian gelding with renal dysplasia shows focal, irregular thinning of the cortex *(arrowheads)* resulting in a nodular surface and poor delineation of the corticomedullary junction. **B**, Histologic section of same kidney (Masson trichrome stain; original magnification ×35) reveals an immature glomerulus *(large arrowhead)* and primitive tubules *(arrows)* surrounded by persistent mesenchyme. (From Ronen N, van Amstel SR, Nesbit JW et al: Renal dysplasia in two adult horses: clinical and pathological aspects, *Vet Rec* 132:269, 1993.)



17.1.3.2

RENAL CYSTS

One or more renal cysts occasionally are discovered as incidental findings on necropsy examination. The cysts may arise from any portion of the nephron but more often occur in the cortex than in the medulla. The pathogenesis is not known, but a defect in the basement membrane that allows tubular dilation is suspected. Renal cysts vary in size from microscopic to as large as the organ itself and routinely have a clear to slightly opaque wall and contain a thin, clear fluid. Congenital cysts are differentiated easily from acquired cysts (following obstruction) by the extensive scarring that accompanies the latter. Renal cysts also may develop as a consequence of drug therapy (i.e., long-acting corticosteroids) or exposure to certain chemicals.[25,30]

17.1.3.3

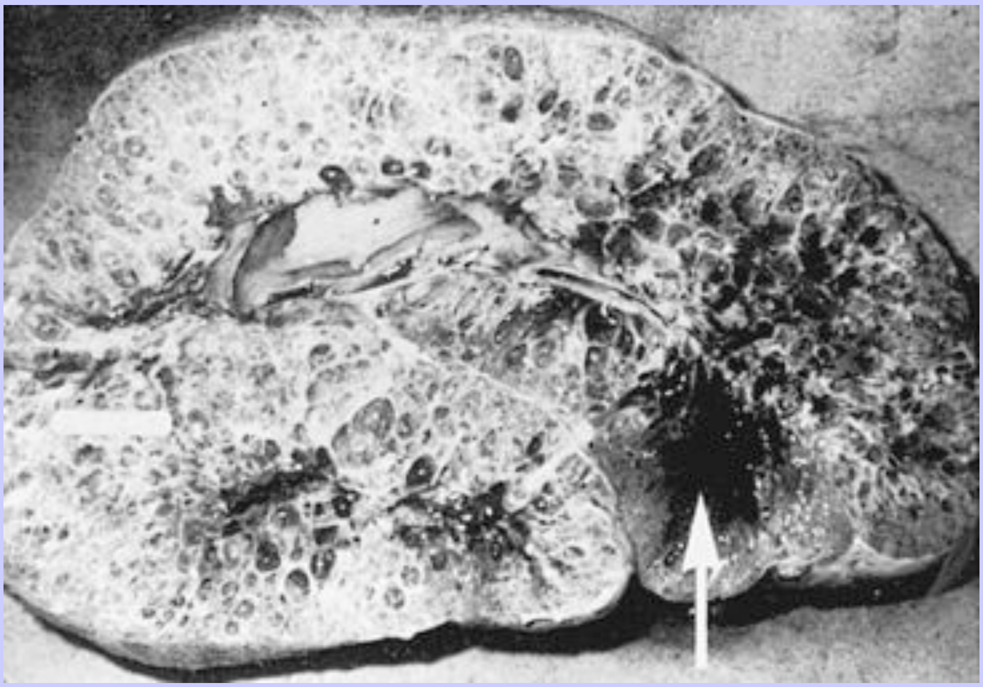
POLYCYSTIC KIDNEY DISEASE, GLOMERULOCYSTIC DISEASE, AND OTHER HEREDITARY NEPHROPATHIES

Polycystic kidney disease (PKD) is a disorder in which numerous, variably sized cysts are found throughout the cortex and medulla. With glomerulocystic disease, cysts are microscopic and limited to Bowman's spaces. Cysts of the bile duct and pancreas also may occur with PKD, and both conditions have been described in stillbirths in many species, including foals.[25] The two major types of human PKD are (1) a rare

congenital or infantile form inherited as an autosomal recessive trait (which may be found in stillbirths) and (2) a more common adult form inherited as an autosomal dominant trait that leads to renal insufficiency in later life in association with dramatically enlarged, cystic kidneys.[41,42] The latter form of PKD develops because of mutations in genes encoding for polycystins, integral membrane proteins responsible for cell-to-cell interaction.[43] Autosomal dominant PKD also has been documented in Persian cats and related breeds and 1175
in bull terriers.[44-46] The genetic defect in Persian cats is thought to be similar to the most common defect in 1176
human beings (*PKD1* gene) and leads to end-stage renal disease by 3 to 10 years of age.[45] As in human beings, the disorder is detectable by renal ultrasonographic screening of juvenile cats and preventable by avoiding subsequent mating of affected animals. Nevertheless, because of heavy inbreeding the prevalence of PKD in Persian cats and related breeds is between40% and 50%.[44,45]

Ramsay, Rothwell, Gibson, et al. described polycystic kidneys in a 9-year-old Thoroughbred mare that exhibited anorexia and weight loss. Clinicopathologic assessment revealed chronic renal failure, and euthanasia was performed. At necropsy, the kidneys were grossly enlarged and each weighed 12 kg (Figure 17.1-6).[47] A similar case of bilateral PKD was described in a 15-year-old pony with a 4-week history of hematuria and moderate weight loss. Evaluation revealed azotemia and presence of large masses in the area of both kidneys on rectal examination, and dramatically enlarged polycystic kidneys weighing 11.4 and 9.1 kg, respectively, were found at necropsy.[48] Bertone, Traub-Dargatz, Fettman, et al. reported a third case of adult PKD in a 10-year-old Paint gelding with weight loss.[49] The horse was mildly azotemic, and several 2- to 15-cm diameter cysts were imaged in both kidneys during ultrasonographic examination. In human beings, polycystic kidneys are believed to result in renal failure as cysts expand (sometimes under pressure) and compress adjacent normal renal tissue. Altered compliance of tubular basement membranes and proliferation of renal tubular epithelium result in outflow obstruction and proximal ballooning, leading to renal cyst formation.[42] In some human cases, pressure within cysts may be 5 to 10 times higher than surrounding interstitial tissue pressures. Bertone, Traub-Dargatz, Fettman, et al. found no increase in pressure in several cysts catheterized percutaneously in a gelding with polycystic kidney disease, but differences in sodium concentrations suggested that the sampled cysts had arisen from different segments of the renal tubule.[49] Euthanasia was performed after a prolonged hospital course(235 days), and the kidneys were not grossly enlarged except where distorted by large cysts. Although not well documented, PKD has been described anecdotally in two additional Paint horses, suggesting that an inherited form of PKD may occur in that breed. A recent report also documented PKD in an 11-year-old Andalusian gelding.[50]

Figure 17.1-6 Longitudinal section of the left kidney (35 cm long, 25 cm wide, 20 cm deep, and weighing 12 kg) from a9-year-old Thoroughbred mare with polycystic kidneys. A calculus is located in the renal pelvis, and the arrow demonstrates the only grossly normal-looking renal parenchyma. (From Ramsey G, Rothwell TLW, Gibson KT et al: Polycystic kidneys in an adult horse, *Equine Vet J* 19:243, 1987.)



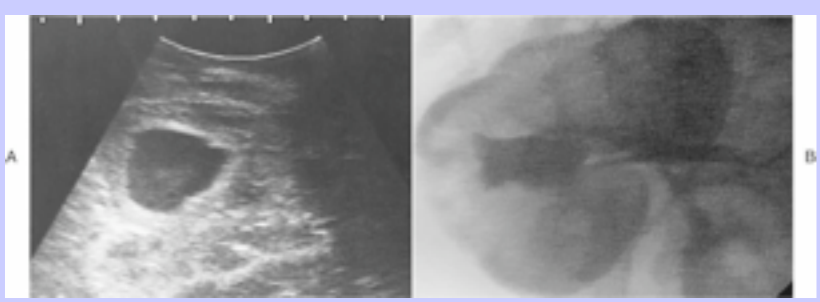
In addition to PKD, a variety of other hereditary nephropathies have been described in human beings.[48] Similar disorders are starting to be recognized in domestic animals, including hereditary nephritis in bull terriers, Samoyeds, and English cocker spaniels. Analogous to Alport's syndrome in human beings, a defective molecular structure of type IV collagen, an important component of the glomerular basement membrane, appears to be the cause of hereditary nephritis in these dog breeds.[46] Similarly, a syndrome of renal tubular dysplasia with autosomal recessive pattern of inheritance recently has been described in a population of highly inbred Japanese black cattle,[51–53] as has a syndromeof suspected hereditary renal oxalosis in Beefmaster calves.[54] Similar hereditary nephropathies are likelyto occur in horses but to date the only one documented is a syndrome of nephrogenic diabetes insipidus in Thoroughbreds.[55]

17.1.3.4

VASCULAR ANOMALIES

Anomalies of the vascular supply to the equine urinary tract are rare but may result in hematuria, hemoglobinuria, partial ureteral obstruction, or hydronephrosis.[30,56] Latimer, Magnus, and Duncan described a distal aortic aneurysm and associated extrarenal arterioureteral fistula in a 5-month-old colt presented for intermittent hematuria, colic, and lameness. Partial ureteral obstruction and hydronephrosis were observed on the affected side.[57] Intrarenal vascular anomalies, termed *renal arteriovenous malformations*, are similarly rare (reported frequency of 0.04% in human beings).[58] Interestingly, these vascular malformations may be silent until later in life, when varying degrees of hematuria and flank pain may ensue. The anomalous vessels are often tortuous and may be enlarged focally and devoid of elastic tissue. Hematuria and hemoglobinuria 1176
are thought to arise from areas where the anomalous vessels lie close tothe collecting system.[58,59] With 1177
vascular anomalies, one should attempt to determine the extent of the defect (unilateral or bilateral) via ultrasonographic examination, contrast radiographic studies, or cystoscopy (visualization that hematuria is coming from only one ureteral orifice). When a unilateral defect is documented in the absence of azotemia, unilateral nephrectomy or selective renal embolization has been recommended to prevent possible fatal exsanguination through the urinary tract[56,57]; however, one may consider conservative treatment if the urinary tract bleeding is minor and has not resulted in anemia.

Figure 17.1-7 Ultrasonographic image of the right kidney of a 9-day-old Quarter Horse colt shows a 2 × 3 cm hypoechoic cavity on a dorsal oblique view. **A**, A swirling pattern, similar in appearance to blood in the ventricles of the heart, was consistent with an arteriovenous malformation. **B**, In a selective nephrogram of the right kidney of the same colt at 20 daysof age, immediately after injection of contrast a dilated vascular space was demonstrated (contrast appears dark because of reverse gray scale), and the renal cortical tissue abaxial to this structure appeared to have reduced capillary phase contrast. (From Schott HC, Barbee DD, Hines MT et al: Renal arteriovenous malformation in a Quarter horse foal, *J Vet Intern Med* 10:204, 1996.)



A large vascular anomaly resulting in transient hemoglobinuria has been reported in a Quarter Horse colt.[60] Over several weeks the large anomalous vascular structure (Figure 17.1-7) spontaneously filled with a thrombus so that specific treatment (a nephrectomy) was not pursued. Severe adult-onset, idiopathic renal hemorrhage also has been described in horses.[61] Whether this latter syndrome may have been a consequence of congenital renal vascular malformations has not been determined (see Section 17.8). Occasionally gross hematuria with passage of blood clots can accompany omphalitis or bladder rupture.[62] One usually can detect these problems during ultrasonographic examination of the umbilical structures, and sometimes can image tissue echogenicity within the bladder that is attributable to a blood clot.

17.1.3.5 PENDULANT KIDNEY

A pendulant kidney is a rare anomaly in the horse.[63] Rectal examination reveals an extremely mobile kidney attached to the dorsal body wall by a thin band of tissue. Although a pendulous kidney could result from extreme weight loss, hydronephrosis, or perirenal trauma, the condition usually is thought to be congenital. The abnormality is an incidental finding unless displacement or rotation leads to partial or complete ureteral obstruction. As an example, the author has palpated the entire right kidney of one mare immediately craniad of the pelvic canal, and ultrasonographic imaging revealed normal size and structure of the anomalously located kidney.

17.1.3.6 ECTOPIC URETER

Although ureteral ectopia occurs rarely in the horse,[64] the condition is the most commonly reported developmental anomaly of the equine urinary tract.[64–82] Ectopic ureters may develop when (1) the ureteric bud (metanephric duct) fails to be incorporated into the urogenital sinus or fails to migrate craniad to the bladder neck, or (2) the mesonephric duct fails to regress. In the former case, the ectopic ureter opens near the urethral papilla in females or into the pelvic urethra near the colliculus seminalis in males, whereas in the latter, the ureter may open anywhere along the vagina, cervix, or uterus (but only in females because this 1177
portion of the mesonephric duct becomes the wolffian duct system in males). In 118 reported cases of ectopic 1178
ureter in horses, 105 (89%) were females[65–68,72–77,79–82]; however, this sex distribution may reflect easier recognition in females of the presenting complaint of urinary incontinence rather than a true sex predilection. Incontinence is recognized more often in females because urine entering the pelvic urethra in males may pass retrograde into the bladder. Although a genetic predisposition for ectopic ureter exists for several dog breeds,[83] no breed predilectionhas been established in horses. However, Quarter Horses may be at greater risk because the condition has been reported in five Quarter Horses, three Standardbreds, two Thoroughbreds, two Appaloosas, an Arabian, a Clydesdale, a Shire, a Fresian, a Foxtrotter, and a Warmblood. The author also has seen the condition in two Quarter Horse fillies (one unilateral and one bilateral), yielding a total of 20 cases.

In horses with ureteral ectopia, urinary incontinence is generally apparent from birth, and affected animals are presented for extensive scalding of the hindlimbs. With unilateral ectopia, horses also void normally, because the other ureter enters the bladder in the appropriate location. Renal function is usually normal, but the affected ureter may be greatly dilated. Urine pooling in the vagina and uterus was a complicating factor in one case.[73] To determine the site of the ectopic ureteral orifice(s), one initially visually examines the vestibule and vagina (using a blade speculum) to look for intermittent urine flow from the area of the urethral papilla. Ectopic ureteral openings usually are not apparent unless urine flow is visible. Endoscopy may be

helpful in females (while inflating the vestibule and vagina with air and using a hand to form a seal at the vulva) and is required in males to visualize the ectopic ureteral opening. Intravesical placement of methylene blue dye was performed in one filly to provide evidence for ureteral ectopia. Continued dribbling of clear urine (from the ectopic ureter) followed by passage of blue, discolored urine indicated that only one ureter emptied into the bladder but provided no information on the location of the opening of the ectopic ureter.[66] Intravenous administration of dyes—including sodium fluorescein (10 mg/kg intravenously; yellow-green), indigotindisulfonate (indigo carmine, 0.25 mg/kg intravenously; blue-purple), azosulfamide (2.0 mg/kg intravenously; red), or phenolsulfonphthalein (1.0 mg/kg intravenously; red)—to discolor the urine may help locate ectopic ureteral openings.[84] Contrast radiography (excretory urography or retrograde contrast studies via catheterization of the bladder and ureters) has been used to detail renal architecture and the course of the ureters in some affected animals; however, results of intravenous urograms are frequently inconclusive in foals weighing more than50 kg (contrast agent is poorly imaged). In a recent report, ultrasound-guided pyelography, in which contrast agent was injected directly into the renal pelvis using a spinal needle, proved to be a more effective technique than imaging after intravenous administration of contrast agent to detail the course of an ectopicureter, and one should consider this technique in future cases.[82]

Treatment has included ureterocystostomy (surgical reimplantation of the ectopic ureter or ureters into the bladder) or unilateral nephrectomy. Before surgery, one must determine whether the condition is unilateral or bilateral, which side is affected if unilateral, and whether urinary tract infection is present. Further, one should attempt to rule out other anomalies, especially of the reproductive tract. If the problem is bilateral (8 of 20 cases), one should establish the presence of a normal micturition response by measuring the intravesicular pressure response to progressive distention until the fluid infused is voided spontaneously. This procedure provides an estimate of bladder volume and ensures competency of the urethral sphincter before reimplantation. Among 14 cases in which surgical correction was pursued, ureterocystostomy was successful in establishing a functional urinary system in nine published cases[67,68,75,76,81,82] and one foal seen by the author, but four died of postoperative complications.[68,75,82] In contrast, all four cases treated by unilateral nephrectomy had a favorable outcome.[72,73,79] Because affected ureters often are dilated and tortuous, surgical reimplantation can be difficult and may not result in a functional ureteral orifice. Consequently, when the problem is unilateral, nephrectomy of the affected side may be the preferred treatment option.[85,86]

17.1.3.7 URETERAL DEFECTS OR TEARS(URETERORRHEXIS)

Retroperitoneal accumulation of urine and uroperitoneum has been described in seven foals with unilateral or bilateral ureteral defects[87–93] and has been observedin three additional foals by the author. These included seven male and three female foals of several breeds(five Standardbreds, two Thoroughbreds, one Belgian, one Oldenburg, and one Appaloosa). Clinical signs (decreased nursing, depression, abdominal distention, diarrhea, and muscle twitching or other signs of neuromuscular irritability) and clinicopathologic abnormalities (hyponatremia, hyperkalemia, hypochloremia, and azotemia) are similar to those in horses with bladder rupture but may have a slightly later onset (4 to 16 days of age). Mild protrusion of the vagina may occur in fillies in which the peritoneum has remained intact.[94] In affected foals, ultrasonographic 1178
examination may reveal dilation of the renal pelvis and affected ureter and fluid accumulation around the 1179
kidneys or farther caudad within the retroperitoneal space. As with ectopic ureters, excretory urography generally has been an unrewarding diagnostic procedure, but contrast pyelography was used successfully to image leakage of contrast agent from a proximal ureteral defect in a recent report.[93] Contrast radiography has not been pursued routinely because exploratory celiotomy generally was performed shortly after a diagnosis of uroperitoneum. Catheterization of the ureters via a cystotomy and retrograde injection of methylene blue

allowed localization of the defect(s), and surgical correction was performed successfully in four cases by suturing the defect around an indwelling catheter.[89,90,93] Although ascending urinary tract infection should be an expected complication with a stent, repair of a defect in one foal without use of an indwelling catheter resulted in further urine leakage from the ureter, prompting a nephrectomy 4 days after the initial surgery.[91] Of the remaining five foals, one died after three unsuccessful attempts at surgical repair,[88] and euthanasia was performed in four cases without attempting repair.[87,92]

At surgery or necropsy a single defect was found in six foals, whereas bilateral defects were found in four foals and multiple defects were apparent in one ureter. In most cases the defects have been located in the proximal third of the ureter near the kidney. Interestingly, distended, tortuous ureters, occasionally accompanied by hydronephrosis, also were described in three affected foals,[88,91,93] and distal obstruction of the ureters at the bladder was suspected in two of these cases, prompting ureteroneocystostomy. Although several reports suggest that these ureteral defects may be anomalies of development, the actual cause of these ureteral defects is not known. Traumatic disruption was suggested in the initial report in which histologic examination of the marginsof the defect revealed hemorrhage and proliferation of immature connective tissue.[87] A traumatic cause was further supported by a subsequent report in which histologic examination of the defects revealed absence of transitional epithelium and inflammation in a foal that had been attacked by dogs.[92] Inflammation and granulation tissue also were visible in the apparently obstructed distal ureter in one of the foals with ureteral distention, again suggesting an acquired lesion. Blunt abdominal trauma, often sustained during automobile accidents, can cause retroperitoneal accumulation of urine and uroperitoneum in human beings.[95] Disruption of the ureter is usually near the kidney, and this complicationof trauma may not be recognized for several days following injury. In one foal evaluated by the author, multiple rib fractures found at necropsy suggested that these ureteral tears actually could be a complication of foaling trauma.

17.1.3.8 RECTOURETHRAL AND RECTOVAGINAL FISTULAE

If the urorectal fold fails to separate completely the primitive hindgut from the urogenital sinus, a rectourethral fistula may be found in a colt or a rectovaginal fistula or a persistent cloaca may be found in a filly.[96] These anomalies are rare in horses and when present usually are associated with atresia ani and other anomalies, including agenesis of the coccygeal vertebrae and tail, scoliosis, adherence of the tail to the anal sphincter area, angular limb deformities, and microphthalmia.[70,71,97–102] Affected foals usually are presented for atresia ani, although one also may observe signs of colic and straining. Evidence for a fistula is passage of fecal material from the vulva or urethra. In fillies one may detect rectovaginal fistulae by digital palpation of the dorsal vestibule and vagina, but in colts a definitive diagnosis usually requires contrast radiographic procedures such as a barium enema or a retrograde urethrogram (Figure 17.1-8). Surgical correction of atresia ani and fistulae has been performed successfully in several foals, but multiple surgical procedures may be required. Because ascending urinary tract infection may be a complication, one should submit a sample of urine collected via bladder catheterization (preferably during surgery) for bacterial culture.[96] In human beings the evidence suggests that these anomalies are hereditary, and in one report several foals born with atresia ani were sired by the same stallion.[97] Consequently, affected horses should not be used for breeding after surgical correction of the anomalies.

Figure 17.1-8 A positive contrast urethrogram in a 3-day-old burro that had atresia ani and intermittent passage of fecal material from the urethra. A catheter passed via the urethra and contrast agent injected into the catheter resulted in accumulation of a large amount of contrast agent in the rectum and a lesser amount in the intrapelvic portion of the urethra. A small amount of contrast agent is visible in the urethrorectal fistula *(arrow)*.

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A urethrorectal fistula resulting in passage of urine from the anus also has been described in a 3-year-old Thoroughbred gelding.[103] The fistula in this gelding was thought to be acquired following trauma or straining because no other developmental problems were detected and the edges of the defect were irregular and inflamed when examined with a speculum inserted into the rectum. 1180

17.1.3.9 BLADDER DEFECTS

Uroperitoneum may result from bladder rupture during parturition in foals (most commonly males)[104] or as a consequence of urachal leakage following infection ofthe umbilical structures.[105,106] In addition, Wellington described uroperitoneum in two foals that were full brothers.[107] Urine entered the abdomen from a dorsal

defect in both colts, and smooth margins to the defects combined with a lack of appreciable inflammation provided evidence in favor of anomalous development rather than trauma. Other authors have suggestedthat some cases of uroperitoneum likely are associated with anomalous bladder defects because of the size, location, or lack of apparent inflammation of the margins of the defects.[70,108–111] For example, Bain[108] described uroperitoneum in a foal in which the ventral portion of the bladder was absent between the lateral ligaments (umbilical artery remnants) from the umbilicus to the urethra.

Anomalous fusion of the bladder to the inner umbilical ring (absence of the urachus) has been describedin one foal.[112] The malformation precluded normal contraction and evacuation of the bladder, and a megavesica —a greatly enlarged bladder—developed. The clinical appearance was similar to that of uroperitoneum, and surgical separation of the bladder from the umbilical ring restored normal anatomic and functional integrity of the bladder. A similar case with a greatly distended bladder was reported in a foal evaluated for abdominal distention[70] that was attributed to an adhesion of the bladder to the urachus or umbilical remnant. An enlarged, flaccid bladder also was described in a foal undergoing exploratory celiotomy for suspected urinary tract disruption.[105] Adhesions to the abdominal wall were not reported, and the foal survived following the surgery during which 50% of the distended bladder was resected. In addition to bladder distention, persistent attachment of the bladder to the area of the umbilicus via a urachal remnant was reported to cause pollakiuria and dysuria in a 15-month-old Thoroughbred filly.[113] The author also has seen postpartum bladder rupturein a mare in which a persistent urachal attachment was suspected to be a contributing factor.

Excessive bladder distention or megavesica has been described further in four stillborn foals[114] and one neonatal foal.[115] In the latter foal and in another report,[116] chronic bladder distention appeared to lead to loss of smooth muscle in the dorsal bladder wall and replacement with collagen. The result was bladder rupture during parturition. Although these reports are similar to an early report by Rooney describing the dorsal bladder wall as the anatomic weak link and likely area for rupture,[104] they differ in that chronic distention of the bladder in utero with smooth muscle loss is not recognized in more typical bladder ruptures in neonatal foals. Why bladder distention should occur in utero without obstruction of the lower tract (not found in these cases) is not clear. Although an excessively long umbilical cord (longer than 85 cm) may lead to urachal obstruction,[114,117] urine produced in utero alternatively could drain into the amniotic cavity via the urethra. Thus this form of megavesica remains poorly characterized and poorly understood.

Bladder distention also is recognized in some foals with hypoxic-ischemic encephalopathy. Affected foals may posture to urinate frequently, and ultrasonographic examination may reveal an enlarged bladder or incomplete bladder emptying. In recumbent foals, one may note abdominal distention, and temporary use of an indwelling bladder catheter to keep the bladder empty is helpful, but ascending urinary tract infection may be a complication. Cystometrography would be useful to assess detrusor function in affected foals, but no reports describe use of this diagnostic test in equine neonates. Although administration of cholinergic drugs (e.g., bethanechol) to improve detrusor function or α-adrenergic blockers (e.g., phenoxybenzamine or acepromazine) to decrease urethral sphincter tone has been described anecdotally to be of benefit, no reports describe the efficacy of these medications in foals with this problem.

17.1.3.10 PATENT URACHUS

The urachus is the conduit through which fetal urine passes from the bladder into the allantoic cavity. Normally, the urachus closes at the time of parturition, but incomplete closure is the most common malformation of the equine urinary tract. Patent urachus occurs more commonly in foals than in other

domestic species.[30] Greater than average length or partial torsion of the umbilical cord has been suggested to cause tension onthe attachment of the umbilical cord to the body wall. The result is dilation of the urachus and subsequent failure to close at birth.[70,71,114,117,118] Patent urachus results in a persistently moist umbilicus after birth, from which urine may leak as drips or as a stream during micturition. One must distinguish this malformation from septic omphalitis, which also can result in urine leakage from the umbilicus within a few hours to days after birth. Patent urachus has been referred to as a congenital problem 1180
and the latter as an acquired one, but both may result in urine leakage from the urachus from birth. Neither is 1181
life threatening, but local sepsis often is accompanied by more severe illness, including septicemia or localized infection, particularly in joints.

The congenital patent urachus traditionally has been treated with frequent (2 to 4 times daily) chemical cauterization of the urachus with swabs dipped in a concentrated phenol or 7% iodine solution or with silver nitrate applicators.[119] Because the urachus may close spontaneously in a number of cases, and because these agents desiccate and irritate tissue (and may predispose to infection), the rationale for this approach has been questioned.[118] In a study comparing the effects of disinfectant solutions on the bacterial flora of the umbilicus of normal foals, use of a 7% iodine solution was observed to cause rapid desiccation of the umbilical tissue and subsequent development of a patent urachus when the stump fell off a few days later.[120] Consequently, in the absence of apparent infection, no local treatment may be indicated specifically, but affected foals frequently are given antibiotics prophylactically. For acquired patency (which may be associated with local infection or septicemia), broad-spectrum antibiotic therapy is indicated, and resolution of the systemic disease may be accompanied by elimination of the umbilical infection and closure of the urachus. Chemical cauterization is contraindicated with local sepsis because it may increase the risk of urachal rupture and development of uroperitoneum.[121] If one observes no decrease in urine leakage after 5 to 7 days of medical therapy or if ultrasonography reveals abnormalities of multiple structures in the umbilicus,[122,123] surgical exploration and resection of the urachus and umbilical vessels may be indicated. In a retrospective study of 16 foals treated for sepsis of umbilical cord remnants, six of nine (67%) survived after surgical resection and antibiotic treatment, whereas only three of seven (43%) survived after antibiotic treatment alone.[124] Although this series of 16 foals often is cited in support of surgical intervention, one should note that the series studied a small number of foals and that the cases were evaluated over 10 years (1975 to 1985), during which time many aspects of neonatal care improved. In a more recent retrospective report of 33 foals with umbilical remnant infections, no difference in survival was observed between foals treated with antibiotics combined with surgical resection or with antibiotic therapy alone.[123] Further, emphasis was placed on the insensitivity of palpation of the umbilicus in detection of umbilical remnant infection (compared with ultrasonographic examination) and the poor outcome of cases in which the umbilical vein was involved. In addition to the possibility of omphalitis leading to urachal rupture and development of uroperitoneum, urachal leakage also may occur into the abdominal musculature and subcutaneous tissues and lead to swelling and cellulitis of the ventral abdominal wall.[125] Both instances require surgical intervention. Finally, trauma or tearing of the urachus also can lead to umbilical evagination of the urinary bladder,[126] which can result in partial or complete obstruction of urine flow, and surgical correction is indicated.

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17.2

17.2—Renal Physiology

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The kidneys perform two essential functions in the maintenance of homeostasis: elimination of nitrogenous and organic waste products and control of body water content and ion composition. In addition, the kidneys are important endocrine organs that produce renin, erythropoietin, and the active form of vitamin D, and they also play an important role in the degradation and excretion of a number of other hormones, including gastrin and parathormone. To gain an understanding of the pathophysiologic alterations associated with renal disorders in horses, one must first review some aspects of normal renal physiology in this species.

17.2.1

Production and Elimination of Nitrogenous and Organic Wastes

The two most commonly recognized waste products excreted in urine are urea and creatinine, but many other nitrogenous or organic wastes are produced each day and subsequently are eliminated by the kidneys (Box 17.2-1).[1]

17.2.1.1

UREA METABOLISM

A molecule of urea is produced in the liver from two ammonium ions that are liberated during catabolism of amino acids. For each urea molecule the carbon atom is derived from bicarbonate. One ammonium ion is cleaved from an amino acid via an α-ketoglutarate-dependent transamination coupled to oxidative deamination of glutamate. The second ammonium ion is derived from aspartate in the urea cycle.[2] Urea synthesized in the liver is released into the blood, and clearance by the kidneys represents the major pathway (75% to 100%) of excretion. Extrarenal urea excretion includes losses in sweat and through the gastrointestinal tract. With normal intestinal function, enteric excretion is minimal because of enterohepatic recirculation (reabsorption of ammonia from the degradation of urea by bacterial ureases and subsequent reformation of urea in the liver).[3]

17.2.1.1.1

BOX 17.2-1 COMPOUNDS EXCRETED BY THE KIDNEYS

Urea

Phenols

Indoles

Skatoles

Hormones

Polyamines

Trace elements

Serum proteases

Creatinine

Pyridine derivatives

Guanidino compounds

β_2-Microglobulin

Hippurate esters

Aliphatic amines

Aromatic amines
Middle molecules

In human beings, inborn errors of metabolism leading to deficiency of a specific transaminase or of one of the five enzymes of the urea cycle can result in accumulation of ammonia and other intermediates of amino acid catabolism. These disorders typically are inherited as autosomal recessive traits, and the consequence is moderate to severe mental retardation because the accumulated intermediates can be toxic to the central nervous system (ammonia) or can act as false neurotransmitters (aromatic amines).[1] Because urea production is limited in these disorders, blood urea nitrogen concentration (BUN) is often low.[2] Although such defects in metabolism appear to be rare in domestic animals,[4] development of encephalopathy in association with hyperammonemia has been recognized in horses.[5,6] Furthermore, in one report of two related Morgan weanling fillies, persistent hyperammonemia was suspected to be caused by a defect in a mitochondrial ornithine transporter similar to an autosomal recessive syndrome of hyperornithinemia, hyperammonemia, and homocitrullinuria in human beings (HHH syndrome).[7]

Blood urea nitrogen concentration depends on age, diet, rate of urea production, and renal function. For example, a low BUN typically is found in neonatal foals following an anabolic demand for amino acids.[8] Next, investigations of nitrogen use in ponies have demonstrated that urea production is proportional to dietary protein content. Similarly, urinary urea excretion increases in parallel with urea production.[9,10] As a result, with increased levels of dietary protein or when urea is supplemented in the diet, BUN may increase twofold or greater.[11–13]

In human beings and small animals, BUN is routinely higher in samples collected postprandially because diets are typically high in protein.[3] Postprandial elevation of BUN has not been described in horses or other herbivores. However, fasting leads to enhanced protein catabolism to meet energy demands and increased BUN in horses.[14,15] In ponies, however, BUN decreases with fasting.[16] This opposite response suggests 1184
differences in the metabolic responses of horses and ponies to anorexia, consistent with a greater capacity of 1185
ponies to mobilize and use fat during starvation. Other causes of protein catabolism, including fever, infection, trauma, myositis, burns, and corticosteroid therapy, also can produce an increase in BUN.[3] Finally, a decrease in renal blood flow (RBF) or renal function produces an increase in BUN. The former may occur with dehydration or during periods of anesthesia or exercise; the latter is a reflection of renal disease.[3] With short bouts of moderate to intensive exercise, BUN often does not change,[13,17] but during prolonged exercise, BUN can increase by 50% or more because of the combined effects of decreased RBF and protein catabolism.[18,19]

Most renal nitrogen excretion occurs in the form of urea in urine. One must recognize that urea excretionis completely passive and that the high concentrations achieved in urine are merely a consequence of medullary tonicity produced by the countercurrent-multiplier function of the loop of Henle. Thus although variations in dietary protein intake lead to parallel changes in urea excretion, the idea that low-protein diets decrease the work load on the kidney is a fallacy.[3] Urinary urea nitrogen concentrations can vary from as low as 50 mg/dlin neonatal foals or horses with primary polydipsia to greater than 2500 mg/dl in normal horses on high-protein diets. Total daily urea excretion usually ranges between 100 and 300 g per day in horses with normal renal function.

17.2.1.2 CREATININE METABOLISM

Creatinine is produced by the nonenzymatic, irreversible cyclization and dehydration of creatine. Creatine is produced indirectly from three amino acids in the kidney, liver, and pancreas and subsequently is transported to other organs such as muscle and brain, where it is phosphorylated to store energy in the form of phosphocreatine.[3,20] In human beings, 1.5% to 2% of the creatine pool is converted to creatinine daily and results in fairly constant excretion of creatinine within a given individual.[3] With normal renal function, a direct relationship exists between daily creatinine production, serum creatinine concentration (Cr), and creatinine excretion, all three being proportional to total muscle mass. The fact that Cr is 30% higher in human males than in females and that urinary creatinine excretion is correlated to body size across a wide range of animal species supports this relationship.[3,21] Creatinine is excreted principallyin urine, but sweat and the gastrointestinal tract are secondary routes of excretion.[3] In contrast to urea, enterohepatic recycling of creatinine does not occur, and the gastrointestinal tract may represent a major route of excretion when renal function is compromised. For example, in a group of azotemic human patients, between 15% and 65% of radiolabeled creatinine was found to be excreted through the intestine.[22] Creatinine excreted by this route is degraded rapidly by bacteriaso that little is found in feces.

Like BUN, Cr can vary with age, activity level, and renal function. In contrast, dietary protein intake has little influence on Cr in horses.[11] Newborn foals routinely have Cr values 30% to 50% higher than those measured in the mare, and values as high as 20 to30 mg/dl have been measured in some premature or asphyxiated foals.[8] These high values may result from limited diffusion of creatinine across the placenta. For example, the Cr in equine amniotic fluid collectedat term is proportionately much greater than urea nitrogen concentration (Cr, 10.1 mg/dl; urea nitrogen, 38.8 mg/dl).[23] If the foal appears healthy and all other laboratory values are within reference ranges, a serumCr value in the range of 5 to 15 mg/dl should not cause alarm. In most healthy foals with normal renal function, Cr decreases to values below 3.0 mg/dl within the first 3 to 5 days of life.[22] After the first few days of life, Cr is usually lower in foals than in adults[12] because of the combined effect of rapid growth and the fact that skeletal muscle comprises a smaller percentage of body weight in foals than in adult horses. Other nonrenal factors that may influence Cr include fasting, rhabdomyolysis or muscle wasting caused by disease, and exercise. Although fasting can increase the measured value for Cr, a substantial portion of this increase actually is due to other compounds (possibly ketones) that increase during fasting and are measured as noncreatinine chromagens in the commonly used Jaffe's colorimetric assay for Cr determination (see Chapter 17.3).[12,14,24] In contrast, the increase in Cr (up to 80% in some reports) associated with various types of exercise is likely the combined result of increased release of creatine from muscleand decreased urinary creatinine excretion during the exercise bout.[12,14,17–19]

Creatinine is filtered freely at the glomerulus and is concentrated to values of 100 to 300 mg/dl in equine urine, which results in a total daily urine excretion of 15 to 25 g of creatinine.[25,26] In comparison to urea, creatinine excretion is responsible for only one tenth as much urinary nitrogen excretion. Minor species and sex differences have been reported for renal tubular handling of creatinine with a weak proximal tubular secretory mechanism in human beings and male dogs (accounting for 7% to 10% of total urinary creatinine excretion).[3,20] To determine whether tubular secretion of creatinine occurs in equine kidneys, Finco and Groves fitted anesthetized ponies with ureteral catheters and performed simultaneous inulin and exogenous creatinine clearance studies.[27] Because inulin is filtered freely at the glomerulus and neither secreted nor 1185
reabsorbed by renal tubules, inulin clearance (Cl_{In}) provides a standard of comparison for creatinine 1186

clearance (Cl_{Cr}). Tubular secretion of creatinine should result in a greater value for Cl_{Cr} than for Cl_{In}, whereas the opposite should occur with tubular reabsorption of creatinine. To magnify any minor tubular secretion of creatinine, stop-flow studies were performed by temporarily occluding the ureteral catheters. During obstruction, tubular lumen pressure increased and tubular flow decreased. As a consequence, fluid remained in contact with tubular epithelium for a prolonged period, enhancing local tubular secretory or resorptive processes. Analysis of a series of urine samples collected after release of ureteral occlusion revealed no differences in tubular handling of inulin or creatinine, leading to the conclusion that creatinine neither was reabsorbed nor secreted by equine kidneys. In contrast, simultaneous measurement of endogenous Cl_{Cr} and Cl_{In} in several horses with chronic renal failure (author's unpublished observations) has revealed higher values for Cl_{Cr}, indicating that tubular secretion of creatinine may develop in horses as renal function declines (see Chapter 17.5). Whether significant excretion of creatinine occurs in sweat or through the gastrointestinal tract has not been investigated in horses.

17.2.1.3

METABOLISM OF OTHER NITROGENOUSAND ORGANIC COMPOUNDS

Although the kidneys excrete a number of nitrogenous and organic wastes in addition to urea and creatinine (see Box 17.2-1), these compounds are quantitatively unimportant in terms of nitrogen balance.[1] Two of the more commonly recognized molecules are ammonia and uric acid. In proximal tubular epithelial cells, ammonium ions and α-ketoglutarate are produced from glutamine. Subsequent metabolism of α-ketoglutarate results in generation of two bicarbonate molecules that are returned to the systemic circulation. Ammonium ions are secreted in exchange for sodium into the tubule lumen, where they remain trapped, because tubules are relatively impermeable to ammonium ions. Furthermore, because the pK_a for ammonia is greater than 9.0, most of the tubular ammonia remains in the form of ammonium ions, even in alkaline equine urine. Although ammonium ion excretion is of little significance in overall nitrogen excretion, it plays an important role in acid (hydrogen ion) excretion. In fact, glutamine metabolism and ammonium ion excretion can increase severalfold in response to metabolic acidosis.[28] Although urinary ammonium concentration is not measured routinely, one can estimate it because it is directly related to the urinary anion gap ($[Na^+ + K^+] - Cl^-$) in human patients with normal anion gap metabolic acidosis.[29] More important, impairment of this proximal tubular acid secretion pathway contributes to development of metabolic acidosis in patients with renal insufficiency.

Uric acid is a product of purine nucleotide degradation and is the major nitrogenous waste product formed in amphibians and reptiles. In mammals, however, uric acid excretion (mostly in the ionic form of urate) is unimportant in terms of overall nitrogen excretion.[30] Uric acid metabolism has received little attention in veterinary species with the exception of Dalmatian dogs. This breed exhibits high urate excretion rates and is predisposed to uric acid stone formation; however, this problem results from decreased hepatic uricase activity rather than any abnormality in renal urate handling.[31] Finally, hyperuricemia (leading to gout in human beings) also can be attributed to a lack of uricase activity in human tissues and greater renal reabsorption of urate compared with other mammalian species. Thus crystallization of urate in tissues appears to be limited to human beings.[30] Urate metabolism has been studied little in horses, although Keenan observed that plasma concentrations increased dramatically in response to exercise (from less than 1 μmol/L at rest to 150 to 200 μmol/L 1 hour after racing) and that these increases were accompanied by a transient increasein urinary urate excretion (from less than 40 μmol/L at rest to 250 to 1270 μmol/L after racing).[17]

The proximal tubule is also the major site of excretion (by tubular secretion) of a number of endogenous organic anions and cations.[30] The anions share the common pathway measured by *p*-aminohippurate clearance, the substance traditionally used to measure effective renal plasma flow (because more than 90% is excreted via this pathway). A number of exogenous compounds also are excreted via these pathways—acetazolamide, furosemide, probenecid, penicillin G, sulfadiazine, salicylate, atropine, cimetidine, and neostigmine. Thus administration of these compounds can interfere with tubular secretion of endogenous organic wastes or other exogenous products by healthy kidneys.[32] More important, pharmacokinetics of these products varies widely in patients with renal insufficiency. Combined with the fact that anion binding to plasma proteins is decreased with azotemia, dosing protocols of many medications may need to be readjusted for patients with renal failure.

17.2.2 Body Water and Electrolyte Balance

17.2.2.1 BODY FLUIDS: VOLUME AND COMPOSITION

Water accounts for at least 60% of total body mass, equivalent to 300 L in a 500-kg horse.[29–35] About 200 L of total body water is intracellular fluid, and the remaining 100 L is extracellular fluid. Extracellular fluid is divided between plasma (4% to 6% of body mass, ≈25 L), interstitial fluid and lymph (10% to 12% of body mass, ≈45 L), and transcellular fluid (6% to 10% of bodymass, ≈30 L, most of which is in the lumen of the 1186
gastrointestinal tract). Despite significant differences in ion composition (Table 17.2-1), the extracellular 1187
fluid and intracellular fluid compartments exchange water freely to maintain osmotic equilibrium.[36]

TABLE 17.2-1 Approximate Ionic Compositions (mEq/L) of Plasma, Interstitial Fluid, and Intracellular Fluid (Skeletal Muscle)

ELECTROLYTE	PLASMA	INTERSTITIAL FLUID	SKELETAL MUSCLE CELL
CATIONS			
Na^+	140	143	10
K^+	4.0	4.1	142
Ca^{2+}	2.5	2.4	4.0
Mg^{2+}	1.1	1.1	34
Total	147.6	150.6	190
ANIONS			
Cl^-	100	113	4
HCO_3^-	25	28.2	12
$H_2PO_4^-$, HPO_4^{-2}	2.0	2.3	40
Protein	14	0.0	50
Other	6.6	7.1	84[*]
Total	147.6	150.6	190

Modified from Rose BD: Physiology of body fluids. In *Clinical physiology of acid-base and electrolyte disorders,* ed 3, New York, 1989, McGraw-Hill.

* This largely represents organic phosphates such as adenosine triphosphate.

From the values in Table 17.2-1 one can estimate the total amount of exchangeable sodium, potassium, and chloride in the body fluids of a 500-kg horse: approximately 16,000 mEq, approximately 28,800 mEq, and approximately 10,800 mEq, respectively (including gastrointestinal fluid ion contents). These values are accurate except for that of sodium, which may be twice as great; however, 40% to 50% is sequestered in bone and is not readily available to buffer sodium alterations in body fluids.[33–35] Thus the 16,000-mEq estimate is accurate for the exchangeable sodium content in body fluids. Similarly, one can estimate body fluid contents of calcium, magnesium, and phosphorus at approximately 1000 mEq (20 g), approximately 6875 mEq (84 g), and approximately 8150 mEq (140 g), respectively (excluding gastrointestinal fluid ion contents, because these vary with the amount and solubility of the dietary source). As for sodium, the values underestimate the total body content of calcium, magnesium, and phosphorus, because more than 99%, 70%, and 85% of these elements, respectively, are contained in the skeleton.[37]

17.2.2.2

WATER BALANCE

Appropriate water balance maintains plasma osmolality in a narrow range (270 to 300 mOsm/kg) and is achieved by matching daily water intake with water loss.[38–40] Water is provided from three sources: (1) free water intake (drinking), (2) water in feed, and (3) metabolic water (Table 17.2-2). Horses consume most of

the water by drinking (about 85%), but feed and metabolic water provide about 5% and 10% of daily water, respectively. Water can be lost by three routes: (1) in urine,(2) in feces, and (3) as insensible losses (evaporation) across the skin and respiratory tract (Table 17.2-3). Investigations of water balance have revealed a maintenance water requirement of 60 to 65 ml/kg/day or 27 to30 L/day for a 500-kg horse.[38,41] These values are consistent with traditional recommendations that 5 to 10 gallons/day of fresh water be provided to a stabled horse under mild environmental conditions.[42] Urinary and fecal water losses range from 20% to 55% and 30% to 55%, respectively, of the total daily water loss.[38,41,43,44] The remaining (insensible) 1187
loss accounted for up to 15% to 40% of daily water loss, despite mild ambient conditions and the lack of 1188
observed sweating in most studies of water balance.

TABLE 17.2-2 Water Balance in Hay-Fed Horses in a Cool Climate

WATER INTAKE (L)		WATER LOSS (L)	
Consumption	23.6	Feces	14.0
Hay	1.1	Urine	4.9
Metabolic	2.7	Insensible	8.5
Total	27.4	Total	27.4

Data from Tasker JB: Fluid and electrolyte studies in the horse. 3. Intake and output of water, sodium, and potassium in normal horses, *Cornell Vet* 57:649, 1967.

TABLE 17.2-3 Water and Electrolyte Balance in Horses Receiving a Low-Sodium Diet (Alfalfa–Timothy Hay)

	INTAKE	URINARY LOSS	FECAL LOSS	UNMEASURED*
TASKER†				
Water (L)	27.4	4.9	14	8.5 (31%)
Sodium (mEq)	329	7	116	206 (63%)
Potassium (mEq)	3930	2196	993	741 (19%)
GROENENDYK, ENGLISH, ABETZ‡				
Water (L)	27.6§	9.9	7.2	10.5 (38%)
Sodium (mEq)	986	527	253	206 (21%)
Potassium (mEq)	3320	2661	504	155 (5%)
Chloride (mEq)	3008	2347	174	487 (16%)

* Unmeasured losses include insensible water losses and electrolyte losses thought to occur in sweat; value in parenthesis is the percentage represented by these unmeasured losses.

† Tasker JB: Fluid and electrolyte studies in the horse. 3. Intake and output of water, sodium, and potassium in normal horses, *Cornell Vet* 57:649, 1967.

‡ Groenendyk S, English PB, Abetz I: External balance of water and electrolytes in the horse, *Equine Vet J* 20:189, 1988.

§ Water intake includes imbibed water (23.6 L), water in feed (1.1 L), and metabolic water (2.9 L).

Water drinking and urine production are the mechanisms by which water balance is finely tuned; however, they can vary widely between individual horses and also are affected by age, environmental conditions, level of exercise, and diet. Often, for example, neonatal foals consume milk in excess of 20% of their body massdaily,[45] which equates to fluid intake approaching250 ml/kg/day. Next, water intake by horses increased 15% to 20% when ambient temperature increased from 13° to 25° C.[46] Under conditions of high ambient temperature and humidity, urine concentration also may increase to conserve water, whereas fecal water content tends to remain fairly stable, at about 75% of fecal weight. Exercising horses, especially endurance horses and racing horses treated with furosemide, can increase water consumption by 100% to 200% to replace body water lost in sweat (and urine). Horses and ponies on all-roughage diets also drink more and have greater daily fecal water loss (because of greater daily fecal volume) than animals fed a large amount of concentrate or complete pelleted diets.[43,44] Diets high in nitrogen (protein) and calcium, such as legume hays, typically increase urine volume by 50% or more and are associated with a similar increase in urinary nitrogen excretion. These diets are also more digestible, so that fecal water excretion generally decreases because of a decrease in total fecal material.[9,14,43,44] Although high dietary levels of salt have been suggested to increase drinking and promote diuresis, no increase in water consumption or urine volume was observed in ponies fed 5 to 10 times the daily salt requirement (equivalent to about 350 g of sodium chloride for a 500-kg horse).[47] The effects of water access, continuous versus intermittent, have received less attention, although a recent study showed no difference in water balance in horses provided water 3 times daily compared with horses that had continuous access to water.[48] Furthermore, horses drink the most water within the hour after feeding,[49] and feral horses and ponies often drink only once or twice daily.[50] Thus horses are unlikely to require continuous access to water. An obvious exception is a patient with renal insufficiency that should have access to fresh water at all times.

Two main stimuli for thirst are increased plasma osmolality and hypovolemia or hypotension.[51] The former is mediated through osmoreceptors in the hypothalamus that have a high threshold for activation (about 295 mOsm/kg) in human beings. Hemodynamic stimuli are mediated by low- and high-pressure baroreceptors. Osmotic and hemodynamic stimuli can produce their dipsogenic effect in part by activating a local renin-angiotensin-aldosterone system in the central nervous system.[52,53]

Renal water reabsorption is controlled principally by the action of arginine vasopressin (antidiuretic hormone) on the collecting ducts.[54] Vasopressin is produced in the neurosecretory neurons of the supraoptic nuclei, packaged in granules, and transported down axons for storage in the neurohypophysis (pars nervosa or posterior pituitary). As for thirst, increases in plasma osmolality and hypovolemia or hypotension are the stimuli for vasopressin release. Osmoreceptors for vasopressin release also are located in the hypothalamus, adjacent to the osmoreceptors mediating thirst. Activation of these receptors is the signal for vasopressin release from the neurohypophysis. Furthermore, these osmoreceptors are not equally sensitive to all plasma solutes. For example, increases in plasma sodium concentration and infusion of mannitol are potent stimuli, whereas increases in plasma glucose and urea concentrations are weak stimuli. These differences have led to the suggestion that osmoreceptor activation is caused by an osmotic water shift that produces cell shrinkage (which would be greater for sodium and mannitol than for glucose or urea). Activation of osmoreceptors signaling vasopressin release also appears to have a threshold value; however, this threshold appears to vary highly between individuals. In addition, the threshold for vasopressin release in human beings is significantly

lower (270 to285 mOsm/kg) than that for thirst. Thus vasopressin release can be thought of as the initial line of defense against a mild increase in plasma osmolality, whereas thirst and drinking are secondary responses to even greater increases.

Studies in horses, ponies, and donkeys have demonstrated that increased plasma osmolality (induced by water deprivation or infusion of hypertonic saline) and hypovolemia (induced by furosemide administration) are stimuli for thirst.[48,54–58] Furthermore, after a period of water deprivation, dehydrated ponies, horses, and donkeys appear to be able to replace water deficits within 15 to 30 minutes of gaining access to water. The increases in plasma osmolality and vasopressin concentration associated with water deprivation also are corrected in this same period of time, indicating that imbibed water is absorbed rapidly fromthe gastrointestinal tract.[55] Although increases in plasma vasopressin concentration have been measured in horses and ponies during water deprivation,[55,59] vasopressinalso appears to be a "stress hormone" in equids, because substantially greater concentrations (tenfold greater than those induced by water deprivation) have been measured after application of a nose twitch, nasogastric intubation, or exercise.[60,61] Thus increases in plasma vasopressin concentration following water deprivation would be expected to vary in horses, and separating osmotic effects from stress effects may be difficult sometimes.

Once released, vasopressin acts on V_2-receptors on the basolateral membrane of collecting duct epithelial 1188

1189 cells, leading to insertion of water channels (transmembrane proteins) in the apical membrane.[51] These channels increase the water permeability of the apical membranes and lead to increased water reabsorption. Action of V_2-receptors is mediated by activation of adenyl cyclase and a stimulatory transmembrane G protein. Interestingly, V_2-receptor activation can be antagonized by activation of adjacent α_2-adrenoceptors and by a prostaglandin E_2–mediated effect on an inhibitory G protein.[62,63] Although effects of these antagonists vary with species and have not yet been studied in horses, the diuresis associated with administration of α_2-agonists to horses[64,65] likely may be attributable to vasopressin antagonism at the collecting duct.

As mentioned previously, most water drinking in equids occurs periprandially; thus feeding practices affect timing of water intake.[49] If a horse eats a large meal once or twice daily, both increased plasma sodium concentration and decreased plasma volume (because of a shiftof fluid into the bowel) stimulate thirst and vasopressin release. The result is a simultaneous increase in water intake and a decrease in urine output.[66] In addition, hypovolemia further stimulates activation of therenin-angiotensin-aldosterone system, which leads to enhanced renal sodium conservation as an additional means of restoring plasma volume. Although the increase in plasma sodium concentration with meal feeding is rather small (1% to 3%), the decrease in plasma volume is much greater (5% to 25%). The magnitudeof this fluid shift (and the degree of activation of the renin-angiotensin-aldosterone system) can be attenuated largely by feeding small meals 4 to 6 times throughout the day.[67,68] Thus more frequent feeding causes less perturbation of body fluids and likely has a protective effect against development of some forms of colic.

Although balance of daily water intake and outputis critical for maintenance of homeostasis, it warrants mention that equids tolerate water deprivation well.[69–75] For example, after horses were deprived of water for72 hours (which resulted in body weight loss in excess of 10%), most of the weight lost (90% of which was assumed to be water) was recovered within the first hour of being provided access to water.[72] Similarly, even greater body weight losses (approaching 20%) induced by water deprivation and desert walking in donkeys and burros were replaced largely within the first few minutes after water was provided.[70,71] Thus in

terms of water balance, equids (especially donkeys and burros) truly can be considered desert-adapted animals.[75,76] An important reason for their ability to tolerate water deprivation appears to be a substantial intestinal reserve of water and electrolytes that they call on during periods of dehydration for the maintenance of plasma volume.[77,78] Despite rapid fluid replacement by equids that have been dehydrated by water deprivation, horses that become dehydrated because of prolonged exercise or diarrheal disease (colitis) often do not drink. This behavior can be attributed to the fact that these conditions produce loss of body water and osmoles in the form of sweat or diarrhea. As a result, plasma osmolality does not increase and osmotic thirst stimulus is not produced. In human endurance athletes this state of mild to moderate dehydration that does not induce thirst has been called "voluntary" and "involuntary" dehydration,[79,80] and although less well-documented, a similar response appears to occur in endurance horses.[81] Another form of involuntary dehydration, which may be accompanied by increases in plasma osmolality and protein concentration, also has been described anecdotally in mares after foaling.

17.2.2.3 ELECTROLYTE BALANCE

Intake and loss of electrolytes also must be matched appropriately to maintain body content of electrolytes within narrow ranges. This balance is most important for the exchangeable ions (Na^+,K^+, and Cl^-) because these have minimal tissue (skeletal) reserves that can be called on during times of need. An exception is the fluid and electrolyte reserve in the lumen of the gastrointestinal tract, which may be able to provide replacement of 10% or more of the body content of these electrolytes.[78] Three sources provide electrolytes: feed, water (usually minimal amounts), and a number of dietary supplements. Electrolytes also can be lost by three routes: in urine, in feces, and in sweat (insensible losses; see Table 17.2-3). Investigations of electrolyte balance have revealed that most horses that eat predominantly hay or pasture grass ingest excess potassium and chloride. In contrast, sodium intake varies and with some diets may be marginal.[38,40,41] A maintenance requirement for sodium of 0.4 to0.8 mEq/kg/day or 200 to 400 mEq per day (6 to 12 g per day) for a 500-kg horse has been suggested[41,57]; however, exercising horses that may lose 500 to 1000 mEq of sodium per hour in sweat or are treated with furosemide have greater dietary requirements to replace such losses.[40] Thus addition of 50 to 75 g of common table salt (which provides 850 to 1275 mEq, because 1 g NaCl provides ≈17 mEq Na^+) is a safe and economical method of providing daily supplemental sodium and chloride to athletic horses.

The data from the water and electrolyte balance studies performed by Tasker[38] and by Groenendyk, English, and Abetz[41] (see Table 17.2-3) provide a good illustration of the capacity of the equine kidneys to conserve sodium when dietary intake is low (see Tasker's data at top) compared with when intake is unlimited (see
Groenendyk, English, and Abetz's data at bottom). Furthermore, these studies demonstrate that urinary 1189
excretion is the major route for loss of potassium and chloride. Although dietary intake of potassium is 1190
usually excessive, equine kidneys do not appear to have a great capacity to conserve potassium during periods of food and water deprivation or with anorexia associated with disease.[38,40,80] Thus urinary potassium concentration and total excretion can remain substantial in the face of decreased intake. Consequently, with decreased feed intake horses can develop significant total body potassium depletion and often benefit from supplemental dietary potassium (25 to 50 g per day of KCl provides 375 to 750 mEq, because 1 g KCl provides ≈15 mEq K^+).

In horses the stimuli for electrolyte intake (salt appetite) have received much less attention than stimuli for drinking. Houpt, Northrup, Wheatley, et al. found that horses that had marginal sodium intake (250 mEqper

day) and that were treated with furosemide ate more salt in the hours after treatment than did placebo-treated horses on the same diet[57]; however, salt intake (which was comparable for eating salt from a block or drinking a 0.9% sodium chloride solution) was excessive in both treatment groups (in excess of 100 g). Thus salt appetite, unlike water intake, is less closely regulated to balance intake with losses. In fact, when salt is available ad libitum, horses appear to consume more than their maintenance needs. The excess is eliminated by increased urinary sodium excretion. Although this apparently excessive salt appetite may seem inappropriate, one could consider it advantageous for exercising horses, which have a much greater daily salt requirement.[82]

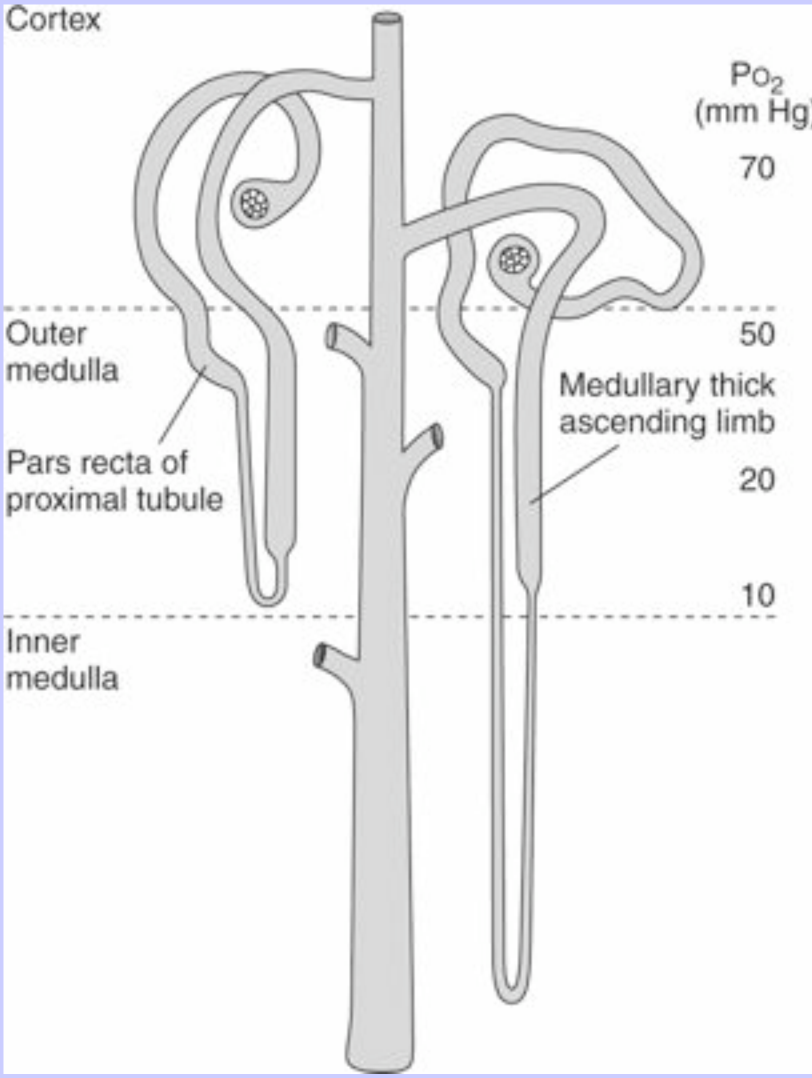
17.2.3 Renal Regulation of Body Water Content and Ion Composition

The kidneys are the organs responsible for fine tuning body water content and ion composition within narrow ranges. The important components of renal regulation of water and ion content include renal blood flow, glomerular filtration, and tubular modification of glomerular filtrate to produce the final urine.

17.2.3.1 RENAL BLOOD FLOW

At rest, the kidneys receive about 15% to 20% of the cardiac output, or about 7.5 to 10 L per minute for an average-size horse.[83,84] This high tissue perfusion, 500 to 600 ml/min per 100 g of kidney compared with 50 to 100 ml/min per 100 g of brain tissue, is necessary for the kidney to function as an effective filter and as a regulator of extracellular fluid composition. Furthermore, tubular reabsorption of glomerular filtrate requires energy. Because more than 99% of the filtrate is reabsorbed, the metabolic rate of the kidney is high (second only to that of the heart), and despite the fact that the kidneys account for less than 1% of body weight, they are responsible for about 10% of whole body oxygen use.[85] Next, RBF is distributed preferentially to the renal cortex. In fact, renal medullary blood flow, which is derived largely via the vasa recta that arise from the efferent arterioles of juxtamedullary glomeruli, accounts for less than 20% of total RBF.[84] Consequently, the renal medullary tissue normally functions in a hypoxic environment. Medullary hypoxia has been described as “an inevitable accompaniment of efficient urinary concentration” as a consequence of countercurrent exchange.[86] Although the latter mechanism would suggest that oxygen tension should decrease progressively toward the inner medulla, the lowest values, often no greater than 10 mm Hg, are found in the inner portion of the outer medulla, termed the *inner stripe* (Figure 17.2-1).[87] This finding can be explained by the substantial metabolic activity of epithelial cells lining the medullary thick ascending limb (mTAL) of the loop of Henle in the inner stripe. The sodium-potassium-adenosinetriphosphatase pumps (Na^+,K^+-ATPase) in the basal membrane of these cells are responsible for the greatest ATP use (and thereby oxygen consumption) in the medulla.[88] The combined effects of low oxygen delivery and a high rate of use 1190
produce the lowest oxygen tension in the inner stripe. Fortunately, several “protective” mechanisms exist to 1191
preserve medullary blood flow and tissue oxygenation during periods of renal hypoperfusion and include a preferential reduction in cortical blood flow along with redistribution of renal blood flow to the corticomedullary region, accumulation of adenosine with depletion of ATP, and production of prostaglandins (PGE_2 and PGI_2) and nitric oxide.[86–88] Of clinical importance is that the use of nonsteroidal antiinflammatory drugs in patients with poor renal perfusion can exacerbate tissue hypoxia because PGE_2 acts as a vasodilator and an inhibitor of Na^+,K^+-ATPase. In fact, the earliest lesions of analgesic nephropathy include degeneration and necrosis of mTAL cells before development of overt papillary necrosis.[88]

Figure 17.2-1 Schematic drawing of a nephron: in the outer medulla is the site of lowest intrarenal oxygen tension *(Po_2)* in the final *(straight)* portion of the proximal tubule and the medullary thick ascending limb of Henle's loop. Limited medullary blood flow and high metabolic rates make these outer medullary nephron segments the most susceptible to damage during periods of renal hypoperfusion.



Renal blood flow of horses has been measured bya variety of techniques including *p*-aminohippurate clearance (Cl_{PAH}, by classic clearance techniques involving timed urine collections and by plasma disappearance curves), clearance of radionuclides, microsphere injection, and use of ultrasonic Doppler flow

probes placed around the renal artery (Table 17.2-4).[83,84,89–105] The latter technique does not provide absolute blood flow values but rather measures changes in RBF from a baseline value.[102] Recently, plasma disappearance curves for ^{131}I-orthoiodohippuric acid and ^{99m}Tc-mercaptoacetyltriglycine have been validated in normal horses in an attempt to establish radionuclide techniques for rapid and noninvasive measurement of RBF in hospitalized horses.[100,105] Although values for RBF determined by these 1191
radionuclide techniques compared well with previous data (Table 17.2-5), their future use in a clinical setting 1192
likely will remain limited because of moderate expense and the need to perform serial measurements to provide clinically relevant information in patients with reduced renal function.[106]

TABLE 17.2-4 Reported Values for Effective Renal Plasma Flow and Renal Blood Flow in Horses and Ponies

NUMBER OF ANIMALS	METHOD[a]	EFFECTIVE RENAL PLASMA FLOW (MEAN ± SD/SE ml/min/kg [RANGE])	RENAL BLOOD FLOW (ml/min/kg)[b]	REFERENCE
1♀ horse	$Cl_{Diodrast}$	6.91 ±0.81	13.2 ± 1.6	89
1 ♀ pony	$Cl131_{I\text{-}o\text{-}HA}$	Bolus injection: 12.85 ± 1.81	21.7 ± 3.1	90[c]
		Constant infusion: 11.45 ± 1.25	19.4 ± 2.1	
3 ♀ horses	$Cl131_{I\text{-}o\text{-}HA}$	Bolus injection: 11.97 ± 2.63	20.2 ± 4.4	
		Constant infusion: 9.56 ± 1.84	16.2 ± 3.1	
6 ♀ ponies	ClPAH	12.09 ± 0.34 (7.86–21.62)	20.4 ± 0.6	91
2 ♀ horses		9.59 ± 0.86 (4.75–19.78)	16.2 ± 1.4	
5 ♀ and gelding horses	$Cl131_{I\text{-}o\text{-}HA}$	8.24 ± 2.88 (5.66–12.89)	13.9 ± 4.9	92
8 ♀ horses	Cl_{PAH}	Bolus injection: 12.0 ± 1.7	20.3 ± 2.9	95
4-day-old foals		Bolus injection: 15.2 ± 1.5	25.7 ± 2.7	
5 horses (3 ♂, 2♀)		Infusion (Cl-plasma): 18.2 ± 2.0	30.8 ± 3.4	
3 ponies (1 ♂, 2♀)		Infusion (Cl-urine): 11.9 ± 1.9	20.1 ± 3.2	
6 ♀ horses	Cl_{PAH}	8.5–10.8d	14.4–18.3	96
6 ♀ horses	Cl_{PAH}	11.9 ± 1.0	20.0 ± 1.7	97
6 horses[e]	$Cl131_{I\text{-}o\text{-}HA}$	6.26 (4.33–6.80)	10.58 (7.32–11.50)	98
8 ♀ horses	Cl_{PAH}	9.65 ± 0.84 (5.60–12.54)	16.31 ± 1.42	100
	$Cl131_{I\text{-}o\text{-}HA}$	11.32 ± 1.03 (7.82–15.71)	19.14 ± 1.74	
6 pony foals (3 ♀, 3 ♂)	Cl_{PAH}	16.63 (15.61–17.26)	28.11	103
10 horses (4 ♀, 6 gelding)	$Cl99_{mTc\text{-}MAG\text{-}3}$	7.92 ± 1.51 (5.58–10.62)	13.39 ± 2.55	104
4 ponies[e]	Microspheres	—	208 ± 58[f, g]	84
11 ponies[e]	Microspheres	—	548 ± 87[f]	83
8 ponies[e]	Microspheres	—	483 ± 79[f]	93
11 ponies[e]	Microspheres	—	670 ± 50[d, f]	94
3 horses (♀, ♂, and gelding)	Microspheres	—	535 ± 93[f]	99

9 horses[e]	Microspheres	—	589 ± 50[f]	101
4 gelding ponies	Microspheres	—	428 ± 49[f]	105

a $Cl_{Diodrast}$, Clearance of 3,5-diiodo-4-pyridine-N-acetic acid; $Cl131_{I\text{-}o\text{-}HA}$, clearance of ^{131}I-*o*-iodohippurate; Cl_{PAH}, clearance of *p*-aminohippurate; $Cl99_{mTc\text{-}MAG\text{-}3}$, $Cl99_{Tc\text{-}MAG3}$, clearance of ^{99m}Tc-mercaptoacetyltriglycine.

b Renal blood flow (RBF) values presented have been calculated from effective renal plasma flow (ERPF) data using extraction ratios (ERs) of 0.80 for diodrast and 0.91 for ^{131}I-*o*-iodohippurate and *p*-aminohippurate: RBF = (ERPF/ER)/(1-hematocrit); hematocrit was assumed to be 0.35.

c Other horses and ponies also were studied following bolus injection of ^{131}I-*o*-iodohippirate and yielded ERPF values of 16.93 ± 6.05 and 10.65 ± 2.73 ml/min/kg for ponies and horses, respectively; these values corresponded to a RBF of 8.6 ± 10.2 ml/min/kg in ponies and 18.0 ± 4.6 ml/min/kg in horses.

d Values estimated from figure.

e Sex not reported.

f Renal blood flow values for microsphere studies are expressed in units of milliliters per minute per 100 g of kidney tissue; a value of 500 ml/min/100 g would correlate to an RBF value of 18 ml/min/kg (or 3.6 and 9 L/min for a 200-kg pony and a 500-kg horse, respectively).

g Values reported are for ponies under general anesthesia (halothane in oxygen); these authors also reported that renal medullary blood flow was 2.6% to 18.8% of total RBF in two ponies.

TABLE 17.2-5 Reported Values for Glomerular Filtration Rate in Horses and Ponies

NUMBER OF ANIMALS	METHOD[a]	GLOMERULAR FILTRATION RATE (MEAN ± SD/SE ml/min/kg [range])	Cl_{CRend}/ClIn RATIO[a]	REFERENCE
Not reported[b]	Cl_{In}	0.83 ± 0.13[c,d]	1.02	Ketz et al (1956)[b]
	Cl_{CRend}	0.85 ± 0.22[c,d]		
1 horse[e]	Cl_{In}	1.4	—	Poulson (1957)[b]
12 ♀horses	Cl_{In}	1.66 ± 0.33[c](1.17–2.28)	0.88 ± 0.11	89
	Cl_{CRend}	1.46 ± 0.24[c](1.10–1.65)		
1 pony	Cl ^{125}I-iothalamate	Bolus injection: 5.43 (1 study)	—	90[f]
		Constant infusion: 6.10 ± 1.27	—	
3 ♀horses	Cl ^{125}I-iothalamate	Bolus injection: 4.20 ± 1.13		
		Constant infusion: 3.14 ± 0.53		
13 ponies[e]	Cl_{CRend}	1.93 ± 0.37[c](1.36–2.70)	—	114
9 (no NaCl supplement)		2.06 ± 0.34[c](1.64–2.70)		
4 (NaCl supplement)		1.63 ± 0.27[c](1.36–1.99)		
7 ♀ horses	Cl_{CRend}	3.68 ± 1.18[c](2.07–4.99)	—	115
4 ♀ horses	Cl_{In}	1.65 ± 0.07[c](1.34–2.04)	0.96 ± 0.02	116
	Cl_{CRend}	1.62 ± 0.03[c](1.29–2.15)		
6 ♀ ponies	Cl_{In}	1.92 ± 0.06[c](0.64–3.37)	0.86[c]	91[g]
	Cl_{CRend}	2.24 ± 0.06[c](1.04–4.15)		
2 ♀ horses	Cl_{In}	1.86 ± 0.14[c](0.71–3.68)	1.11[c]	
	Cl_{CRend}	1.67 ± 0.13[c](0.68–3.09)		
12 ♀ and gelding horses	Cl ^{99m}Tc-DTPA	1.93 ± 0.27 (1.39–2.53)	—	92
4 gelding horses	Cl_{CRend}	1.34 ± 0.51[c](1.01–2.10)	—	18
1 ♀ pony	Cl_{CRend}	1.15 ± 0.08	—	118
4 horses (1 gelding, 3 ♀)	Cl_{CRend}	1.45 ± 0.21[c]		
10 gelding horses	Cl_{CRend}	1.88 ± 0.46	—	25

12 ♀horses	Cl_{CRend}	1.48 ± 0.04	—	119[h]
1 ♂ pony	$Cl\ 14_{C\text{-}In}$	1.74 ± 0.15	0.61 ± 0.11	27[i]
	Cl_{CRend}	1.06 ± 0.10		
4 ♀ ponies (2 ♂, 2)	$Cl14_{C\text{-}In}$	1.66 ± 0.38[c](1.34–2.22)	1.02 ± 0.07[c]	
	Cl_{CRex}	1.70 ± 0.39[c](1.43–2.27)		
6 ♀ horses	Cl_{CRend}	1.92 ± 0.51 (1.49–2.74)	—	26
2 ♀ horses	Cl_{CRend}	Awake horses: 2.65[c]	—	120[j]
		During anesthesia: 1.32[c]		
		Following anesthesia: 2.50[c]		
8 ♀ horses	Cl_{In}	Bolus injection: 1.63 ± 0.33		95
4-day-old foals	Cl_{CRend}	2.81 ± 0.55	1.00[c,k]	
5 horse (3 ♂, 2 ♀)	Cl_{In}	Bolus injection: 2.30 ± 0.34		
3 pony (1 ♂, 2♀)		Infusion (Cl-plasma): 2.56 ± 0.30		
		Infusion (Cl-urine): 2.82 ± 0.32		
6 ♀ horses	Cl_{CRrex}	2.56 ± 0.60[c]	—	121
6 ♀ horses	Cl_{In}	1.88 ± 0.67	—	97
8 ♀ horses	Cl_{In}	1.83 ± 0.21 (0.89–2.95)	—	100
	$Cl99m_{Tc\text{-}DTPA}$	1.79 ± 0.18 (1.08–2.51)		
12 ♀horses	Cl_{In}	1.55 ± 0.04[c](0.98–2.22)	—	122
	$Cl99m_{Tc\text{-}DTPA}$	1.47 ± 0.27[c](0.91–1.82)		
	$Cl99m_{Tc\text{-}DTPA(cam)}$	1.55 ± 0.22[c,l]		
30 horses (7♂, 23 ♀)	Cl_{In}	1.73	1.03[c]	123
	Cl_{CRend}	1.79		
6 pony foals (3 ♀, 3 ♂)	Cl_{In}	3.21 ± 0.36 (2.73–3.64)	0.60[c]	103
	Cl_{CRend}	1.92 ± 0.14 (1.60–2.14)		
5 horses	$Cl99m_{Tc\text{-}DTPA}$	3.3 ± 0.4	—	125
6 ♀ horses	Cl_{CRend}	1.20–1.87	—	105a

a Cl_{In}, Inulin clearance; Cl_{CRend}, endogenous creatinine clearance; $Cl125_{I\text{-}iothalamate}$, clearance of ^{125}I-iothalamate; $Cl99m_{Tc\text{-}DTPA}$, clearance of ^{99m}Tc-diethylenepentaacetic acid; $Cl14_{C\text{-}In}$, clearance of ^{14}C-inulin; Cl_{CRex}, exogenous creatinine clearance; $Cl99m_{Tc\text{-}DTPA(cam)}$, clearance of

	99mTc-diethylenepentaacetic acid determined by serial imaging at the body surface with a gamma camera.
b	Values taken from Knudsen.89
c	Values presented have been calculated from original data.
d	Low glomerular filtration rate (GFR) values were attributed to rapidly declining plasma inulin concentrations (nonsteady state conditions) during the urine collection periods.
e	Sex not reported.
f	Other horses and ponies also were studied following bolus injection of 125I-iothalamate and yielded GFR values of 5.39 ± 1.79 and 3.44 ± 1.11 ml/min/kg for ponies and horses, respectively.
g	Attempts at measuring GFR by plasma disappearance following bolus injection of inulin were unsuccessful.
h	Value presented is for control group; GFR was not different after phenylbutazone administration (1.36 ± 0.04 ml/min/kg) or phenylbutazone and furosemide administration (1.44 ± 0.12 ml/min/kg) but was reported to increase to 1.75 ± 0.16 ml/min/kg after water loading (25 L) and to 1.77 ± 0.18 ml/min/kg after water loading and phenylbutazone administration.
i	Ponies were anesthetized during the studies.
j	Mares studied before, during, and after 1.2 minimum alveolar concentration halothane anesthesia.
k	Value calculated from urinary clearance values for inulin and creatinine.
l	Despite correction for differences in depth (right kidney closer to lateral body surface than left kidney), the clearance of 99mTc-diethylenepentaacetic acid determined by serial imaging at the body surface with a gamma camera showed a greater (.60% total) GFR by the right kidney compared with the left kidney (.40% of total). Because similar differences have not been demonstrated in microsphere studies of renal blood flow (in which both kidneys receive equal blood flow), this technique requires further refinement before it can be used to provide accurate measures of GFR in horses.

Intrinsic and extrinsic factors play a role in the control of RBF. The former include autoregulation and action of renal nerves; the latter include vasoconstrictors (catecholamines, renin-angiotensin system, arginine vasopressin) and vasodilators (prostaglandins, dopamine, atrial peptides, bradykinin, adenosine, and nitric oxide).[107] Although not unique to the kidney, autoregulation of blood flow is a physiologic response that maintains cortical RBF in the normal range through a rather wide range of perfusion pressures (75 to 180 mm Hg in human beings). This response is thought to be independent of neural or hormonal mechanisms and is attributed to a myogenic response to changes in arterial wall tension. The local action of renal nerves or release of vasoconstrictor substances leads to an increase in renal vascular resistance that may occur in response to disease states (hypovolemic or endotoxic shock), drugs (particularly anesthetic agents), or physical stress (exercise). Renal blood flow may or may not decrease, depending on the degree of vasoconstriction. For example, renal vascular resistance increases during low-intensity exercise to divert a greater portion of the cardiac output to the working muscles. Thus the fraction of cardiac output delivered to 1192
the kidneys decreases; however, because cardiac output also increases in response to exercise, total RBF 1193
remains unchanged.[97] In contrast, during halothane anesthesia in ponies, redistribution of cardiac output occurs without an increase in cardiac output. Under these circumstances, renal vasoconstriction is accompanied by a decrease in renal blood flow to about 60% of the awake value at 1.0 to 1.5 minimal alveolar concentration of halothane. As the plane of anesthesia deepens (to 2.0 minimal alveolar concentration), a greater increase in renal vascular resistance (or degree of vasoconstriction) further

decreases RBF to about 25% of the awake value, likely because of further vasodilatation of other vascular beds and a mild decrease in cardiac output.[93] Although RBF was not measured, results of a recent study of prolonged (18 hours) anesthesia with sevoflurane are of interest in terms of probable renal hypoperfusion and damage.[108] After 10 hours of anesthesia an increase in urine production was accompanied by evidence of tubular dysfunction (e.g., glucosuria and enzymuria). Furthermore, microscopic lesions following anesthesia were limited to the more distal nephron (mTAL and distal tubule), providing support that these normally hypoxic nephron segments may be the first to succumb to prolonged hypoperfusion.

When RBF decreases, counteracting vasodilatory mediators usually are released in an attempt to ameliorate the decrease in RBF. The best studied of these vasodilatory mediators include renal prostaglandins (PGE_2 and PGI_2) and dopamine. Although the role of renal prostaglandins in the control of basal or resting RBF is thought to be insignificant, renal prostaglandins are important mediators of vasodilatation in response to a number of vasoconstrictive stimuli.[109] Furthermore, production of renal prostaglandins is several times
greater in medullary tissue, so that action of these mediators leads to a greater increase in inner cortical 1193
(region of juxtamedullary glomeruli) and medullary blood flow. As mentioned previously, one should not be 1194
surprised that the lesion associated with antagonism of prostanoid production by use of nonsteroidal antiinflammatory drugs is medullary or papillary necrosis.[110,111] With or without renal vasoconstriction, activation of dopamine receptors (DA_1 type) leads to renal vasodilatation. Because the receptors are located on most renal arterioles, blood flow increases in the renal cortex and the medulla. For this reason, dopamine infusions are touted to be of benefit in treating acute renal failure because this catecholamine has been shown to increase RBF and urine output by 30% to 190% in normal horses.[102]

17.2.3.2 GLOMERULAR FILTRATION

Approximately 20% of the blood entering the glomeruli passes through small pores in the filtration barrier into Bowman's capsule. The primary force driving filtration is glomerular capillary transmural hydraulic pressure. A relatively constant pressure across the glomerular capillary wall is maintained by greater resistance in the arteriole leaving the glomerulus (efferent arteriole) than in the arteriole entering the glomerulus (afferent arteriole). This difference in vascular resistance generates the hydraulic pressure that forces plasma water out of the glomerular capillaries.[112] The filtration barrier is made up of three layers: endothelium of the glomerular capillaries, basement membrane, and foot processes of the epithelial cells (podocytes) lining Bowman's capsule. The pore size of the filtration barrier, about 8 to 10 nm in diameter, prevents filtration of cells and larger proteins. As a result, the fluid that enters Bowman's capsule is an ultrafiltrate that is essentially identical to plasma except that it has less than 0.05% of the protein content of plasma. Interestingly, the diameter of albumin is about 6 nm, so its size should not prevent filtration. Glycosaminoglycans containing heparan sulfate and sialic acid residues impart a significant negative charge to the filtration barrier. Thus charge repulsion of albumin (which is similarly negatively charged) may be more important than molecular size in preventing significant loss of albumin into the filtrate; however, metabolic disturbances (metabolic acidosis) can neutralize the glomerular charge barrier, and one can observe transient proteinuria in the absence of structural damage to the glomerular barrier.[113]

By definition, *glomerular filtration rate (GFR)* is the volume of plasma filtered per unit of time and commonly is described in milliliters per minute per kilogram of body mass. The GFR of horses and ponies ranges from 1.6 to 2.0 ml/kg/min, with some authors reporting slightly higher values for ponies. This range is similar to those of other animals and human beings. For a 500-kg horse this value equals 800 to 1000 ml per minute or about 1200 to 1400 L per day. This value represents filtration of the total plasma volume 60 to 70

times per day. Because urine production is about 10 L per day, more than 99% of the glomerular filtrate is reabsorbed.

Like RBF, GFR has been measured in horses bya variety of techniques, including Cl_{In} (by classic clearance techniques), Cl_{Cr}, and clearance or plasma disappearance of radionuclides (see Table 17.2-5).* Plasma disappearance curves for ^{99m}Tc-diethylenetriaminopentaacetic acid (^{99m}Tc-DTPA) have been documented to compare well in normal horses with Cl_{In} (the gold standard).[100] Although this technique is less expensive than ^{131}I-orthoiodohippuric acid clearance for estimating RBF,[106] clinical use is limited by availability of nuclear medicine capabilities and expense (because one must take multiple measurements to assess disease progression or response to treatment). Recently, Gleadhill, Marline, Harris, et al. described use of a three blood sample technique to estimate GFR by plasma disappearance of ^{99m}Tc-DTPA.[124] Interestingly, rather than expressing GFR on the basis of per kilogram of body mass or a body surface area, they suggested that GFR should be compared with extracellular fluid volume. Because one also can use plasma activity of ^{99m}Tc-DTPA to estimate extracellular fluid volume, one can make this estimate of GFR using ^{99m}Tc-DTPA alone. Standardization of GFR based on extracellular fluid volume is attractive and warrants further consideration because it eliminates the effect of variable body composition (e.g., specifically differences in body fat) when expressing GFR based on body mass. The authors subsequently used this method to estimate the decrease in GFR accompanying exercise.[125]

The mechanisms responsible for control of RBF (autoregulation, neural input, hormonal factors) also play a role in control of GFR. In addition, GFR is affected further by factors such as plasma protein concentration (oncotic pressure) and alterations in the filtration barrier. As discussed previously, a balance exists between that action of vasoconstrictor and vasodilator substances during periods of decreased RBF. Interestingly, GFR decreases less than RBF with moderate to severe renal vasoconstriction. This sparing effect on GFR has long been attributed to greater vasoconstrictive effects of angiotensin II on efferent arterioles compared with afferent arterioles.[126] Such a response could increase the glomerular capillary transmural hydraulic pressure driving filtration and would be manifested by an increase in filtration fraction. In fact, the latter response hasbeen documented in exercising horses.[97] More recently, however, other vasoconstrictors (endothelins) and vasodilators (endothelium-derived relaxing factors, nitric oxide) have been shown to play a role in the 1194
control of glomerular capillary hemodynamics and filtration, so that a singular role for angiotensin II is likely 1195
an oversimplified explanation for the sparing effect on GFR.[127]

* References 25–27, 89–92, 95, 97, 100–109, 114–125.

17.2.3.3 RENAL TUBULAR FUNCTION

Once the glomerular filtrate enters the renal tubule, it is modified extensively in the process of becoming the final product excreted into the renal pelvis. A complete review of renal tubular function is beyond the scope of this text; however, a few general concepts warrant mention, and a number of specific aspects are addressed elsewhere in this chapter. First, most glucose, amino acid, electrolyte, and water reabsorption occurs across epithelial cells lining the proximal tubule; however, these substances arenot all reabsorbed to the same extent. For example, this tubule segment is responsible for reabsorption of essentially all filtered glucose and amino acids, about 90% of filtered bicarbonate, about 70% of filtered sodium, and about 60% of filtered chloride.[128] Furthermore, at the end of the proximal tubule fluid is no more concentrated than it was in Bowman's space. Tubular sodium concentration is unchanged, whereas tubular chloride concentration actually has increased (because of preferential bicarbonate reabsorption). Despite limited modification of

these tubular fluid components, net reabsorption of between 60% and 80% of the total filtered load of sodium, chloride, and water occurs within the proximal tubule. Proximal tubular epithelial cells are also responsible for secretion of ammonium ions and a number of organic anions and cations, as described previously.

Tubular fluid passing into the loop of Henle becomes progressively more concentrated (hypertonic) as it travels to the inner medulla because the descending limb is permeable to water, urea, and electrolytes (the latter to a lesser degree).[129] In contrast, the ascending limb is relatively impermeable to water but actively reabsorbs sodium, chloride, and potassium via the apical $Na^+/K^+/2Cl^-$ cotransporter (blocked by furosemide), which is coupled to Na^+,K^+-ATPase on the basolateral membrane. As a result, fluid leaving this nephron segment is actually less concentrated (hypotonic) than the original filtrate. The loop of Henle is responsible for reabsorption of an additional 15% to 20% of filtered sodium and chloride, along with addition of urea to the tubular fluid. More important, Henle's loop is responsible for generation of the medullary osmotic gradient via countercurrent multiplication. This function results from the combined effects of different permeability characteristics of the descending and ascending limbs of the loop of Henle and active removal of sodium and chloride in the ascending limb.

The distal tubule is quantitatively less important in reabsorption of electrolytes and water; however, the distal tubule is the nephron segment in which the final qualitative changes in urine occur.[130] For example, the distal tubule is an important site of calcium, potassium, and acid excretion. The latter two typically are exchanged for sodium under the influence of aldosterone. Tubule fluid passes from the distal tubule into the outer or cortical collecting ducts, which are impermeable to urea. In addition to further modification of fluid in the cortical collecting ducts, tubular urea concentration increases steadily as water is removed (under the influence of vasopressin) as fluid travels to the inner medulla. In contrast, in the absence of vasopressin (as with diabetes insipidus), the collecting ducts are impermeable to water and produce hypotonic urine. The collecting ducts remain impermeable to urea (which accounts for up to 50% of the osmoles in urine) except for the innermost medullary segments, which allow urea to be recycled into the interstitium for maintenance of the medullary osmotic gradient.

Reabsorption of glomerular filtrate by renal tubules requires a close association with the vascular system that carries reabsorbed solute and water to the circulation. Proximal tubules are adjacent to peritubular capillaries, which have a tremendous capacity to accommodate the massive flux of solute and water across proximal tubule epithelial cells. Equally important in maintenance of the medullary osmotic gradient are the vasa recta, hairpin capillaries that travel deep into the renal medulla in association with loops of Henle derived from the population of juxtamedullary nephrons. Blood flow through these capillaries is typically slow, allowing for countercurrent exchange of solute in the medullary interstitium, which is necessary for generation and maintenance of medullary hypertonicity. Urea leaving the descending limb of Henle and being recycled across the innermost portion of the medullary collecting duct is responsible for about half of this medullary hypertonicity.

These basic aspects of tubular function have a number of important clinical implications. First, proximal tubule epithelial cells have a high metabolic rate. Although most of the proximal tubule is in the more highly perfused renal cortex, renal hypoperfusion leads to a relative hypoxia surrounding these cells because of ongoing metabolic activity. Consequently, the proximal tubule is highly susceptible to injury when cortical blood flow is reduced (e.g., with hypovolemia or other states accompanied by a decrease in RBF). Second, as discussed before, the renal medulla receives only a small fractionof the total RBF, leading to a normally hypoxic local environment. Thus any degree of renal hypoperfusion also is accompanied by exacerbation of medullary hypoxia, especially in the inner stripe because of the metabolic activity of epithelial cells lining

the mTAL. In fact, in cases of acute renal failure in human beings, histologic examination of renal tissue 1195
actually may show more severe lesions in the more distal nephron (mTAL) rather than in the proximal 1196
tubule.[131] Recognition of this more distal tubular damage has also led to consideration of therapeutic interventions to reduce damage to this nephron segment during periods of poor renal perfusion (e.g., continuous infusion of furosemide to decrease the metabolic activity of the mTAL). Third, despite the fact that the distal tubule and collecting ducts are responsible for reabsorption of less than 5% of the total glomerular filtrate, a decrease in reabsorption of only 1% to 2% can be quantitatively significant and can lead to dramatic polyuria (see Chapter 17.9). Next, generation of a maximal medullary concentration gradient requires slow flow of tubular fluid for countercurrent multiplication and slow flow of blood through the vasa recta to maximize countercurrent exchange. Thus conditions that increase tubular flow rates (high-volume intravenous fluids) or increase vasa recta blood flow (endogenous PGE_2 and PGI_2 production consequent to renal hypoperfusion) compromise the medullary concentration gradient (partial medullary washout) and lead to production of more dilute urine with increased urinary sodium concentration (and excretion).

A final aspect of tubular function that appears to be unique to horses among the domestic species is excretion of calcium. Equine urine is well recognized as being cloudy and viscid. These qualities can be attributed to the large amount of calcium excreted in normal equine urine, largely in the form of calcium carbonate crystals, and mucus secreted by glands in the renal pelvis and proximal ureter that acts to “lubricate” the lower urinary tract to minimize adherence of crystal to the epithelium lining the ureters, bladder, and urethra. Although the nature of this unique tubular calcium excretion has been studied little in horses, one report of the role of vitamin D in calcium and phosphorous homeostasis in horses suggested that this vitamin/hormone was less important in horses than in other species.[132] This fascinating difference between horses and other species evaluated by large animal internists clearly warrants further investigation.

17.2.3.4 EXCRETION OF SOLUTE AND WATER

Renal function traditionally is thought of in terms of glomerular filtration, tubular modification of the filtered fluid, and excretion of the final urine. This concept accommodates excretion of nitrogenous and organic wastes and the major aspects of regulation of body water content and ionic balance. Urine concentration and volume also are affected by solute excretion, and another way to think about renal function is in terms of total solute and water excretion. For example, a horse could produce 6 L of urine daily with an osmolality of 900 mOsm/kg to excrete 5400 mOsm of solute or, if the solute load were doubled to 10,800 mOsm, the horse could produce 12 L of urine with an osmolality of900 mOsm/kg to eliminate the additional solute. Thus urine osmolality reflects the ability of the kidney to dilute or concentrate the final urine but does not necessarily provide an accurate estimate of the “quantitative ability” to excrete solute or retain water. One assesses these functions by calculating the osmolal (C_{osm}) and free water clearances (C_{H2O}).[133] Like other clearances, these calculations require measurement of urine flow (via timed urine collection) and measurement of plasma and urine osmolality.

These measures of renal solute and water handlingare conceptualized by considering urine to have two components: (1) that which contains all the urinary solute in a solution that is isosmotic to plasma (C_{osm}, usually expressed in milliliters per minute or liters per day), and (2) that which contains free water without any solute (C_{H2O}, also expressed in milliliters per minute or liters per day). The sum of these two components is the actual urine flow rate in milliliters per minute or liters per day. Because urine is typically more concentrated than plasma, C_{H2O} typically has a negative value, indicating water conservation. In fact, the inverse of free water clearance is termed *renal water reabsorption*. Returning to the foregoing example,

excretion of the 5400 mOsm would require production of 18 L of urine that is isosmotic with plasma (using a value of 300 mOsm/kg for plasma). However, because 6 L of concentrated urine actually was produced during the period measured, the kidneys quantitatively have reabsorbed 12 L of free water per day. In contrast, despite production of urine with an identical urine osmolality (900 mOsm/kg), excretion of 10,800 mOsm would require production of 36 L of urine isosmotic with plasma. Free water clearance would be 30 L per day (i.e., 30 L per day of free water would be reabsorbed by the kidneys). Thus although concentrated urine always will have a negative C_{H2O} value, indicating renal water reabsorption, and dilute urine always will have a positive value for C_{H2O}, indicating renal water excretion, quantitative assessment of renal solute and water handling requires measurement of osmolal and free water clearances.

Excretion of free water by the kidney occurs by generation of hypotonic tubule fluid in the ascending limb of Henle's loop, and the amount or volume of free water produced depends on the amount of tubule fluid presented to that segment. Free water consequently is excreted by keeping the collecting ducts relatively impermeable to water (lack of vasopressin). Assessment of C_{H2O} is most helpful in patients with hyponatremia and hypoosmolality that cannot be attributed to another primary disease process (diarrhea or bladder rupture). For hyponatremia to develop, water excretion must be defective. For example, 1196
hyponatremia can develop with prerenal failure (hypovolemia) or with oliguric renal failure following a 1197
reduction in GFR and the amount of filtrate presented to the loop of Henle. Hyponatremia and hypoosmolality may also develop with use of loop diuretics because less free water is generated in the ascending limb of Henle's loop because of blockade of the apical $Na^+/K^+/2Cl^-$ cotransporter (smaller amounts of solute are removed). A final cause of true hyponatremia may be the syndrome of inappropriate vasopressin secretion or syndrome of inappropriate antidiuretic hormone secretion. Although the latter condition has not been documented in horses, occasionally it may play a role in the development of hyponatremia in a foal.[134]

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17.3 17.3—Examination of the Urinary System

Harold C. Schott, II

17.3.1 History and Physical Examination

To begin the evaluation of a horse with urinary tract disease, one should collect a complete history and perform a thorough physical examination. Important historical information includes duration and type of clinical signs, number of horses affected, diet, medications administered, and response to treatment. One also should assess water intake and urine output. For example, owners may mistake pollakiuria (frequent urination) for polyuria (increased urine production), and distinguishing between the two is helpful for forming a diagnosticplan. Pollakiuria frequently occurs in females during estrus or in either sex of horse with cystic calculi or cystitis. In contrast, polyuria more often accompanies renal disease, pituitary adenoma, behavior problems (primary polydipsia), diabetes insipidus, or diabetes mellitus. Astute owners may note increased thirst after exercise or a change in urine character, such as a clearer stream, to support polydipsia and polyuria.

One can determine water intake over 24 hours by turning off any automatic watering devices and providing a known volume of water to the horse.[1] Water intake can vary widely with environmental conditions, level of activity, and diet (see Chapter 17.2), so that repeated measurements over several 24-hour periods may be more rewarding in documenting average daily water consumption. Urine output, which should range between 5 and 15 L in a horse with normal renal function, is more difficult to determine. One can apply a urine collection harness for 24-hour urine collections[2–5]; alternatively, one can use an indwelling Foley catheter attached to a collection apparatus to quantify urine output in mares. Although horses used for research tolerate these devices fairly well, the devices have limited application to clinical patients. One can construct a practical collection device for geldings and stallions by cutting off the bottom of a large plastic bottle, padding it, and fitting it over the prepuce. One covers the opening of the bottle with a rubber tube and clip, and one can remove urine every few hours.[6] During the collection period, horses usually are tied or restrained in stocks to minimize interference with the collection device.

The most common presenting complaints for horses with urinary tract disease are weight loss and abnormal urination. Other clinical signs vary with the cause and site of the problem and may include fever, anorexia, depression, ventral edema, oral ulceration, excessive dental tartar, colic, or scalding or blood staining of the perineum or hindlegs. Although lumbar pain and hindlimb lameness have been attributed to urinary tract disease, a musculoskeletal problem is the usual cause of these clinical signs. Decreased performance may be an early presenting complaint for renal disease, but poor performance likely results from changes associated with uremia (mild anemia and lethargy) rather than from renal pain. Occasionally a horse with urolithiasis or renal neoplasia may have a history of recurrent colic. Prolonged or repeated posturing to urinate and dysuria or

hematuria would be important findings to implicate the urinary tract as the probable source of abdominal painin such patients.

In addition to a thorough physical examination, one should include rectal palpation in the evaluation of all horses with suspected urinary tract disease. One should palpate the bladder to determine size, wall thickness, and presence of cystic calculi or mural masses. If the bladder is full, one should palpate the bladder again after bladder catheterization or voiding. One can palpate the caudal pole of the left kidney for size and texture. The ureters generally are not palpable unless enlarged or obstructed by disease, but one should palpate the dorsal abdomen (retroperitoneal course of ureters) and trigone to determine if they are detectable. Dilation of a ureter may occur with pyelonephritis or ureterolithiasis, and in mares palpation of the distal ureters through the vaginal wall may be more rewarding. One also should palpate the reproductive tract to assess whether a reproductive problem could be causing the clinical signs.

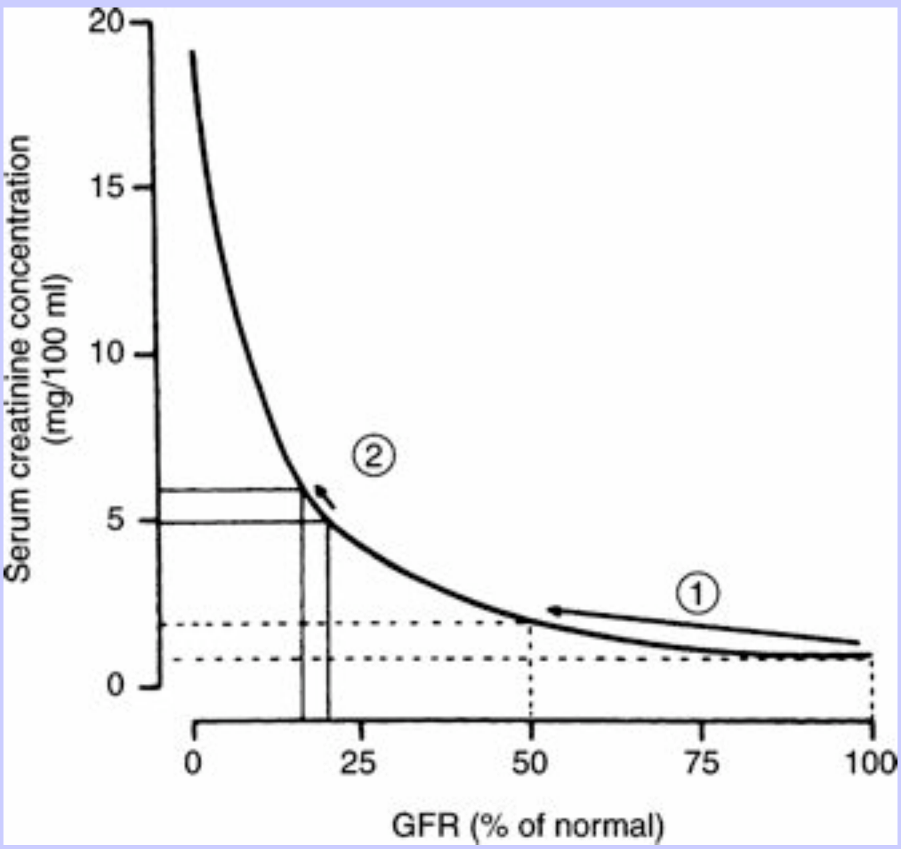
17.3.2 Hematology and Serum Biochemistry

A complete blood count that reveals an elevated white blood cell count and total protein or fibrinogen concentration would support an inflammatory or infectious disease process. One may observe mild anemia (packed cell volume 20% to 30%) consequent to decreased erythropoietin production and a shortened red blood cell life span in horses with chronic renal failure.

Blood urea nitrogen (BUN) and serum creatinine (Cr) concentrations are the most commonly used indexes of renal function, specifically glomerular filtration rate (GFR).[7–9] One must remember that BUN and Cr do not increase until the majority of nephrons (generally considered about 75%) become nonfunctional.[10] Although this commonly used percentage is based on studies of partially nephrectomized laboratory animals, several clinical reports in which unilateral nephrectomy was used successfully to manage disorders of the upper urinary 1200
tract support a similar renal reserve capacity in horses.[11–15] In addition, renal function remained within normal 1201
ranges and animals maintained body weight after experimental unilateral nephrectomy in ponies[16] andin horses (R. DeBowes, personal communication, 1991). Thus measurement of BUN and Cr is of little use in evaluating early or minor changes in GFR. Once elevated, however, small increases in BUN and Cr are more sensitive indicators of further deterioration in GFR, for one can intrepret doubling of BUN or Cr as a 50% decline in remaining renal function (Figure 17.3-1).

One can measure urea by a variety of methods that are categorized broadly as direct or indirect analyses.[7,8] The direct method is the diacetyl monoxime reaction, in which urea reacts with diacetyl after hydrolysis of diacetyl monoxime to diacetyl and hydroxylamine. One determines urea concentration spectrophotometrically by measuring the yield of the yellow diazine reaction product. Indirect analysis is based on enzymatic conversion of urea to ammonia and carbonic acid by urease. Several methods exist for subsequently determining ammonia concentration, and the one used most often is the enzyme-coupled reaction with glutamate dehydrogenase. Although the term "*blood* urea nitrogen" is widely accepted, one must remember that the actual measurement reported is the urea concentration in serum or plasma.

Figure 17.3-1 Relationship between glomerular filtration rate *(GFR)* and serum creatinine. When renal function is normal, a large decrease in glomerular filtration rate (as with acute renal failure) results in a minor increase in serum creatinine *(arrow 1)*. In contrast, when renal function is decreased (as with chronic renal failure), a much smaller decrease in glomerular filtration rate results in a similar increase in serum creatinine *(arrow 2)*. (From Brezis M, Rosen S, Epstein FH: Acute renal failure. In Brenner BM, Rector FC, editors: *The kidney*, ed 4, vol 1, Philadelphia, 1991, WB Saunders.)



One also can assay Cr by several methods, but the one used most often is the Jaffe's reaction, which is a colorimetric assay based on the formation of a complex between creatinine and alkaline picrate.[7–9] Unfortunately, a number of other substances in plasma or serum contribute to the yellow color, which leads to a 20% overestimation of actual Cr in human beings and in horses.[17] These noncreatinine chromagens include glucose, pyruvate, acetoacetate, fructose, uric acid, and ascorbic acid. Interference by noncreatinine chromagens is greatest when Cr is in the normal range, which leads to a high coefficient of variation in repeated

measurements of the same sample. With azotemia, Cr measurement by the Jaffe's reaction becomes more accurate because the contribution from noncreatinine chromagens does not increase significantly (noncreatinine chromagens are responsible for less than 5% of the color development when Cr is greater than 5.0 mg/dl). Noncreatinine chromagens do not interfere significantly with urine Cr measurement.

In addition to the factors discussed in the preceding section that influence urea and creatinine metabolism (see Chapter 17.2), spurious increases in Cr may be reported in various metabolic disorders or after administration of certain cephalosporin antibiotics.[8] When such increases in Cr are thought to be factitious, one can measure *true* Cr by several methods, which include use of an automated analyzer that distinguishes creatinine and noncreatinine chromagens by their different rates of color development or performance of the creatinine imidohydrolase enzyme assay. The latter yields ammonia, which can be quantified by colorimetric methods. As an example, Cr measured by the Jaffe's reaction increased 16% after horses were fasted for 3 days; however, when serum was analyzed by the enzymatic method, no increase in Cr was detected.[17] In addition to spurious increases in Cr, other substances can cause spurious decreases in serum Cr. The most common one is bilirubin, which, when greater than 5.0 mg/dl, may decrease measured Cr by 0.1 to 0.5 mg/dl.[8]

The reporting of serum or plasma urea and creatinine concentrations also varies between different countries. In the United States, BUN and Cr are reported in milligrams per deciliter, whereas in other parts of the world they are reported in standard international units of millimoles per liter and micromoles per liter, respectively. One can convert BUN from milligrams per deciliter to millimoles per liter and Cr from milligrams per deciliter to micromoles per liter by multiplying by 0.357 and 88, respectively.[8]

Azotemia is the term used to describe an elevation in BUN and Cr; thus it is strictly a laboratory abnormality. 1201
Azotemia can be prerenal in origin, because of decreased renal perfusion; may be attributable to primary renal 1202
disease; or may accompany obstructive diseases or disruption of the urinary tract (postrenal azotemia).[10,18] Thus one should interpret BUN and Cr in light of hydration status of the patient, presenting complaint, and physical findings. In general, animals with prerenal azotemia tend to have smaller increases in BUN and Cr than animals with intrinsic renal failure, whereas animals with postrenal failure may have the greatest degree of azotemia.[19] Unfortunately, BUN and Cr can cover a wide range of values for all three categories of azotemia; thus specific ranges do not identify the type of azotemia.[19–22] In an attempt to characterize azotemia better, use of the BUN/Cr ratio also has been examined. In theory the ratio should be higher for prerenal azotemia (because of increased reabsorption of urea with low tubule flow rates) and postrenal azotemia (because of preferential diffusion of urea across peritoneal membranes in cases of uroperitoneum) than for azotemia associated with intrinsic renal failure. As for categoric values for BUN and Cr, BUN/Cr ratios measured in azotemic dogs with naturally occurring diseases were distributed over wide, nondiscriminatory ranges for all three types of azotemia.[19,20] In horses the BUN/Cr ratio more often has been used to separate acute renal failure from chronic renal failure. In the acute form of renal failure, Cr tends to increase proportionately more than BUN, leading to a BUN/Cr ratio of lessthan 10:1.[23] In contrast, with chronic renal failure, the BUN/Cr ratio often exceeds 10:1. Although a clear explanation for this difference has not been established, the difference may be related to different volumes of distribution for urea and creatinine. Urea, a nonpolar molecule, diffuses freely into all body fluids, whereas creatinine, a charged molecule, likely requires longer to diffuse out of the extracellular fluid space. Thus a sudden decrease in renal perfusion leads to a greater increase in Cr than in BUN. Muscle breakdown or damage, as with rhabdomyolysis, may be an additional factor contributing to the rapid increase in serum Cr. Furthermore, the BUN/Cr ratio value provides only a suggestion of the duration of azotemia in horses, for one can find exceptions for acute and chronic renal failure. Finally, the BUN/Cr ratio

also may be useful in assessing adequacy of dietary protein intake in cases of chronic renal failure (see Chapter 17.5).[24]

The terms *prerenal azotemia* and *prerenal failure* describe the reversible increase in BUN and Cr associated with renal hypoperfusion.[10,18,22,25] Although these terms are entrenched firmly in human and veterinary medical literature, they likely contribute to the failure to recognize the renal damage that accompanies a number of medical and surgical conditions. Lack of recognition is attributable to the large renal functional reserve. In fact, in many cases of prerenal failure, one can demonstrate altered glomerular and tubule function by proteinuria and cast formation, impaired concentrating ability, and changes in electrolyte excretion.[26,27] Although such functional alterations are usually reversible, a degree of permanent nephron loss can occur and could explain the finding of microscopic evidence of renal disease in as many as one third of equine kidneys examined.[28] Thus considering prerenal failure as a transient and reversible period of compromised renal function that can lead to permanent but clinically silent decreased renal functional mass may be more appropriate. Furthermore, periods of decreased renal blood flow or prerenal failure are accompanied by a number of compensatory renal responses that are mobilized to preserve renal blood flow (autoregulatory response of the afferent arterioles) and GFR (increase in filtration fraction because of angiotensin II–mediated efferent arteriolar constriction). In addition, increased intrarenal production of vasodilatory prostaglandins (PGE_2 and PGI_2) is an important response to renal ischemia that maintains or even increases medullary blood flow (see Chapter 17.2). Thus one also can consider prerenal failure as a period of decompensation from the numerous renal compensatory responses to hypoperfusion.[29]

Prerenal azotemia traditionally is differentiated from intrinsic renal failure by assessing urinary concentrating ability. With prerenal azotemia, maintenance of urinary concentrating ability is demonstrated by a urine specific gravity greater than 1.020 and urine osmolality exceeding 500 mOsm/kg. In contrast, urinary concentrating ability typically is lost with intrinsic renal failure, and urine specific gravity and osmolality are less than 1.020 and 500 mOsm/kg, respectively, in the face of dehydration.[30] Such assessment is challenging, however, because it is valid only when performed on urine collectedbefore initiation of fluid therapy or administration ofany of a number of medications (α_2-receptor agonists, furosemide) that can affect urine flow and concentration.[31–34] In addition to these measures of urinary concentrating ability, urine/serum ratios of osmolality, urea nitrogen and creatinine concentrations, and fractional sodium clearance may provide useful information to differentiate prerenal azotemia from intrinsic renal failure (Table 17.3-1).[30,31] For example, urine/serum Cr ratios exceeding 50:1 (reflecting concentrated urine) and fractional sodium clearance values less than 1% (indicating adequate tubule function) would be expected in horses with prerenal azotemia, whereas ratios less than 37:1 and clearance values greater than 0.8% were reported in a group of horses determined to have primary renal disease.[30] Although these values can be helpful, the data in Table 17.3-1 illustrate that renal 1202
hypoperfusion is accompanied by a progressive loss of concentrating ability, because the ranges of these ratios 1203
tend to be lower for horses with prerenal azotemia than for clinically normal horses. Thus these data also support the concept that the progression from prerenal failure to intrinsic renal failure is associated with decompensation of the intrarenal responses to hypoperfusion.[29] Clinically, this decompensation is recognized as persistence of azotemia, whereas prerenal azotemia rapidly resolves(by 30% to 50% within 24 hours and completely by72 hours) in response to fluid therapy and other supportive treatments.

TABLE 17.3-1 Diagnostic Indexes That May Be Useful for Separating Prerenal From Renal Azotemia in Horses

DIAGNOSTIC INDEX	NORMAL HORSES	PRERENAL AZOTEMIA	RENAL AZOTEMIA
Urine osmolality (mOsm/kg)	727–1456	458–961	226–495
Uosm/Posm	2.5–5.2	1.7–3.4	0.8–1.7
UUN/PUN	34.2–100.8	15.2–43.7	2.1–14.3
UCr/PCr	2.0–344.4	51.2–241.5	2.6–37.0
FCl_{Na}	0.01–0.70	0.02–0.50	0.80–10.10

Modified from Grossman BS, Brobst DF, Kramer JW et al: Urinary indices for differentiation of prerenal azotemia and renal azotemia in horses, *J Am Vet Med Assoc* 180:284, 1982.

Uosm, Urine osmolality; *Posm,* plasma osmolality; *UUN,* urine urea nitrogen; *PUN,* plasma urea nitrogen; *UCr,* urine creatinine; *PCr,* plasma creatinine; FCl_{Na}, fractional sodium clearance.

In patients at risk for developing acute renal failure, including horses with serious gastrointestinal disorders, or rhabdomyolysis and in those receiving nephrotoxic medications, serial assessment of urine specific gravity or osmolality, sodium concentration, and fractional sodium clearance may be useful in identifying significant changes in renal function before the onset of azotemia. Similarly, if one determines urine flow rate during a timed urine collection period, assessment of renal water reabsorption (free water clearance; see Chapter 17.2) can be a sensitive predictor of impending renal failure.[35–37] Unfortunately, monitoring of these parameters often is complicated by use of intravenous fluid support in such patients. Although intravenous fluids can complicate interpretation of many of these indexes of renal function, Roussel, Cohen, Ruoff, et al.[31] found that the urine/plasma osmolality ratio remained greater than 1.7:1 in healthy horses receiving 20 L of an intravenous polyionic solution over 4 hours. Thus serial measurement of urine specific gravity or osmolality may provide useful information for patients at high risk for acute renal failure.

One usually suspects postrenal azotemia resulting from obstruction or disruption of the urinary tract based on clinical signs, including dysuria and renal colic. With bladder rupture, however, some affected foals and adult horses continue to void urine although progressive abdominal distention usually accompanies development of uroperitoneum. One most often confirms urinary tract disruption by measuring a twofold or greater value for peritoneal fluid creatinine concentration compared with serum creatinine concentration. Occasionally in a foal with a urachal problem or a gelding with a disrupted urethra, postrenal azotemia may be accompanied by considerable swelling in the abdominal wall or in the prepuce, respectively.

In addition to screening for azotemia and concentrating ability, the laboratory database should include serum electrolyte, protein (albumin and globulin) and glucose concentrations, and muscle enzyme activity.[8,18,21–25] Hyponatremia and hypochloremia are common in horses with renal disease. Serum potassium concentration may be normal or may be elevated in cases of acute or chronic renal failure. Hyperkalemia is often most extreme and most serious with urinary tract disruption and uroperitoneum. Calcium and phosphorus concentrations vary in horses with renal disease. Hypercalcemia and hypophosphatemia often occur in horses with chronic renal failure, especially when they are fed alfalfa hay (see Chapter 17.5), whereas hypocalcemia and hyperphosphatemia are more common with acute renal failure. With protein-losing glomerulopathies,

albumin tends to be lost to a larger extent than the higher-molecular-weight globulin. One can find low total protein and albumin concentrations in severe cases of chronic renal disease, whereas other horses may have an increased globulin concentration consistent with a chronic inflammatory response. Hyperglycemia (values greater than 150 to 175 mg/dl) following stress, exercise, sepsis, pituitary adenoma, or diabetes mellitus can result in glucosuria.[38,39] Finally, when pigmenturia is a complaint, muscle enzyme activity measurements are helpful in differentiating myoglobinuria from hematuria or hemoglobinuria.

17.3.3 Urinalysis

One should perform urinalysis whenever one suspects urinary tract disease. One can collect urine as a midstream catch during voiding, via urethral catheterization, or via cystocentesis in foals. Unlike cows, horses cannot be stimulated easily to urinate, but they often urinate within a few minutes after being placed in a freshly bedded stall. Manual compression of the bladder during rectal palpation may stimulate urination after the rectal examination is completed. One should evaluate color, clarity, odor, viscosity, and specific gravity at the time of collection.[40,41] Normal equine urine is pale yellow to deep tan and often is turbid because of the large amounts 1203
of calcium carbonate crystals and mucus. Urine appearance commonly changes during urination, especially 1204
toward the end of micturition, when more crystals tend to be voided. If pigmenturia or hematuria is present, noting the timing and duration of passage of discolored urine may help localize the source. Pigmenturia throughout urination is most consistent with myonecrosis or a bladder or kidney lesion, whereas passage of discolored urine at the start or end of urination more often occurs with lesions of the urethra or accessory sex glands (see Chapter 17.8).

17.3.3.1 ASSESSMENT OF URINE CONCENTRATION

Urine specific gravity is a measure of the number of particles in urine and is a useful estimate of urine concentration. Although determination of specific gravity with a refractometer is quick and easy (one should not use reagent strips to measure specific gravity in horses),[40] one must recognize that urine concentration is determined most accurately by measurement of urine osmolality because the presence of larger molecules in urine, such as glucose or proteins, leads to overestimation of urine concentration by assessment of specific gravity. Clinically, overestimation is a problem in patients with diabetes or heavy proteinuria.[42] Urine specific gravityis used to separate urine concentration into three categories: (1) urine that is more dilute than serum (hyposthenuria or specific gravity less than 1.008 and osmolality less than 260 mOsm/kg); (2) urine and serum of similar osmolality (isosthenuria or specific gravity of 1.008 to 1.014 and osmolality of 260 to300 mOsm/kg); and (3) urine that is more concentrated than serum (specific gravity greater than 1.014 and osmolality greater than 300 mOsm/kg). Althoughurine of most normal horses is concentrated (3 to 4 times more concentrated than serum with specificgravity of 1.025 to 1.050 and an osmolality of 900 to 1200 mOsm/kg), occasionally a normal horse produces dilute or highly concentrated urine. For example, in response to water deprivation of 24 to 72 hours' duration, horses with normal renal function often produce urine with a specific gravity greater than 1.045 and an osmolality greater than 1500 mOsm/kg.[43–45] In contrast, foals typically have hyposthenuric urine consequent to their mostly milk diet.[46] Although the constant polyuria decreases the ability of the neonate to generate a large osmotic gradient in the medullary interstitium, dehydrated foals still can produce urine with a specific gravity greater than 1.030. With chronic renal insufficiency the ability to produce concentrated (specific gravity greater than 1.025) or dilute (specific gravity less than 1.008) urine is lost. Thus horses with chronic renal failure typically manifest isosthenuria. As discussed previously, urine specific gravity is helpful in differentiating prerenal from renal azotemia in horses that exhibit dehydration or shock following a number of disorders.

17.3.3.2 REAGENT STRIP ANALYSIS

The pH of equine urine is usually alkaline (7.0 to 9.0).[40,41,47] Vigorous exercise or bacteriuria can resultin acidic pH. Bacteriuria can impart an ammonia odor to the urine secondary to breakdown of urea by bacteria with urease activity. Concentrate feeding generally decreases urine pH toward the neutral value.[47] Similarly, the more dilute the urine sample is, the closer the pHis to 7.0. The dilute urine produced by foals typically is neutral to mildly acidic and is relatively free of crystalline material. Interestingly, calcium oxalate crystals are more prevalent in urine of foals than in that of adults.[48] Occasionally, one detects aciduria in a dehydrated or anorectic horse. Although aciduria typically has been attributed to metabolic acidosis, many patients actually may have hypochloremic metabolic alkalosis accompanied by paradoxical aciduria. The mechanism for paradoxical aciduria is likely similar to that described in ruminants with abomasal outflow obstruction.[49] Briefly, after all chloride has been reabsorbed from the glomerular filtrate, further sodium reabsorption occurs by exchange with (excretion of) potassium or hydrogen ions. Thus paradoxical aciduria is most likely to occur with concomitant hypokalemia or whole-body potassium depletion.

Commercially available urine reagent strips can yield false-positive results for protein when one tests alkaline samples. Thus one can assess proteinuria better by performing the semiquantitative sulfosalicylic acid precipitation test or by specific quantification with a colorimetric assay (such as the Coomassie brilliant blue dye method[50] or other assays that are used routinely on cerebrospinal fluid). In normal mares a mean value of 3.2 mg/kg(1.6 g) per day and a range of 3.6 to 22.3 mg/kg(1.8 to 11.2 g) per day for urinary protein excretion have been reported by Schott, Hodgson, and Bayly[51] and by Kohn and Strasser,[52] respectively. These values translate into urinary protein concentrations of less than 100 mg/dl in most normal horses. Comparison of the quantitative protein result (milligrams per deciliter) to urine creatinine concentration (milligrams per deciliter) in the form of a urine protein/creatinine ratio also is recommended. This technique is more practical because it obviates timed urine collection. Although a normal range hasnot yet been reported for horses, values exceeding 1.0:1 and 3.5:1, respectively, are considered above normalfor dogs[53] and indicate nephrotic range proteinuria in human beings.[8] Thus a urine protein/creatinine ratio greater than 2:1 likely supports significant proteinuria in an equine patient. Proteinuria may occur with glomerular disease, bacteriuria, or pyuria or may transiently follow exercise.[51] 1204

1205 Normal equine urine should not contain glucose. Although the renal threshold for glucose has not been evaluated thoroughly in horses, an early study byLink suggested that the threshold may be lower (about 150 mg/dl) than that of small animals and human beings.[54] Thus glucosuria can accompany hyperglycemia associated with the causes described previously or with administration of dextrose-containing fluids or parenteral nutrition products.[38,39] In addition, glucosuria may accompany sedation with α2-agonists or exogenous corticosteroid administration.[32,33] When one detects glucosuria in the absence of hyperglycemia, one should suspect primary tubule dysfunction. Glucosuria more often has been detected in horses with acute renal failure (mostly in experimental models of nephrotoxicity) than in those with chronic renal disease. Unlike ruminants, ketones rarely are detected in equine urine, even in advanced catabolic states or with diabetes mellitus. A positive result for blood on a urine reagent strip can reflect the presence of hemoglobin, myoglobin, or intact red blood cells in the urine sample. Evaluation of serum for hemolysis and of urine sediment for red blood cells, combined with an ammonium sulfate precipitation test to detect myoglobin,[55] can be rewarding in differentiating between these pigments (see Chapter 17.8). Finally, occasionally one detects bilirubinuria on reagentstrip analysis of equine urine. Bilirubinuria is associated with intravascular

hemolysis, hepatic necrosis, and obstructive hepatopathies. In most instances, one more commonly detects hemolysis and hepatic disease by abnormal biochemical data such as elevated serum bilirubin concentration and increased hepatic enzyme activity.

17.3.3.3

SEDIMENT EXAMINATION

Sediment examination is probably the most underused diagnostic technique available for evaluation of urinary tract disorders in horses. In human beings, sediment examination has been demonstrated to be a useful predictor for occurrence and severity of acute renal failure.[56] Unfortunately, a major limitation is that one should examine sediment within 30 to 60 minutes after collection. To perform sediment examination, one centrifuges 10 ml of fresh urine (usually in a conical plastic tube) at 1000 rpm for 3 to 5 minutes. One discards the supernatant urine (or uses it for quantitative protein determination) and resuspends the pellet in the few drops of urine remaining in the tube. One transfers a drop of sediment to a glass slide and applies a coverslip. One first examines the sediment at low power to evaluate for casts and subsequently at high power to quantify erythrocytes, leukocytes, and epithelial cells and to determine whether bacteria are present. Casts are molds of Tamm-Horsfall glycoprotein and cells that form in tubules and subsequently pass into the bladder. They are rare in normal equine urine but may be associated with inflammatory or infectious processes. Casts are unstable in alkaline urine; thus one should evaluate sediment as soon as possible after collection to ensure accurate assessment. One should see fewer than five red blood cells per high-power field in an atraumatically collected urine sample. Increased numbers of urinary red blood cells can result from inflammation, infection, toxemia, neoplasia, or exercise (see Chapter 17.8). Pyuria (more than 10 white blood cells per high-power field most often is associated with infectious or inflammatory disorders and normal equine urine should have few bacteria, if any. The absence of bacteria on sediment examination does not rule out their presence, however, and one should perform bacterial culture of urine collected by catheterization or cystocentesis (foals) when one suspectscystitis or pyelonephritis. Finally, equine urine is rich in crystals. Most of these are calcium carbonate crystals of variable size, but calcium phosphate crystals and occasionally calcium oxalate crystals also are visible in normal equine urine (Figure 17.3-2).[40,41,57] Addition of a few drops of a 10% acetic acid solution may be necessary todissolve crystals for accurate assessment of urine sediment.[40]

17.3.3.4

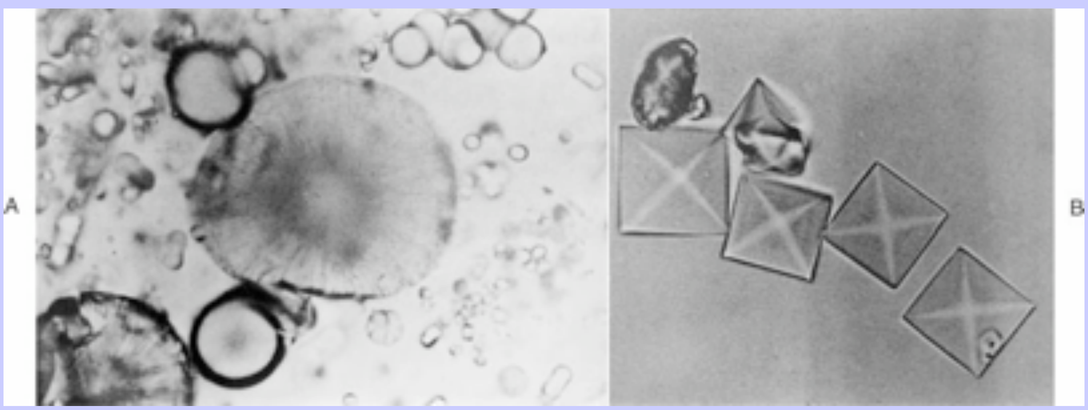
ENZYMURIA

Renal tubules are metabolically active, being responsible for absorption or excretion of a wide range of substances. Transport of these compounds is facilitated by a number of enzymes, which are found in large amounts in lysosomes within or in the brush borders of tubular epithelial cells. Regular turnover of these cells and release of endocytotic vesicles and lysosomes into the tubular lumen results in activity of enzymes in urine (enzymuria).[58] A number of substances that are filtered at the glomerulus (including bile acids, aminoglycoside and cephalosporin antibiotics, mannitol, dextrans, radiographic contrast media, and heavy metals) are taken up via endocytosis into proximal tubular epithelial cells. Endocytotic vesicles combine with lysosomes and substances that are not broken down by lysosomal enzymes subsequently are extruded into the tubule lumen through evacuation of residual bodies.

Inflammation or necrosis of tubular epithelial cells results in elevated urinary activity of lysosomal and brush border enzymes. Because proximal tubular epithelial cells are the most metabolically active of all kidney cells, they are particularly susceptible to ischemic injury. Furthermore, they can be injured similarly by exposure to large quantities of nephrotoxins in glomerular filtrate. As a result, determination of the activities

of certain urinary enzymes can provide evidence of tubular damage several days before azotemia may 1205
develop.[59–62] Additional contributors to increased urinary enzyme activity include (1) low-molecular-weight 1206
enzymes such as amylase that normally are filtered by glomeruli and reabsorbed inthe proximal tubules, (2) postrenal genitourinary tract epithelia that usually contribute a negligible amountto the overall urinary enzyme activity (unless they become neoplastic), and (3) secretions from accessory sex glands. Contributions from the latter explain why intact males tend to have higher urinary activities of lactate dehydrogenase (LDH) and *N*-acetyl-β-D-glucosaminidase (NAG).

Figure 17.3-2 Crystals commonly observed in equine urine sediment (original magnification ×160). **A**, Large, round calcium carbonate crystals (*center* and *lower left*) and smaller calcium phosphate crystals *(oblong)*. **B**, Calcium oxalate dihydrate crystals. (Reproduced from Osborne CA, O'Brien TD, Ghobrial HK et al: Crystalluria: observations, interpretations, and misinterpretations, *Vet Clin North Am Small Anim Pract* 16:45, 1986.)



Although more than 40 enzymes have been detected in urine of different species, only a few appear to be of diagnostic relevance. To be of clinical use, a urinary enzyme must have measurable activity in the kidney; its activity must lie within a fairly narrow range in urine of healthy animals; it must be sufficiently large (molecular weight >60,000) so as not to be filtered freely across the glomerulus; and its activity must increase early enough during the course of renal injury to permit institution of corrective treatment. Finally, the activity of the urinary enzyme should remain fairly stable in urine for several days without the need for special processing. In human beings and dogs, a number of enzymes, includingNAG, LDH, β-glucuronidase, alanine aminopeptidase, alkaline phosphatase (ALP), leucine aminopeptidase,γ-glutamyltransferase (GGT), and kallikrein have been demonstrated to be sensitive indicators of renal damage.[59–61,63,64] With respect to horses, normal values have been established for activities of GGT, ALP, NAG, LDH, and kallikrein.[65–69] Attempts to assay aspartate aminotransferase and alanine aminotransferase activities were unsuccessful in normal horse urine.[66]

Alkaline phosphatase and GGT are membrane-associated enzymes found primarily in the brush border of the proximal tubular epithelium.[60–64,70] Their activity in distal tubular epithelium is negligible.[13] These enzymes have activity in other tissues, but because they are not filtered by the glomerulus, elevated activity in urine in the absence of significant proteinuria is presumed to originate from the kidneys. Measurable activity in normal urine is attributed to cell turnover.[60] Lactate dehydrogenase is a more ubiquitous tubular epithelial enzyme, being as active in the distal tubules and medullary papillae as in proximal tubular epithelium.[63–70] *N*-acetyl-β-D-glucosaminidase is a proximal tubular lysosomal enzyme.[60–70] Only GGT, ALP, LDH, and NAG have been assayed in the urine of horses known to have, or thought to have, some form of renal dysfunction. Determination of NAG activity can be difficult in normal equine urine because of its alkalinity, and normal values may be less than detectable assay limits when one uses a spectrophotometric, rather than a fluorometric, method.[71–74] Published normal activities (expressed per gram of creatinine) for these enzymes in equine urineare GGT: 0-25 IU/g Cr; ALP: 0-28 IU/g Cr; LDH:0-12 IU/g Cr; and NAG: less than 1 IU/g Cr (<2 IU/L urine). Comparison with creatinine concentration, which is relatively constant, results in less volume-related variation and allows interpretation of a randomly collected specimen (compared with a urine sample obtained during a timed collection period).[65,75,76] 1206

1207

Factors one must consider when measuring urinary enzyme activities include conditions under which the urine sample has been stored, urinary pH, diurnal variation, gender- and age-related variations, and other naturally occurring inhibitors or promoters of enzyme activity in urine (albumin, mucoproteins, proteolytic agents, amino acids, and ammonia). Although these factors have received limited study respecting equine urine, it has been documented that freezing can decrease activity of all enzymes, especially GGT. Furthermore, the colder the temperature, the more rapid the loss of enzyme activity.[65] To obtain the most accurate results, one should refrigerate samples (4° C) and assay them within 72 hours of collection. In species that have a slightly acidic or neutral urinary pH, assay of NAG is considered one of the most valuable diagnostic tests available. However, its activity appears to be susceptible to pH changes. In human beings receiving nephrotoxic medications, NAG activity in urine became undetectable at pH values greater than 8.[77] Similarly, NAG activities were undetectable (less than 2 IU/L) in urine of normal horses.[78] In a study of monensin toxicosis in horses, NAG activity increased as urinary pH decreased.[67] Certain amino acids and ammonia further act as inhibitors of lysosomal enzymes such as ALP and NAGin the urine of human beings, dogs, and rats,[79,80] and techniques have been developed to remove these agents from the urine by gel filtration before assay of their activity.[81]

Theoretically, assessment of changes in the urinary activity of selected enzymes may assist the clinician in identifying the segment of the nephron suffering the greatest dysfunction or damage. Although NAG, GGT, and ALP are associated primarily with proximal tubular epithelium, LDH usually is associated with distal tubular epithelial cells. Increases in urinary GGT and ALP activity have been induced experimentally in horses receiving gentamicin and neomycin for 5 to 10 days.[82,83] Increases also have been measured in horses with diarrhea, acute abdominal crises, and endotoxic shock. In the latter instances, enzymuria was assumed to indicate tubular damage following ischemia. Five consecutive days of furosemide administration also produced moderate increases in GGT and ALP activity, with ALP increasing more rapidly.[78] However, 48 hours of water deprivation failed to induce any changes in GGT, ALP, or LDH activity.[69] Similarly, no change in urinary LDH activity was observed in horses administered phenylbutazone (8.8 mg/kg p.o.) daily for six days.[69]

Although increases in urinary enzyme activities generally indicate acute tubular damage, one must interpret urine enzyme/Cr ratios carefully. Threshold values above which elevations are significant have not been well documented, although one study reported that a GGT/Cr value greater than 25 IU/g indicated tubular damage.[65] In contrast, in a study of gentamicin-induced nephrotoxicity in pony mares, Hinchcliff, McGuirk, and MacWilliams[82] measured GGT/Cr values exceeding 100 IU/g several days before measuring an increase in serum creatinine concentration. Furthermore, one frequently may measure GGT/Cr values between 25 and 100 IU/g in horses receiving gentamicin at recommended doses. Similar to gentamicin pharmacokinetics, enzymuria in these horses has been highly variable and these horses have not been recognized to be at risk of developing acute renal failure. Although enzymuria likely reflects a degree of tubular damage in these patients, one should interpret increases in GGT/Cr values between 25 and 100 IU/g with caution, whereas increases greater than 100 IU/g are more likely to be clinically significant.

Horses with chronic renal disease may have normal or reduced enzyme activities that reflect cellular changes that occur in the nephron in response to chronic inflammation. Just as BUN and Cr concentrations may be normal during the early stages of renal disease, urinary enzyme activities may not reflect accurately renal dysfunction later in the disease course when results of blood tests and urinalysis are more likely to be abnormal. A possible reason for this phenomenon is that substantial destruction of tubular epithelium occurring early in the disease leaves fewer epithelial cells to be an ongoing source of elevated enzyme activities. Alternatively, regenerating tubular epithelial cells may be more refractory to the effects of the toxin.

All in all, determination of urinary enzyme activities has failed to gain acceptance as a routine measure of renal tubular damage in most equine hospitals. This lack of acceptance can be attributed to the high sensitivity for detection of subclinical renal tubular damage. For example, although a urine GGT/Cr ratio elevated to a value of 75 supports tubular damage, it does not provide the clinician a quantifiable risk for development of acute renal failure. Thus as a single measurement, the ratio is of limited use in deciding whether one should discontinue use of a nephrotoxic medication (e.g., gentamicin). In contrast, more dramatic elevations of the GGT/Cr ratio may precede development of azotemia and could be a useful warning that one may need to discontinue a medication or, at a minimum, prolong the dosing interval. Until values for urinary enzyme activities are reported for a larger number of equine patients with various diseases, the true value of assessing urinary enzyme activity will remain unclear.

17.3.3.5 FRACTIONAL CLEARANCE OF ELECTROLYTES

Urinary electrolyte losses, which reflect tubular function, can be expressed as excretion rates (total amount of 1207
electrolyte excreted during a given time period, expressed as milliequivalents per minute) or as clearance 1208
rates. Determination of clearance rates uses the same clearance concept to measure GFR. In brief, a clearance rate (Cl_A) is a measure of the volume of plasma that is cleared completely of the substance in question (A) during a given time period. One calculates the Cl_A by performing a timed urine collection (to determine urine flow in milliliters per minute) and measuring the concentration of the desired substance in plasma and urine (creatinine or inulin for determination of GFR)[8]:

$$Cl_A = \frac{\text{Urine [A]}}{\text{Plasma [A]}} \times \text{Urine flow}$$

As with protein, urinary clearance of many substances, including electrolytes, often is compared with that of creatinine.[8] Basically, a substance that is filtered mostly across the glomerulus but is neither reabsorbed nor secreted by renal tubules (inulin) will have a clearance rate similar to that of creatinine. In contrast, a substance that is poorly filtered (larger molecule) or reabsorbed to a great extent by renal tubules (sodium or chloride) will have a lower clearance value than that of creatinine. Similarly, clearance values for substances that are eliminated by filtration and tubular secretion (potassium) may exceed that measured for creatinine. An advantage of comparing the clearance of a substance (A) to creatinine clearance (expressed as a fraction of creatinine clearance) is that it obviates the need to perform a timed urine collection, for the urine flow factor is cancelled out in the calculation:

$$\frac{Cl_A}{Cl_{Cr}} = \frac{\dfrac{\text{Urine [A]}}{\text{Plasma [A]}} \times \text{Urine flow}}{\dfrac{\text{Urine [Cr]}}{\text{Plasma [Cr]}} \times \text{Urine flow}}$$

that, by rearrangement and expression as a percentage becomes

$$\frac{Cl_A}{Cl_{Cr}} = \left(\frac{\text{Plasma [Cr]}}{\text{Urine [Cr]}} \times \frac{\text{Urine [A]}}{\text{Plasma [A]}}\right) \times 100$$

This calculation is called the fractional creatinine clearance value.[8,84] More often, however, the term *fractional excretion* has been used to describe this value. Although most sources recommend that blood and urine samples be collected at the same time for determination of fractional clearance values, serum electrolyte and creatinine concentrations are usually stable (except in patients with prerenal azotemia or acute renal failure), so one can use blood values measured within a few days of the urine sample in the clearance calculations. Consequently, leaving a specimen cup for the client to use to collect a voided sample is acceptable and obviates bladder catheterization in many cases.

As previously discussed (see Chapter 17.2), the equine kidneys function to conserve more than 99% of filtered sodium and chloride ions. In contrast, potassium ions are conserved poorly except during periods of whole-body potassium depletion (anorexia, prolonged exercise). Thus normal fractional clearance values areless than 1% for sodium but are considerably higher for potassium (Table 17.3-2).[30,41,45,52,85–94] Increases in fractional sodium and chloride clearance values may reflect an appropriate renal regulatory response to dietary excess, as with psychogenic salt consumption.[95] Alternatively, increases in fractional clearance values specifically for sodium and phosphorous also can be early indicators of renal tubule damage[30,96–98]; however, one must interpret results of these calculations in light of fluid therapy because fractional clearance values can be increased artifactually in horses receiving intravenous polyionic solutions.[31] Similarly, medication (furosemide) or light exercise also can result in increased urine flow and fractional sodium and chloride clearance values.[99]

The kidneys play an important role in equine calcium and phosphorus homeostasis, and renal loss of these ions varies with dietary intake. Thus fractional clearances of calcium and phosphorous also have been used to assess adequacy of dietary intake.[86–88,90,92,100–105] Although diet is evaluated more appropriately on a herd basis by analyses of hay and concentrates, fractional clearances may be useful in individual animals or when feed analysis is impractical (e.g., forage consists of pasture). Determination of fractional calcium and

phosphorus clearances has received limited study with a focus on young racing horses.[100–105] For example, excessive dietary phosphorus intake (which can lead to nutritional secondary hyperparathyroidism) leads to increased fractional clearance of phosphorus. Evaluation of fractional calcium clearance is hampered by the fact that most of the calcium in equine urine is in the form of calcium carbonate crystals. To measure the urinary calcium concentration reliably, one must collect the entire contents of the bladder during voiding or via catheterization to ensure collection of the initial crystal-poor and final crystal-rich fractions. Subsequently, one treats a well-mixed aliquot of urine with acetic acid or nitric acid to solubilize the crystals.[92] In one report, fractional calcium and phosphorus clearance values greater than 2.5% and less than 4% were considered consistent with adequate dietary intake (adequate calcium intake with phosphorus intake that was not excessive).[101] Unfortunately, because the ranges for fractional calcium and phosphorus clearances can be wide in normal horses (see Table 17.3-2), measurement of these clearances may not be sensitive enough to detect minor dietary imbalances.

Determination of fractional electrolyte clearance values also has been advocated in the evaluation of horses 1208
with recurrent rhabdomyolysis.[106–108] Low sodium and potassium clearances have been reported in some 1209
affected horses. Whether these low fractional clearance values reflected total body electrolyte depletion (as a consequence of repeated bouts of exercise in hot weather or repeated furosemide administration) or a true physiologic predisposition to rhabdomyolysis was not determined. Nevertheless, low fractional clearance values document the need for electrolyte supplementation in equine athletes. Harris and Snow also described another population of horses that exhibited recurrent rhabdomyolysis that had increased fractional phosphorus clearance and was reported to respond to dietary supplementation with ground limestone.[107] Thus although determination of fractional electrolyte clearances may be helpful in the evaluation of horses with recurrent rhabdomyolysis, only a small portion of affected horses are likely to show significant clinical improvement in response to dietary electrolyte supplementation alone.

TABLE 17.3-2 Fractional Electrolyte Clearance Values for Horses and Ponies

SODIUM	POTASSIUM	CHLORIDE	PHOSPHORUS	CALCIUM	REFERENCE
ADULTS					
0.16 ± 0.24	27.0 ± 14.6	0.17 ± 0.11	NR	NR	85[*]
0.02–1.00	15–65	0.04–1.60	0.00–0.50	NR	86
0.11–0.87	10.8–28.5	NR	0.07–0.74	NR	88
0.01–0.70	NR	NR	NR	NR	30
0.27 ± 0.02	38.52 ± 7.26	1.01 ± 0.24	NR	1.49 ± 1.58	89
0.0–0.46	23.9–75.1	0.48–1.64	0.04–0.16	NR	52
0.032–0.52	23.3–48.1[†]	0.59–1.86	0–20[†]	0.0–6.72[†]	90
0.034 ± 0.095	42.4 ± 9.8	0.352 ± 0.190	0.710 ± 0.250	NR	45
0.04–0.52	35–80	0.70–2.10	0.00–0.20	NR	106
0.0002–2.43	1.0–42.7[‡]	0.012–3.47	0.023–2.77	NR	41
NR	NR	NR	0.115–0.302	NR	100
NR	NR	NR	0.08–5.53[†]	2.10–4.60[†]	101
NR	NR	NR	0.61–0.75	11–33	102[§]
FOALS					
0.31 ± 0.18	13.26 ± 4.49	0.42 ± 0.32	3.11 ± 3.81	2.85 ± 3.26	91
AFTER FUROSEMIDE ADMINISTRATION					
12.0	207		9.5		86

NR, Not reported.

* Values calculated from data provided.

† Fractional clearance of potassium may exceed upper limit on high potassium diets; a fractional clearance of phosphorus exceeding 4% suggests excessive dietary intake; and a fractional clearance for calcium should exceed 2.5% with adequate intake.

‡ Low range attributed to low urine potassium concentrations determined by ion specific electrodes.

§ Fractional clearance value for magnesium reported at 7 to 30.

Finally, the methodology used to determine fractional electrolyte clearances should be standardized because several factors may lead to erroneous results. For example, a note of caution is warranted when ion-specific electrodes (instead of a flame photometer) are used to measure urinary potassium concentration, for components of animal urine can interfere with the ion-specific electrode and lead to spurious low values. One usually can avoid this problem by performing the analysis on urine diluted with water.[109] Next, as mentioned

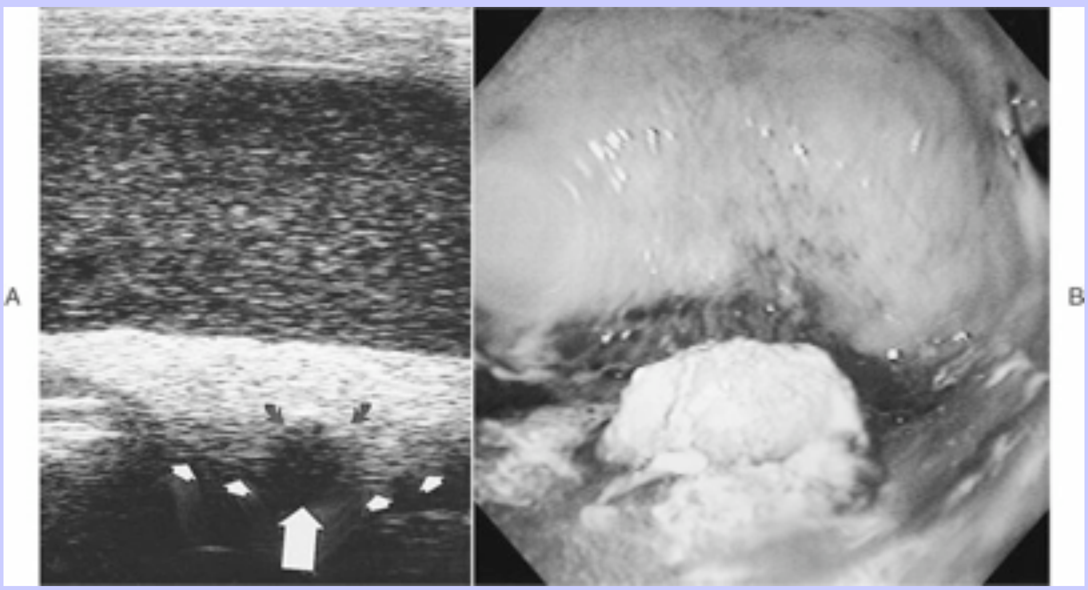
previously, light exercise may increase urine flow rate and sodium excretion.[99] Thus "spot" urine samples are best collected in the morning before feeding and exercise. Recently, McKenzie, Valberg, Godden, et al. compared fractional electrolyte clearance values between single-sample spot urine collections to 24-hour volumetric urine collections over 3 days in horses receiving diets varying in cation-anion balance.[94] They found substantial variation in fractional clearance values within individual horses over the 3-day study despite feeding of a consistent diet. When they assessed the effect of the differing diets, fractional clearance values for sodium, potassium, and chloride were generally similar when calculated using urine electrolyte concentrations determined in spot urine samples or well-mixed 24-hour collection samples. However, they found greater variability with fractional clearance values for calcium and magnesium. The fact that they detected considerable variability should not be surprising when one remembers that fractional clearance 1209
values are a calculation using four measurements (creatinine and electrolyte concentrations in plasma/serum 1210
and urine). Thus small variations in each measured value have the potential to magnify error in the final calculation. All in all, this study should serve as a reminder that determination of fractional electrolyte clearances is only one of the diagnostic tools one should use to evaluate patients with renal disease or recurrent rhabdomyolysis or to assess diet in a group of horses.

17.3.4 Imaging Techniques

17.3.4.1 ULTRASONOGRAPHY

One can perform ultrasonographic examination of the urinary tract transrectally or transabdominally.[110–117] Bladder imaging is performed best transrectally using a 5-MHz probe. One must remember the character of equine urine while imaging the bladder, for the urine will be an inhomogeneous, echogenic fluid because of the presence of mucus and crystals. The latter can appear as echogenic sediment in the ventral aspect of the bladder, and ballotting the bladder may cause this echogenic material to swirl in the bladder. One also can confirm the presence of a cystic calculus because calculi have a highly echogenic surface and produce an acoustic shadow (Figure 17.3-3). Similarly, one may image and palpate masses in the bladder wall during the examination.

Figure 17.3-3 Transrectal ultrasonographic and cystoscopic images of the bladder of a Miniature horse mare with recurrent cystitis and urolithiasis. **A**, The ultrasonographic image shows a layer of echogenic crystalline material in the ventral aspect of the bladder (*small, white arrows* outline ventral bladder wall) and presence of a small cystolith (outlined by *small black arrows* and *large white arrow*). **B**, After lavage of the urine sediment, cystoscopy confirmed presence of a small urolith, which was amenable to removal by digital manipulation.

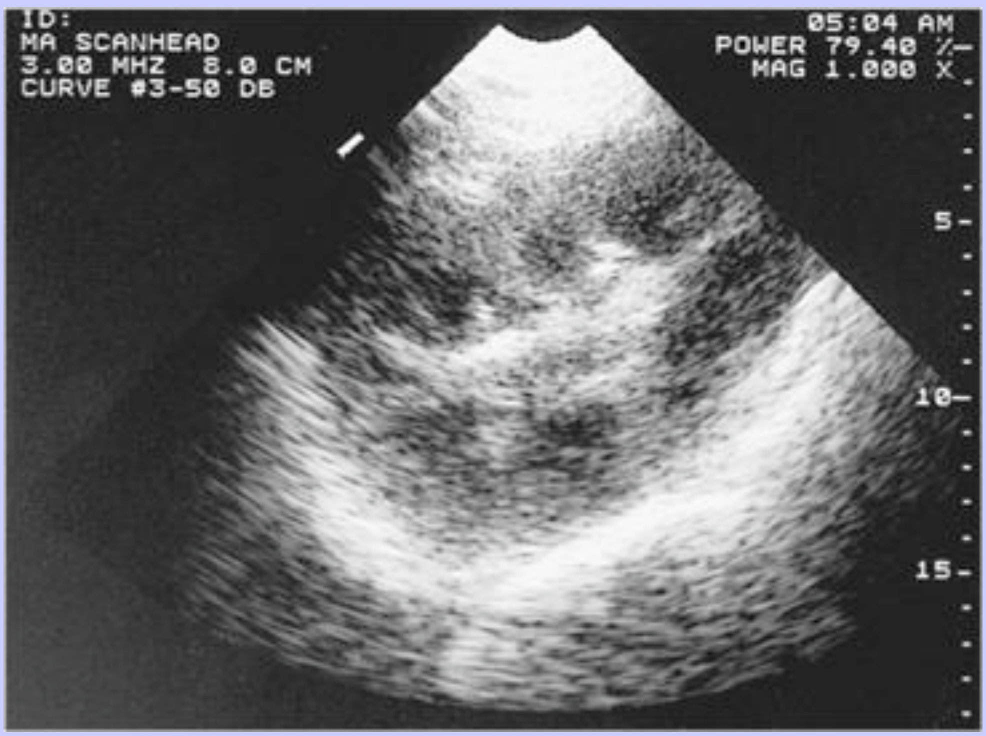


The right kidney is triangular or horseshoe shaped and is imaged best transabdominally via the dorsolateral extent of the last two or three intercostal spaces (Figure 17.3-4). The left kidney is somewhat a more typical bean shape, lies deep to the spleen, and can be imaged via the last two intercostal spaces or via the paralumbar fossa. Because the left kidney is deeper than the right kidney, it can be difficult to image completely and is examined best with a 2.5- or 3-MHz probe. One should assess systematically the size and shape of both kidneys, architecture, and echogenicity of the parenchyma, including imaging the kidneys in dorsal, sagittal, transverse, and transverse oblique anatomic planes.[115] In a full-size horse the right kidney should not measure more than 15 cm in the longest axis, whereas the left kidney may measure up to 18 cm if imaged in longitudinally.

In acute renal failure the kidneys may be normal or enlarged, and abnormalities of parenchymal detail are not often detected. When present, abnormal findings may include perirenal edema, widening of the renal cortex, and loss of a distinct corticomedullary junction.[111,112,114,116–118] Chronic renal failure can result in decreased kidney size, irregular surfaces, and increased echogenicity because of renal fibrosis. Cystic or mineralized areas in renal parenchyma can be associated with chronic renal disease or congenital anomalies. 1210

Although uncommonly recognized, distinct curvilinear hyperechoic bands in the outer renal medulla parallel to the corticomedullary junction have been imaged in foals and adults horses with renal disease attributable to acute or chronic phenylbutazone toxicity.[117,119,120] This finding, termed the *medullary rim sign*, is thought to resultfrom damage (and possible secondary mineralization) to the inner stripe of the outer medulla, the location of highly metabolically active nephron segments including the distal extent of the proximal tubule and the thick ascending limb of Henle's loop. Calculi in the renal pelvis generally cast an acoustic shadow and can resultin hydronephrosis of the affected kidney (Figure 17.3-5).[111,112,114] Occasionally, one cannot image one or both kidneys because of a gas-filled bowel between the kidney and abdominal wall. Reexamination at a later time generally is required for successful imaging in such cases. In addition, administration of high rates of fluid therapy, especially in foals, can lead to iatrogenic pyelectasia (fluid distention of the renal pelvis).[117,121] One should use caution so as not to interpret this mild distention of the renal pelvis falsely as evidence of ureteral or lower urinary tract obstruction. 1211

Figure 17.3-4 Transabdominal ultrasonographic image of a normal right kidney: the renal medulla is more echolucent than the renal cortex, except for the renal pelvis, which varies in echogenicity.



17.3.4.2 RADIOGRAPHY

Radiography rarely is used to evaluate urinary tract disease in adult horses. One usually can obtain diagnostic radiographs of the urinary tract only in foals or Miniature horses. Excretory urography using intravenous contrast material or pyelography using contrast agent delivered into the renal pelvis under ultrasonographic

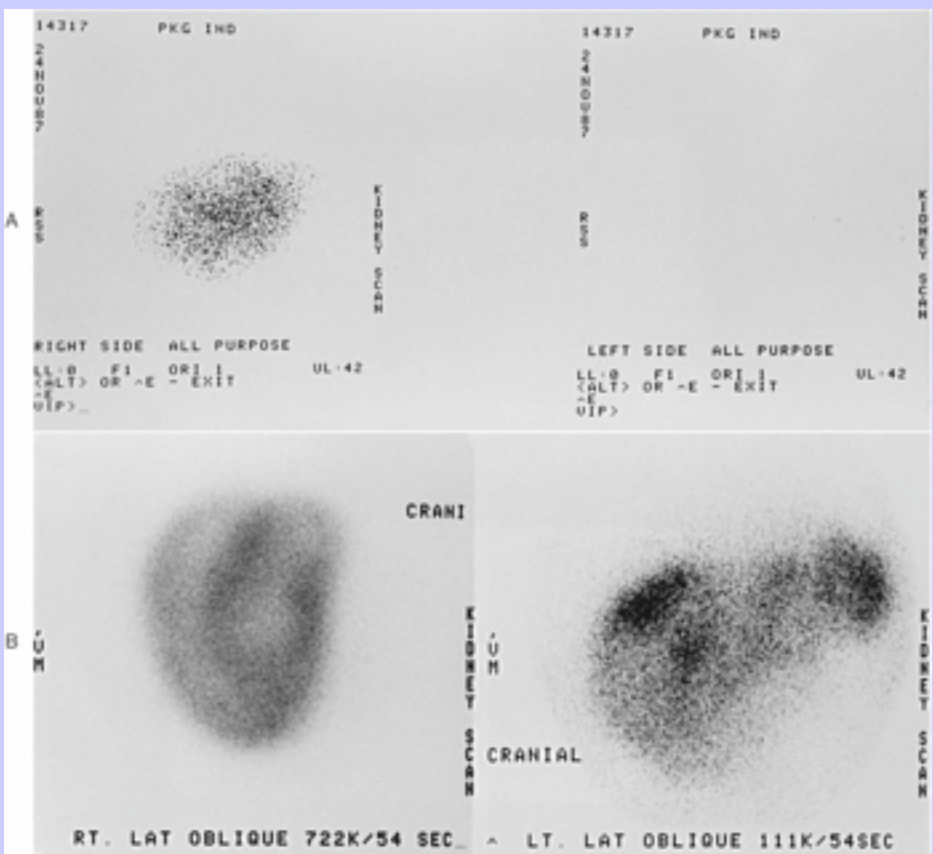
guidance may be useful to identify a nonfunctional or hypoplastic kidney or an ectopic or torn ureter.[122,123] The latter procedure may be more rewarding, but both require general anesthesia to be performed safely. One also can use retrograde contrast studies to evaluate the ureters in mares[124]; however, they have been used most often in foals suspected of having an ectopic ureter or a ruptured bladder. Contrast radiographic studies alsocan help to identify strictures or masses in the urethra or bladder, but endoscopy is generally more useful for these problems. In small animals, abdominal survey radiographs are most useful for assessing kidney size and shape, whereas ultrasonography provides more information about parenchymal changes associated with renal disease.[125] Thus use of a standardized protocol for ultrasonographic evaluation of the equine kidneys should provide essentially the same amount of information as the combined use of survey radiography and ultrasonography in small animal patients.[115]

Figure 17.3-5 Transrectal ultrasonographic image of the left kidney of a mare with nephrolithiasis and hydronephrosis. The nephrolith has an echogenic surface and produces an acoustic shadow. The echolucent crescent moon-shaped structure is a fluid-filled remnant of the renal parenchyma, consistent with hydronephrosis.

17.3.4.3 NUCLEAR SCINTIGRAPHY

Nuclear scintigraphy is an additional imaging modality often used to assess renal anatomy and function in human beings and small animals. One can use various radionuclides and pharmaceuticals, depending on the type of scintigraphic examination being pursued.[126–128] In fact, scintigraphy is used routinely for quantitative measurement of GFR in these species. Walsh and Royal compared use of renal scintigraphy (using 99-metastable technetium tagged to diethylenetriaminopentaacetic acid, which is similar to inulin in that it is neither secreted nor reabsorbed after filtration) for measurement of GFR in horses but found greater 1211
variability compared with GFR values measured by plasma disappearanceof inulin or the same radionuclide 1212
(^{99m}Tc-DTPA).[129] Nevertheless, renal scintigraphy using ^{99m}Tc-DTPA can provide qualitative information about renal function and is the only method currently available for assessing split renal function (assessing individual kidney function) in horses. Renal scintigraphy also has been performed with ^{99m}Tc tagged to glucoheptanate (taken up by the proximal tubule epithelial cells to provide anatomic detail) or mercaptoacetyltriglycine (MAG_3, which is similar to *p*-aminohippurate because it is eliminated by proximal tubular secretion) to provide qualitative information about renal anatomy and function (Figure 17.3-6).[130,131] 1212
Thus one may use renal scintigraphy to document the presence of a functional kidney in horses when 1213
multiple ultrasonographic examinations have been complicated by interfering bowel or when one desires information about individual kidney function.

Figure 17.3-6 Renal scintigraphic images of horses with renal disease. **A**, Renal scintigraphic image using *99m*Tc-diethylenetriaminopentaacetic acid revealed absence of functional left renal tissue, compared with an image of the right kidney, in a stallion with chronic renal failure. A nonfunctional hypoplastic left kidney was found on necropsy examination. **B**, Renal scintigraphic image using Technetium-99m-glucoheptonate (^{99m}Tc-GH) in a gelding with unilateral left-sided pyelonephritis revealed inhomogeneous uptake of the radionuclide and lesser count emission over the same time compared with the normal right kidney. The scintigraphic study, which provided anatomic and functional information, supported pursuit of unilateral nephrectomy, rather than prolonged antibiotic administration, as the treatment of choice for this horse.



17.3.4.4

ENDOSCOPY

Endoscopy of the urinary tract is a useful diagnostic aid when the complaint is abnormal urination.[40,132–134] A flexible endoscope with an outside diameter of 12 mm or less and a minimum length of 1 m is adequate for examination of the urethra and bladder of an adult horse of either sex. One should sterilize the endoscope before endoscopy of the lower urinary tract. Tranquilization of the patient is recommended, and one should clean the distal end of the penis or the vulva thoroughly. One passes the endoscope as one passes a catheter, using the air control intermittently to inflate the urethra or bladder. Normal urethral mucosa is pale pink with longitudinal folds. When dilated with air the mucosa flattens and may appear redder than normal, and a prominent submucosal vascular pattern may be apparent. Passage of a catheter before endoscopy (for sample collection orto empty the bladder) can produce mild irritation and erythema of the urethral mucosa, and one should not mistake these for abnormal findings. One should examine the regions of the ischial arch (where the urethra begins to widen into the ampullar portion) and of the colliculus seminalis (in the roof of the pelvic urethra just distal to the urethral sphincter) closely, for these are common sites of posturination or postbreeding hemorrhage in geldings or stallions (see Chapter 17.8). Subsequent passage of the endoscope through the urethral sphincter and air distention allows evaluation of the bladder for calculi, inflammation, and masses (see Figure 17.3-3). Viewing the ureteral openings in the dorsal aspect of the trigone can help determine the source of hematuria or pyuria (see Chapter 17.8). A small volume of urine should pass from each ureteral opening approximately once each minute or more frequently if the horse is well hydrated or has been sedated with an α_2-agonist. One can perform ureteral catheterization to obtain urine samples from each kidney by passing sterile polyethylene tubing via the biopsy channel of the endoscope. Additionally, one can take biopsy samples of masses in the bladder or urethra.

17.3.5

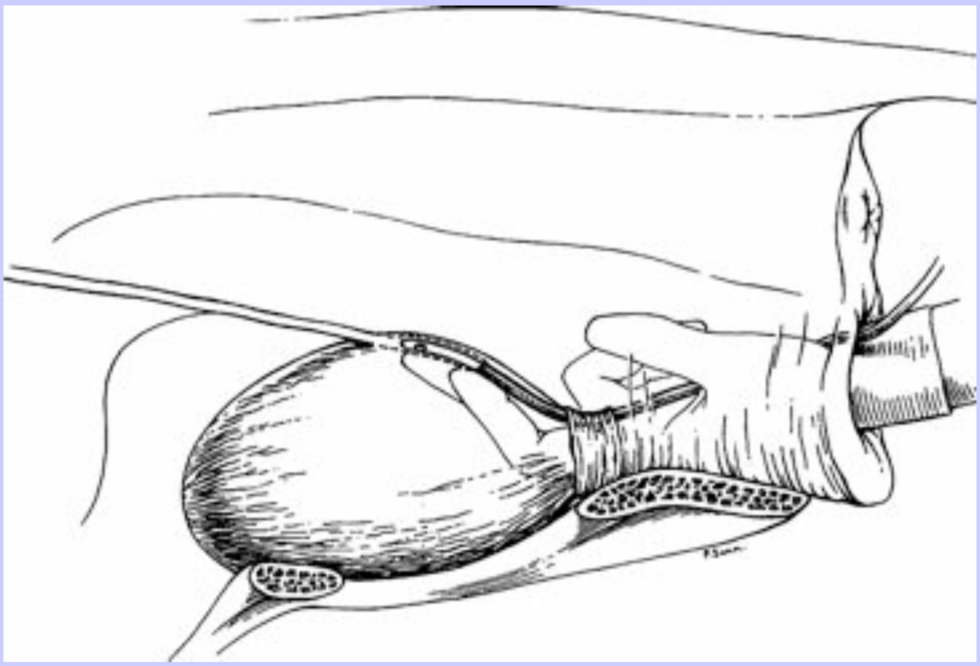
Specialized Diagnostic Techniques

17.3.5.1

URETERAL CATHETERIZATION

With development of high-resolution videoendoscopic equipment, retrograde instrumentation of the bladder, ureter, and renal pelvis rapidly is replacing surgical exploration for diagnostic evaluation and therapeutic management of urinary tract disorders in human beings and dogs.[135–138] Similarly, retrograde ureteral catheterization and instrumentation can be a valuable technique for evaluation and treatment of horses with unilateral disorders of the upper urinary tract.[139–141] In addition to localization of unilateral renal hemorrhage, pyelonephritis, and neoplasia, ureteral catheterization further enables retrograde pyelography.[124] As mentioned previously, one may perform this technique successfully in male and female horses during cystoscopic examination.[40,132–134]

Figure 17.3-7 Manual placement of a polypropylene catheter into the ureter of a mare. (From Schott HC, Hodgson DR, Bayly WM: Ureteral catheterisation in the horse, *Equine Vet Educ* 2:140, 1990.)



In mares, one also can catheterize the ureters manually without endoscopic guidance.[142] After preparation of the vulva, one catheterizes the bladder and drains the urine. One removes the bladder catheter and dilates the urethra manually until two fingers can be passed intothe bladder. One can palpate the ureteral orifices dorsally as small, soft projections. This done, one places a catheter between the fingertips, passes it through the urethra, and directs it into the ureter (Figure 17.3-7). A relatively rigid catheter with a rounded end (No. 8 to 10 French polypropylene catheter) facilitates passage into the ureter. After advancing the catheter 5 to 10 cm into the ureter, one attaches a syringe to the other end of the catheter and collects a urine sample. During sampling, one holds the catheter in place and occludes the ureteral opening with the fingertips to minimize loss of urine around the catheter.

17.3.5.2 WATER DEPRIVATION AND THE ANTIDIURETIC HORMONE CHALLENGE TEST

Water deprivation is a simple test to determine whether hyposthenuric polyuria is caused by a behavior problem such as primary (psychogenic) polydipsia or results from central or nephrogenic diabetes insipidus.[6] 1213
One should not perform a water deprivation test in an animal that is clinically dehydrated or azotemic. One 1214
should perform a baseline urinalysis (sample collected by catheterization to empty the bladder at the start of the test) and measure BUN, Cr, and body weight before removal of water (food does not necessarily need to be removed, but this may help prevent gastrointestinal complications of water deprivation). One measures urine specific gravity and weight loss after 12 (usually overnight) and 24 hours. Horses with normal renal

function typically produce urine with a specific gravity greater than 1.045 and an osmolality greater than 1500 mOsm/kg in response to water deprivation of 24 to 72 hours in duration.[43–45] Practically, one can stop the test when urine specific gravity reaches 1.025 or greater. Furthermore, one should stop the test if more than 5% of body weight is lost or clinical evidence of dehydration becomes apparent. With long-standing primary polydipsia, affected horses may not be able to concentrate urine fully (to a specific gravity greater than 1.025) because of partial washout of the medullary interstitial osmotic gradient. Extending the test period beyond 24 hours for such patients offers little benefit; however, affected horses should respond more favorably to water deprivation (producing urine with a higher specific gravity) after a period of partial water deprivation (termed a *modified water deprivation test*) during which daily water intake is restricted to 40 ml/kg for several days, which should allow time for restoration of the medullary interstitial osmotic gradient.[6] Horses with central or nephrogenic diabetes insipidus cannot concentrate urine in response to a water deprivation test.[6,143–145] When one suspects these problems, one should monitor patients every few hours, for significant dehydration may ensue within6 hours of water deprivation.

In the absence of azotemia or clinical signs of early renal failure, inability to concentrate urine in responseto water deprivation supports a diagnosis of diabetes insipidus; however, the test does not distinguish between the neurogenic and nephrogenic forms of the disorder. One can differentiate these by exogenous administration of vasopressin (antidiuretic hormone).[143–145] Until recently, the most commonly used form of exogenous antidiuretic hormone was a water insoluble tannate of the antidiuretic factor arginine vasopressin that was extracted from the posterior pituitary and suspended in peanut oil (Pitressin tannate). However, this preparation is no longer available. Currently, two approaches exist for performing an antidiuretic hormone challenge test. First, one can administer aqueous synthetic vasopressin (20 U/ml for intramuscular or subcutaneous injection) as an intravenous infusion (5 U [0.25 ml] added to 1 L of a 5% dextrose solution and administered intravenously at a rate of 2.5 mU/kg over 60 minutes [~250 ml to a 500-kg horse]) or an intramuscular injection (0.5 U/kg). An increase in urine specific gravity to 1.020 or greater after 60 to 90 minutes would be the expected response, whereas failure to increase urine concentration would support nephrogenic diabetes insipidus. Second, one can use desmopressin acetate (DDAVP), a synthetic analogof arginine vasopressin. Administration of desmopressin acetate is considered the safer diagnostic technique in small animals because the change in structure of this vasopressin analog has decreased pressor actions and less effect on visceral smooth muscle compared with an enhanced antidiuretic effect. One microgram of desmopressin acetate has an antidiuretic activity of 4 U of arginine vasopressin. The author and coworkers recently validated use of desmopressin acetate as a replacement for the antidiuretic hormone challenge test in normal horses. In this study, we diluted the nasal spray form of desmopressin acetate (0.1 mg/ml DDAVP) in sterile water and administered 0.05 μg/kg intravenously (25 μg, equalto 100 U of antidiuretic activity, to a 500-kg horse) to horses that had polyuria induced by repeated nasogastric intubation with water for 3 days preceding desmopressin acetate challenge. Urine was collected for 8 hours after desmopressin acetate administration and an increase in urine specific gravity to values greater than 1.020 was observed from 2 to 7 hours after desmopressin acetate administration (Figure 17.3-8, unpublished data). Furthermore, desmopressin acetate administration had no effect on heart rate or systemic blood pressures. These data demonstrate that intravenous administration of desmopressin acetate is a safe and useful diagnostic tool for evaluation of horses with diabetes insipidus.

A final test for evaluating polyuria and polydipsia isan intravenous challenge with hypertonic saline (Hickey-Hare test).[146] The goal is to produce an increase in plasma osmolality, which should trigger release of endogenous vasopressin. One protocol for the test would be to measure plasma osmolality and endogenous vasopressin concentrations before and within 30 minutes after administration of 1 to 2 ml/kg of a 7.5% sodium chloride solution. A normal response, expected in horses with primary polydipsia, would be

concurrent increases in plasma vasopressin concentration and urine specific gravity. Horses with nephrogenic diabetes insipidus also would respond with an increased plasma vasopressin concentration; those with neurogenic diabetes insipidus would not. Urine specific gravity would not be expected to increase in response to hypertonic saline administration with either form of diabetes insipidus. One could evaluate plasma vasopressin concentrations similarly before and at the end of a water deprivation test.[147,148] Unfortunately, however, assays for plasma vasopressin concentration are not currently available at commercial laboratories. Thus pursuit of this diagnostic test requires cooperation of a research laboratory. 1214

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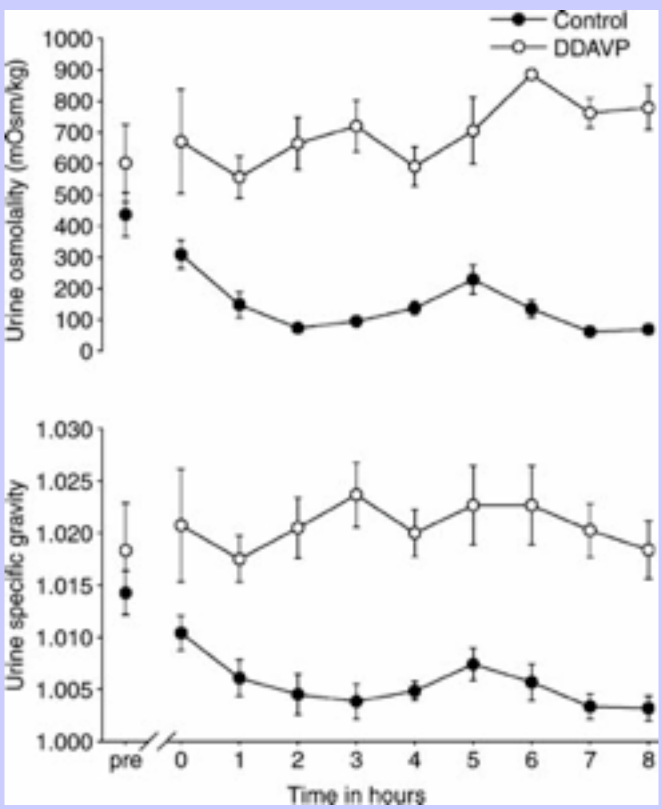
Figure 17.3-8 Urine osmolality and specific gravity in six horses administered 0.05 μg/kg desmopressin acetate (DDAVP, open circles) or placebo (filled circles) intravenously at time 0. The horses had polyuria induced by repeated nasogastric intubation with water (40 ml/kg) twice daily for 3 days preceding desmopressin acetate challenge and again 4 hours after administration of desmopressin acetate. Urine was collected for 8 hours after treatment, and an increase in urine specific gravity to values greater than 1.020 was observed from 2 to7 hours after desmopressin acetate administration (author's unpublished data).



17.3.5.3 QUANTITATIVE MEASURES OF RENAL FUNCTION

As mentioned previously, azotemia does not develop until more than 75% of nephrons cease to function; therefore measurement of BUN and Cr, although readily available, provides a poor reflection of smaller declines in renal function. A number of methods are available to quantitate renal function in horses.[40,149] Basically, these tests can be separated into plasma disappearance curves or clearance studies involving use of timed urine collections. Generation of plasma disappearance curves requires collection of an initial blood sample, intravenous administration of one of a number of compounds (inulin, creatinine, sodium sulfanilate, phenolsulfonphthalein [phenol red], or radionuclides), and collectionof another series of blood samples over the subsequent 60 to 90 minutes.[129,150–155] One can express resultsin terms of an elimination half-life in minutes or as clearance values in milliliters per kilogram per minute. Mean elimination half-lives of 39.5 ± 4.4, 32.8 ± 4.1, and 16.4 ± 2.3 minutes have been reported for sodium sulfanilate, $^{99m}Tc\text{-}MAG_3$, and phenolsulfonphthalein, respectively, in healthy horses.[150,151,155] The difference in these values can be explained in part by the fact that $^{99m}Tc\text{-}MAG_3$ and phenolsulfonphthalein are eliminated mostly by tubular secretion and consequently are cleared more rapidly from plasma than is sodium sulfanilate (eliminated mostly by glomerular filtration). Plasma elimination half-times are most useful when measured sequentially throughout the course of renal disease;for example, Bertone, Traub-Dargatz, Fettman, et al.[156] reported a progressive increase in the sodium sulfanilate elimination half-time from 90 to 150 minutes during a 240-day course of chronic renal failure in a horse with polycystic kidney disease.

When one uses plasma disappearance curves of a substance to estimate GFR, the compound being used must meet all the requirements of a filtration agent[152–154]: (1) no significant binding to plasma proteins, (2) ability to be filtered freely across the glomerulus, and (3) absence of tubular reabsorption or secretion. The gold standard compound is inulin More traditionally, GFR has been measured by timed urine collection periods (to measure urine flow rate) and measurement of plasma and urine concentrations of a test compound that meetsthe listed requirements.[8,149] Several protocols exist for performing urine clearance studies. Ideally, the urine collection period should span 24 hours, although for practicality, one may use shorter collection periods.[6,149] For all protocols, one documents the bladder as being empty at the start of the collection period by catheterization or observation of voiding. One collects all urine produced during the study period and pools it into one sample. One again should empty the bladder via catheterization at the end of the collection period, record the total volume of urine produced, and assay an aliquot of the pooled urine sample for the test substance. Similarly, one measures the concentration of the test substance in a blood sample collected near the midpoint of the urine collection period. For urine collections of 12 to 24 hours, endogenous creatinine is the test substance used because it is the only one that does not have to be given by steady intravenous infusion during the collection period. GFR is calculated as the clearance of creatinine,

$$\mathrm{GFR} = \frac{\mathrm{Urine}\ [\mathrm{Cr}]}{\mathrm{Plasma}\ [\mathrm{Cr}]} \times \mathrm{Urine\ flow}$$

with the modification that the result usually is divided by body mass (in kilograms) to express GFR in term of milliliters per minute per kilogram. Although endogenous creatinine is a convenient test substance, its use typically underestimates GFR because noncreatinine chromagens in serum artifactually increase the value in 1215

the denominator.[149] Similarly, significant tubular secretion of creatinine can be one of the compensatory 1216
responses to renal failure. This can lead to overestimation of GFR calculated using endogenous creatinine clearance. Despite these limitations, the endogenous creatinine clearance technique for measurement of GFR

can provide useful information, especially when it is performed on several occasions during the course of renal failure (see Chapter 17.5).

To avoid the limitations of endogenous creatinine clearance as a measure of GFR, one can administer a number of filtration markers (inulin, creatinine, radionuclides) as an intravenous infusion throughout the urine collection period.[153,154,157–162] One starts the infusion as a bolus to increase the plasma concentration of the test substance to the desired level (for example, the desired plasma concentration for exogenous creatinine is 5 to10 mg/dl, to minimize the influence of noncreatinine chromagens) and subsequently continues a steady infusion throughout the remainder of the collection period. One usually performs this type of study for a shorter period with the horse restrained in stocks. One should empty the bladder of the horse at the start of the study and collect urine after two or three 30-minute intervals. One should pass a catheter and empty the bladder completely at the end of each 30-minute period. Alternatively, one can use an indwelling bladder catheter for the entire collection. After one measures urine volumes, one assays an aliquot of each urine sample and a blood sample collected at the midpoint of each urine collection period for the test substance and calculates GFR as the mean value for the two or three collection periods. For all practical purposes, these types of GFR measurements (with the exception of exogenous creatinine clearance) usually are limited to research studies, because commercial laboratories do not offer inulin assays. One also can use this shorter protocol for GFR determination using endogenous creatinine as the test substance, without the need for an intravenous infusion. The results of a number of studies measuring GFR in normal horses were presented in the previous section of this chapter (see Chapter 17.2).

17.3.5.4 RENAL BIOPSY

Renal biopsy is a useful diagnostic technique for identifying the affected region of the nephron, the type of lesion, and the chronicity and severity of disease.[8,163–166] Although biopsy is a safe procedure when performed with ultrasonographic guidance, it has risks, including perirenal hemorrhage or hematuria and, less commonly, penetration of bowel. In human beings, perinephric hematomas are common and have been detected in 57% to 85% of patients the day after biopsy. Microscopic hematuria occurs in virtually all patients for the first couple of days after biopsy, and gross hematuria occurs in 5% to 10% of patients. Most of these complications are inconsequential, but in 1% to 3% of patients the complications have resulted in the need for postbiopsy transfusions.[8] Similarly, in a group of seven normal horses subjected to renal biopsy, postbiopsy macroscopic and microscopic hematuria occurred in five animals. Furthermore, perirenal hemorrhage was a prominent finding during necropsy examination of five of these animals (including one examined 27 days after biopsy tissue was collected).[166] Thus renal biopsies remain controversialin human and equine renal failure patients.[167–169] Renal biopsies should be approached with caution and are indicated only when the results would alter the therapeutic plan or prognosis. Information about the effect of renal biopsy results on therapy and outcome of renal disease in human beings is limited; however, in one prospective study, biopsy results were found to influence physicians' decisions on about half of cases when the technique was performed.[170] In general, renal biopsy is pursued more aggressively in human beings with acute renal insufficiency than in those with chronic renal insufficiency, especially when it is difficult to determine the type of renal disease based on results of urinalysis and sediment examination.[8] In the equine patient, one performs a renal biopsy with the horse sedated and restrained in a stocks. Penetration of the needle (a Tru-cut biopsy needle or, preferably, a triggered biopsy device) into the renal parenchyma is imaged sonographically by triangulating the ultrasound beam with the biopsy instrument and the kidney or by determination of the site and depth of biopsy needle placement via ultrasonographic imaging immediately before biopsy. One should place the tissue collected in formalin for histopathologic and electron microscopic

evaluation. One can collect additional samples for bacterial culture and for immunofluorescence testing (placed in Michel's medium or quick frozen after coating with a preservative such as Tissue-Tek). One should determine appropriate sample processing beforehand by contacting the pathologist who will examine the biopsy samples.

Although renal biopsy results could provide useful diagnostic and prognostic information about the type of renal disease in horses with acute renal failure (e.g., glomerulonephritis, tubular necrosis, and interstitial nephritis), they have been used more often to document the presence of chronic disease in horses with chronic renal failure. In most cases of chronic renal failure, one cannot detect the inciting cause unless it can be associated with a historical event or immunofluorescence testing is pursued. This limitation can be attributed to the fact that significant renal disease develops before onset of azotemia. Pathologic lesions are widespread at this point, and involvement of all nephron segments and the interstitium often leads to the 1216
interpretation of end-stage kidney disease. In occasional cases the results may help separate infectious 1217
(pyelonephritis) or congenital (renal dysplasia) causes from nonspecific causes of renal failure. Although such results could be useful in the therapeutic approach to these patients, one should consider the limitations and risks of renal biopsy before performing this technique in horses with chronic renal failure.

17.3.5.5 URODYNAMIC PROCEDURES

Cystometrography and urethral pressure profiles are used to evaluate detrusor and urethral muscle function, respectively. Both techniques involve measurement of intraluminal pressure during inflation of the bladder or urethra. These techniques have been useful for diagnosis of myogenic and neurogenic disorders of the bladder and urethra in dogs and human beings.[171] The procedures have been performed experimentally in normal horses and ponies,[172–174] but little information is available about use of these techniques in clinical cases (see Chapter 17.12).

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17.4 17.4—Acute Renal Failure

Warwick M. Bayly

Acute renal failure (ARF) is a clinical syndrome associated with abrupt reduction in glomerular filtration rate (GFR). Sustained reduction in GFR is associated with failure of the kidneys to excrete nitrogenous wastes causing azotemia and with disturbances in fluid, electrolyte, and acid-base homeostasis. The human medical literature is full of various terms and definitions for different forms of ARF. Veterinary medical definitions are simpler. Basically, ARF can result from decreased renal perfusion without associated cell injury (prerenal failure); obstruction or disruption of the urinary outflow tract (postrenal failure); or ischemic or toxic damage to the tubules, tubular obstruction, acute glomerulonephritis leadingto a primary reduction in the filtering capacity of the glomeruli, or tubulointerstitial inflammation and edema. Any of these intrarenal causes can be associated with intrinsic renal failure. Prerenal azotemia and ischemic tubular insults or necrosis represent a continuum, the former resulting in the latter when perfusion is compromised sufficiently to result in death of tubule cells.[1] Classically, ARF has been associated with oliguria and occasionally with anuria, and these are certainly the most commonly noted clinical signs associated with this disease in horses; however, nonoliguric forms of ARF, particularly intrinsic renal failure, exist and are characterized by slower development of azotemia, lower peaks in serum creatinine concentrations, more subtle increases in urinary sodium clearance, and a more rapid recovery of renal function in response to treatment. Nonoliguric renal failure seems to be diagnosed rarely in horses, although it is not uncommon to recognize mildly azotemic horses that have apparently normal urine output. In some cases, localized proximal tubule damage and reduced solute reabsorption actually may lead to enhanced distal delivery of filtrate, which may result in polyuric ARF.

In horses, ARF is usually prerenal or renal in origin and most often is caused by hemodynamic or nephrotoxic insults.[2] With the exception of bladder rupture in the neonate, postrenal failure is uncommon in horses. Identification and correction of the cause of ARF is important, for in the early stages of failure renal dysfunction is frequently reversible, whereas established ARF often requires extensive supportive care and carries a guarded prognosis. By identifying patients at increased risk and attempting to interrupt the cycle of events leading to ARF, one possibly may reduce the incidence of this condition.

17.4.1 Causes

Prerenal failure is associated with conditions that result in decreased cardiac output or increased renal vascular resistance, or both, and is the most common cause of reversible azotemia. In horses the most common causes of reduced cardiac output (and therefore reduced renal perfusion) are associated with diarrhea, endotoxemia, acute

blood loss, septic shock, and prolonged exercise. The resultant reductions in renal blood flow (RBF), GFR, and urine output usually result in azotemia and retention of water and electrolytes. Anesthesia also may decrease cardiac output enough to result in a degree of prerenal azotemia. Nonsteroidal antiinflammatory drugs (NSAIDs) also can precipitate prerenal azotemia in patients with decreased RBF.[3] Although prostaglandins play only a minor role in maintenance of RBF in the normal state, PGE_2 and PGI_2 are important vasodilatory mediators of RBF under conditions of reduced renal perfusion. Thus administration of NSAIDs to dehydrated or toxemic patients may contribute further to renal hypoperfusion by exacerbating a decrease in RBF. In some cases this may be sufficient to produce ischemic renal parenchymal damage also, thus causing intrinsic renal failure. Generally, the parenchymal lesion associated with NSAID toxicity is medullary crest or papillary necrosis. Such lesions develop because the renal medulla normally receives much less blood flow than the renal cortex, and consequently is much more susceptible to NSAID-induced changes in RBF.

In human beings, intrinsic renal diseases that lead to ARF generally are categorized according to the primary site of injury: tubules, interstitium, glomeruli, or vessels.[1] Acute tubular necrosis (ATN) is the form of intrinsic renal failure recognized most often in horses, interstitial and primary glomerular disease being recognized occasionally and vascular disease almost never. Ischemia, especially when associated with microvascular coagulation (which often leads to irreversible cortical necrosis[4]), and nephrotoxins probably are the most common causes of equine ATN. Important nephrotoxins include aminoglycoside antibiotics and NSAIDs. Less commonly, ATN develops following exposure to endogenous pigments (myoglobin or hemoglobin), heavy metals such as mercury (contained in some counterirritants), or vitamin D or K_3.[5–8] Use of aminoglycosides, particularly gentamicin, is a common cause of equine ATN.[9] Toxicity is a result of damage to proximal tubular epithelial cells that is mediated by impaired cell organelle function. Administration of potentially nephrotoxic agents such as NSAIDs or furosemide (which can exacerbate hypovolemia) can increase the risk of aminoglycoside nephrotoxicity. 1221

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Myoglobinuria and hemoglobinuria have been associated with development of ARF in horses (pigment nephropathy).[10] Myoglobinuric nephrosis can follow exertional rhabdomyolysis, heat stroke, or extensive crush injuries. Causes of intravascular hemolysis and hemoglobinuria include incompatible blood transfusions, immune-mediated hemolytic anemia, fulminant hepatic failure, and toxicosis from ingesting onions (*Allium* species) or withered red maple leaves *(Acer rubrum)*. Although the mechanism of pigment-induced renal injury is still ill understood, increased hydroxyl radical formation associated with reduction of ferrous iron compounds and tubular obstruction by casts of heme proteins are likely contributing factors. That pigment nephropathy is uncommon in well-hydrated horses suggests a possible link to renal perfusion. Myoglobin and hemoglobin have been suggested to induce renal vasoconstriction.

Acute interstitial nephritis often is not recognized but is believed usually to result from an allergic reaction to drugs such as the β-lactam and sulfa antibiotics. Autoimmune and embolic or ascending bacterial infections also may be associated with the condition, which is characterized by edema and inflammatory cell infiltration of the interstitium. Tubules frequently contain white and red blood cells, which pass into the lumen through the disrupted tubular basement membrane.

Glomerulonephritis often is identified post mortemin aged horses[11,12] and apparently is most often immune mediated. Glomerulonephritis often results in subacute or nonoliguric renal failure. Although the condition is theoretically reversible with immunosuppressive agents, in horses such undertakings are usually impractical and rarely are tried for long.

Postrenal obstructive failure can develop following disease of the renal pelves, ureters, bladder, or urethra. The severity of the failure depends on the extent of the obstruction. Frequently, problems are not recognized in horses until urine output obviously is reduced or renal function is impaired to the point that systemic problems are manifested. Although a neurogenic bladder can cause a functional obstruction, postrenal failure in horses most often results from intraluminal blockage by uroliths, which can cause obstruction anywhere in the urinary outflow tract.[13,14] Other possible intraluminal causes include neoplasia or stricture formation. Extraluminal obstructive lesions such as retroperitoneal tumors or adhesions or bladder displacements also occasionally are associated with development of postrenal failure.

17.4.2 Pathophysiology

The pathophysiology of equine ARF has receivedlittle study, and the mechanisms at work are assumed essentially to be the same as those identified in experimental studies of other animal species. Several mechanisms have been demonstrated to be involved in the development of ARF, the actual pathogenesis being complex and depending somewhat on the cause of the disease. Multiple factors probably operate in different combinations at different times and in different nephrons. These different mechanisms are discussednext in relation to the type of failure with which theyare associated (i.e., prerenal, intrinsic, and postrenal failure).

The pathophysiology of prerenal failure and ischemic ARF tend to involve the same processes. Toxins that cause ATN also share many pathophysiologic features with ischemic ARF.[15] The heterogeneity of intrarenal blood flow is an important factor in the development of this condition. The kidneys are particularly susceptible to ischemic and toxic injury because of their unique anatomic and physiologic features. Although they receive approximately 20% of the cardiac output, only about 10% to 20% of total RBF reaches the medulla via the vasa recta. This low medullary blood flow is necessary to ensure a functional countercurrent mechanism in this region of the kidney; however, low blood flow also creates a large corticomedullary oxygen gradient and renders the renal medulla hypoxic and highly susceptible to ischemic injury. Conversely, the renal cortex receives 80% to 90% of total RBF and is particularly susceptible to toxins.

Hypovolemia triggers compensatory systemic and renal responses. The systemic responses include activation of the autonomic nervous system and renin-angiotensin system and release of antidiuretic hormone. Peripheral vasoconstriction is one of the effects of these responses. Renal responses to decreases in circulating blood volume have several phases. Initially, tubular reabsorption of sodium and water increases and is mediated by nerves and hormones. Reabsorption usually is associated with reduced clearance of urea and an associated increase in serum urea nitrogen concentration in the face of preservation of GFR, which in turn maintains the plasma creatinine concentration in the normal range. More severe hypovolemia overcomes renal autoregulation, RBF being redistributed from the cortex to the medulla and GFR thus declining. The renal circulatory changes further enhance tubule solute reabsorption in the face of the decreased GFR. The net effect is production of small amounts of concentrated urine, a high urine/plasma creatinine ratio, and low fractional sodium clearance.[16]

A further reduction in RBF results in a syndrome considered intermediate between the prerenal and intrinsic
ischemic forms of ARF. Urine concentrating ability apparently is disrupted earlier than is sodium reabsorptive 1222
capability, with the result that urine osmolality decreases and output increases, but fractional sodium clearance 1223
stays low. Patients may appear to be mildly polyuric.[17] With more severe or prolonged renal hypoperfusion and ischemic insult, urinary sodium and fractional sodium clearance start to increase and the animal develops nonoliguric, and then oliguric, ARF. These changes are associated with progressively more severe tubule necrosis as the ARF progresses from prerenalto renal. The more severe the insult, the poorer the prognosis.

Intrarenal vasoconstriction is caused by an imbalance between vasoconstrictive and vasodilating factors (systemic or local) that act on small renal vessels in particular. These mediators include the vasodilator nitric oxide and the constrictor endothelin. Hypercalcemia is associated with increases in free calcium in the vascular smooth muscle and leads to enhanced vascular tone. Vasopressin and angiotensin II also have been shown to induce significant vasoconstriction under certain experimental conditions, as have endotoxins and myoglobin. Some nephrotoxins—gentamicin, heavy metals, and radiographic contrast agents—can cause renal vasoconstriction in addition to having direct toxic effects on the proximal tubules.[18–20]

Prostaglandins E_2 and I_2 are potent mediators that are responsible for a critical vasodilating response in the face of reduced RBF when activation of the renin-angiotensin system alone would result in further vasoconstriction. Increases in circulatory concentrations of angiotensin II stimulate synthesis of these renal prostaglandins. Consequently, the vasoconstrictive effects of stimulating the renin-angiotensin system usually are blunted somewhat by concomitant increases in PGE_2 and PGI_2; however, in the face of increased angiotensin II and simultaneous inhibition of prostaglandin synthesis (e.g., because of an NSAID), a significant increase in renal vascular resistance results.[21]

The reduction in GFR associated with reduced renal perfusion is associated with a number of mechanisms involving the glomeruli, vasculature, and tubules. In the initial phases a reduction in glomerular capillary hydrostatic pressure is associated with a net drop in RBF and a rise in renal vascular resistance. The latter phenomenon usually is associated with afferent arteriolar vasoconstriction and efferent arteriolar vasodilation and initially is reversible with volume expansion. Later (2 days or more), restoration of RBF does not necessarily improve GFR, and a drop in RBF is associated with a disproportionately greater drop in GFR.[22] Even when vasoconstriction is reversed, GFR may not improve; this reflects loss of RBF autoregulation. This disproportionate reduction in GFR suggests a fall in the ultrafiltration coefficient of the glomeruli following a reduction in total filtering area. The mechanism for this is unclear butmay be associated with increases in angiotensin II concentration, for this agent is known to induce mesangial contraction and a drop in the ultrafiltration coefficient.[23]

In addition to the aforementioned vascular changes that can affect GFR, the possible effects on GFR in the juxtamedullary region of even mild changes in RBF warrant specific mention. The glomeruli and adjacent straight portion of the proximal tubule and thick ascending limbs of Henle's loops in this region are apparently particularly susceptible to hypoxia (i.e., ischemia) because of their high oxygen requirements. Swelling of endothelial and tubule cells following prolonged ischemia in this region results in increased vascular resistance and continued compromise of the medullary circulation, even when cortical RBF has been restored.[15,24]

Glomerular filtration rate also can be compromised significantly by obstruction of tubular lumina by casts of cellular debris and inflammatory cells. Increased intratubular pressure decreases the net driving pressure for glomerular filtration in the same way that obstruction of the urinary outflow tract ultimately can lead to a lower GFR. One of the reasons that agents that accelerate the solute excretion rate (e.g., furosemide and mannitol) may be therapeutic adjuncts has been that they are believed to help disperse these luminal blockages.

Tubuloglomerular feedback is a regulatory mechanism that lowers GFR whenever solute (most notably sodium chloride) concentrations at the macula densa are increased. In ARF, impaired transport in the thick ascending limb of the loop of Henle in the context of preserved glomerular response to signals from the macula densa, results in a decrease in GFR. This feedback is a normal protective mechanism mediated principally by the renin-angiotensin system, although prostaglandin, intracellular calcium, and adenosine may play a role in signal transmission or regulation. In essence the mechanism serves to prevent massive fluid losses associated with a

reduction of tubular reabsorptive capacity; however, in cases of renal hypoperfusion and ischemia, the effect is the opposite, for basically the feedback mechanism exacerbates the effects of already reduced RBF.

Proximal tubule cells undergo morphologic changes early in cases of ischemia. They lose their brush borders and polarity, and the integrity of their tight junctions is disrupted, probably following alterations in the actin and microtubular cytoskeletons.[25,26] Under these conditions the glomerular filtrate is able to leap back to the peritubular circulation, thus reducing the net (or effective) GFR. This mechanism is thought to be an important source of GFR reduction only in more severe cases of ischemia or nephrotoxin exposure. 1223

Tubule cells involved in solute reabsorption have a high metabolic rate and a high demand for oxygen. The existence of the corticomedullary oxygen gradient makes these cells vulnerable to the effects of hypoxia and ischemia, the thick ascending limb of the loop of Henle in the outer medulla being most susceptible.[27] Early in ischemic and toxic ARF, decreases in adenosine triphosphate (ATP) and adenosine diphosphate tissue levels occur with associated elevations in adenosine monophosphate and inorganic phosphate concentrations. Much of the adenosine monophosphate is broken down further to adenosine and then to xanthine. Adenosine is a potent constrictor of cortical blood flow and probably enhances the effect of the tubuloglomerular feedback system. Depletion of ATP in tubular cells inhibits cell volume regulation, and the resultant swelling probably contributes to luminal obstruction and increased vascular resistance. Redistribution of Na^+,K^+-ATPase from the basolateral to the apical membrane of the tubule cells reduces the ability of the cells to extrude sodium into the peritubular fluid and circulation.[28] Redistribution of integrins to the apical surface contributes to the breakdown of tight junctions.[29] Depletion of ATP in tubule cells also leads to an increase in cytosolic calcium concentration. In addition to being a vasoconstrictor, calcium activates proteases and phospholipases, interferes with mitochondrial energy metabolism, and can break down the cytoskeleton.[1,30] Administration of calcium channel blockers has helped ameliorate ARF in some experimental situations.[31] 1224

Reperfusion of renal tissue after a period of ischemia is associated with rapid production of oxygen free radicals and with significant tissue damage. Xanthines, neutrophils, phospholipase A_2, mixed-function oxidases, and mitochondrial electron transport are associated with the production of these oxidants.[32] Phospholipase A_2 hydrolyzes phospholipids in cell and mitochondrial membranes to free fatty acids and lysophospholipidsand produces arachidonic acid. Arachidonate in turn is converted to eicosanoids that are vasoconstricting and chemotactic for neutrophils.[33] Cell membranes are particularly susceptible to phospholipase activity following reperfusion. Reperfusion injury is a major consideration in transplant surgery; however, the role it playsin the pathogenesis of prerenal and ischemic ARF isnot clear.

Many of the cellular biochemical and structural changes that occur along with the ischemic form ofARF are also important parts of the pathogenesis ofATN associated with exposure to nephrotoxins. Toxin-associated dysfunction and necrosis of cells result in increased tubular pressure and a decreased glomerular capillary hydrostatic pressure gradient. Loss of reabsorptive capability triggers tubuloglomerular feedback and further reduces GFR by lowering RBF following vasoconstriction and a decrease in the glomerular ultrafiltration coefficient. Transepithelial backleak of solutes into the circulation further interferes with the excretory function of the kidneys.

17.4.2.1 ACUTE TUBULAR NECROSIS

Many agents are recognized to have potential nephrotoxic effects.

Aminoglycoside-induced renal toxicity results from accumulation of these agents within the renal cortex. Streptomycin is the least nephrotoxic of the aminoglycosides, whereas gentamicin and kanamycin are intermediate nephrotoxins. Neomycin is the most nephrotoxic. Most cases of aminoglycoside toxicity are associated with conditions that cause reduced renal perfusion because the healthy kidney usually can tolerate some degree of aminoglycoside overdosing. After filtration at the glomerulus, aminoglycosides bind to phospholipase on the brush border of proximal tubules and subsequently are reabsorbed by pinocytosis. Accumulation of these antibiotics interferes with lysosomal, mitochondrial, and Na^+,K^+-ATPase function by inhibiting phospholipase A activity. Binding to the brush border is saturable with the result that sustained exposure of the proximal cells to the drug (as with multiple daily dosing regimens) results in greater accumulation of the drug and increased nephrotoxicity. As a result, once daily dosing may attenuate the risk of nephrotoxicosis while maintaining or improving therapeutic efficacy (because it results in higher peak concentrations).[34,35] Many mild cases of aminoglycoside toxicity notably are associated with nonoliguric ARF and therefore may go unrecognized in horses.

Some cephalosporins, such as cephaloridine, have considerable nephrotoxic potential. These agents cause necrosis because of mitochondrial toxicity following accumulation of the antibiotic in the cell.[36]

Not all cases of ATN are caused by direct toxic changes in tubule cells. For instance, whether myoglobin and hemoglobin are truly nephrotoxic is still debatable. The principal characteristics of pigment nephropathy caused by these agents are tubule obstruction and reduced RBF (caused by the direct vasoconstrictor effects of the pigment). Whether obstruction is physical, caused by pigment accumulation, or reflects aggregation of sloughed cells is not clear. Myoglobin tends to be associated with nephropathy more frequently than hemoglobin. Patients often quickly become oliguric, presumably because of the widespread tubule obstruction.

17.4.2.2 ACUTE INTERSTITIAL NEPHRITIS

To distinguish between drug-induced ATN and acute interstitial nephritis can be difficult, and the latter rarely is diagnosed in horses. Making a distinction may be a particular problem when continued use of 1224
antibiotics is indicated. With ATN one may alter the dosing regimen, whereas with interstitial nephritis, 1225
short-term corticosteroid therapy is often of benefit in human beings. Interstitial nephritis often is marked by eosinophiluria and eosinophilia and is more likely to be associated with the presence of red cells than is ATN. The exact immune mechanism by which interstitial nephritis develops is unclear, although it is thought most likely to be caused by delayed cell-mediated hypersensitivity or the presence of antitubule basement membrane antibodies.[37] The prognosis is usually grave in horses with acute interstitial nephritis.

17.4.2.3 ACUTE GLOMERULONEPHRITIS

Acute glomerular nephropathy is rare in horses, but when it occurs, it usually is manifested by the nephrotic syndrome, although hematuria and oliguria sometimes are apparent. Deposits of γ-globulin and complement are found along the basement membrane (global form) or in the mesangial area (mesangioproliferative form).[38] Group C streptococcal antigens have been identified along with equine glomerulonephritis, and equine infectious anemia viral antigen-antibody complexes have been recognized in the glomeruli of horses that were not in renal failure.[12,39] Deposition of immune complexes activates the complement cascade. Formation of C_3b and C_5a causes platelet aggregation and attracts neutrophils. Tissue damage results from deposition of

complement per se and from inflammation associated with neutrophil activation and release of reactive oxygen radicals, proteases, elastases, and other lysozymes. These enzymes, plus platelet-activating factor and leukotriene B_4, increase vascular permeability and upregulate expression of adhesion molecules, thus promoting further inflammation.[33] Severe reduction in GFR results from large drops in the glomerular permeability coefficient, which is associated with the widening of Bowman's spaces following inflammation and deposition of immune complexes.

The pathophysiology of postrenal ARF was referred to previously. Basically, increases in ureteral pressure for any reason result in GFR reduction because of a drop in the glomerular capillary hydrostatic pressure gradient, some tubular backleaking, a decrease in the glomerular permeability coefficient, and ultimately, a reductionin RBF.

17.4.3 Clinical Signs

In most horses with hemodynamically mediated (i.e., prerenal or ischemic) ARF, clinical signs are usually referable to the primary problem, such as acute colic or enterocolitis, sepsis, coagulopathies, rhabdomyolysis, or heavy metal poisoning, rather than to renal dysfunction. Therefore the predominant clinical signs are often dehydration (with or without diarrhea), depression, and anorexia. Other signs can include tachycardia, hyperemic mucous membranes, pyrexia, mild abdominal pain, and laminitis. Because clinical signs usually relate to the inciting problem, ARF may not be suspected or detected unless the veterinarian specifically evaluates renal function as part of the workup for a more obvious disease. In general, the clinical manifestations of ARF reflect the systemic effects of toxic substances usually excreted in the urine (i.e., uremia generally is reflected by anorexia and depression), urinary tract dysfunction, and derangements of fluid, electrolyte, and acid-base balance. One may observe signs of encephalopathy in horses with severe azotemia.

Although oliguria is considered the hallmark of ARF, urine production in horses varies. Oliguria frequently occurs in the early stages of hemodynamically mediated ARF and is the most frequently reported clinical sign that is related directly to urinary tract dysfunction. As outlined in the section on pathophysiology, however, nonoliguric and polyuric stages of prerenal and intrinsic ARF also can be associated with renal hypoperfusion. Anuria is rare. Nonoliguric or polyuric ARF also can be associated with exposure to nephrotoxins (ATN), and polyuria is common during the recovery phase of ARF, regardless of its cause. The magnitude of azotemia tends to be lower in nonoliguric than in oliguric ARF, possibly indicating less severe damage in the former condition. Similarly, nonoliguric ARF is associated with a more favorable prognosis.

Patients with ARF often are treated initially with large volumes of intravenous or oral fluids for the primary disease. In these cases, oliguria may progress to polyuria. When significant renal damage has been sustained, persistence of oliguria in the face of fluid administration usually is manifested as failure to produce a significant volume of urine in response to fluid therapy. The degree of change of azotemia also is minimal in the initial 24 to 36 hours of treatment. If one does not monitor these patients carefully, fluid retention may lead to development of subcutaneous and pulmonary edema. Soft feces caused by fluid retention also may be apparent in patients with oliguric ARF.

Postrenal or obstructive uropathy usually is characterized by mild to severe abdominal pain and pollakiuria and stranguria (see Chapter 17.7).

17.4.4 Diagnosis

Increases in plasma urea nitrogen and creatinine concentrations (i.e., azotemia) are frequently the initial findings that suggest compromised renal function. Azotemia simply reflects a reduction in GFR; it has almost no differential diagnostic value. After establishing recent development of azotemia, the equine internist must proceed systematically to differentiate between six possible syndromes associated with ARF: prerenal ARF, ischemic ARF, ATN, acute interstitial nephritis, acute glomerulonephritis, and obstructive (postrenal) ARF. A useful way to go about this is first to try to rule out pre- and postrenal ARF. If this is possible, the patient must have a type of intrinsic ARF and one can direct further diagnostic efforts to identifying the disease subtypes. 1225 1226

As described in greater detail later (see Urolithiasis), the diagnosis of postrenal obstructive disease usually is based on a combination of clinical signs, history, and the results of rectal palpation, ultrasonography, and urinary tract endoscopy. The frequency and volume of urination can vary with these cases, and total obstruction causes anuria. Salt and water reabsorption often are impaired as the problem persists, which results in hyponatremia (i.e., plasma sodium concentrations in the low normal range). Although the mechanisms for this sodium wasting arenot completely clear, the wasting apparently is related to impaired function of the ascending limb of Henle's loop and an increased medullary blood flow following prostaglandin release. Both of these events greatly diminish the magnitude and effect of the medullary countercurrent concentrating mechanisms. Prostaglandin also directly inhibits the effect of antidiuretic hormone.[40]

Measurement of specific gravity in the azotemic horse is a commonly practiced means of detecting prerenal ARF. In these cases the value is usually greater than 1.025 and often as high as 1.055. In human beings, one can use a number of reliable indexes to differentiate renal azotemia from prerenal azotemia[41–43] that are basedon urine osmolality and the ratio of urine to plasma osmolality (U/P_{osm}), urine sodium concentration and fractional sodium clearance, and ratios of urine to plasma urea concentrations (U/P_{UN}) and urine to plasma creatinine concentrations (U/P_{Cr}). Fractional sodium clearance in particular is a good indicator of solute reabsorption and proximal tubule function, whereas U/P_{Cr}, and to a lesser extent U/P_{UN}, are useful indexes of the ability of the tubules to reabsorb water. The utility of these indexes in horses also has been investigated and has been shown to have considerable differential diagnostic value.[16] Although these tests are discriminatory, some degree of overlap occurs between them respecting prerenal and parenchymal or intrinsic problems. One also must bear in mind that these indexes do not allow differentiation between intrinsic and postrenal disease. A number of horses with prerenal (nonoliguric) ARF have urine osmolality less than 360 mOsm. A small percentage of horses in prerenal ARF have U/P_{Cr} less than 30 (usually greater than 50) and fractional sodium clearance greater than 0.80% (usually less than 0.50%), most likely because of the unrecognized existence of tubule damage before volume depletion or because of a natriuretic effect of some treatments such as diuretics or intravenous fluids. Natriuresis is particularly likely to be induced along with bicarbonate administration because sodium cations are lost with unreabsorbed bicarbonate anions. In these cases the determination of the fractional chloride clearance may be a better indicator of the response of the kidney to hypoperfusion.

No parameter reliably differentiates between prerenal and ischemic ARF because of the pathophysiologic continuum between these diseases. At one end is reduced GFR with preserved tubule function and concentrating mechanisms. This form of disease is readily reversible with appropriate therapy. Additional or more prolonged decreases in GFR lead to disturbances in tubule function and slower reversal of damage, until the other end of the spectrum is reached, in which complete and irreversible loss of renal function occurs. Assessment of urine specific gravity before initiation of fluid therapy is helpful in differentiating prerenal from renal failure. As normally functioning kidneys would preserve salt and water maximally in response to a

transient decrease in RBF with prerenal failure, so urine specific gravity and osmolality are greater than the values associated with serum, whereas the urine produced by horses with intrinsic ARF is often isosthenuric (specific gravity less than 1.020). In a clinical situation, assessment of the response to fluid therapy is the most practical way to differentiate prerenal failure from intrinsic forms of ARF. Azotemia caused by prerenal problems should resolve quickly with replacement of fluid deficits and restoration of renal perfusion. In prerenal failure, volume repletion also should restore renal function, with the result that the magnitude of azotemia should decrease by 50% or more during the first day of therapy. In contrast, fluid therapy usually does not lead to prompt resolution of azotemia associated with intrinsic problems. Application of the measurement of U/P_{Cr} and U/P_{UN} ratios is limited to use on urine samples collected before initiation of fluid therapy or the first urine sample voided after fluid therapy has been started.

In prerenal ARF, electrolyte and acid-base abnormalities generally reflect problems caused by a primary disease (e.g., enterocolitis, colic, blood loss). Most frequently, horses are mildly acidotic, hyponatremic, and hypochloremic. Plasma concentrations of potassiumand calcium vary according to what disease is causing renal hypoperfusion. Potassium concentration also is affected to some extent by urine output, hyperkalemia being most common in association with oliguria and anuria.

The technique for biopsy of the left kidney has been well-described.[44] The main indication of kidney biopsyis 1226
to help differentiate between types of intrinsic renal disease when one thinks that this distinction will have 1227
therapeutic and prognostic relevance. One often can diagnose ischemic failure and ATN without biopsy. The primary complication associated with the biopsy procedure is renal hemorrhage, which can be severe. Ultrasonographic guidance and use of a spring-loaded biopsy instrument may reduce the risk of complications. Ultrasonography also allows biopsy of the right kidney. As the knowledge of equine renal physiology and pathology improves and advances in molecular genetics lead to techniques that supplant or supplement standard histopathologic methods, the diagnostic usefulness of renal biopsy may increase. For example, biopsy results may dictate the use of immunosuppressive agents in the treatment of some forms of renal parenchymal disease (e.g., interstitial nephritis).

Identification of subtypes of intrinsic ARF often depends on analysis of urine and urine sediment. The availability of a history of exposure to ischemic insults or potential nephrotoxins such as aminoglycosides certainly helps in this regard, but determination of the severity of the condition and its prognosis still generally relies on the analysis of urine. Ischemic tubule disease is similar to ATN. In both cases a slight to moderate proteinuria with specific gravities usually less than 1.020 and urine osmolality less than 350 mOsm occurs. Fractional sodium clearance is nearly always greater than 1.0, regardless of urine output. Granular casts frequently are visible, particularly with ATN. Enzymuria and phosphaturia are frequently prominent early in the course of ATN.[45,46] Plasma sodium and chloride concentrations are usually low. Plasma concentrations of calcium and inorganic phosphate vary greatly: increases, decreases, and normal values are possible, depending on the diet of the horse, the nephrotoxin, and the location and severity of damage to the nephron.[39,45]

One must remember that GFR is reduced in cases of ATN and is most likely caused by the increase in presentation of sodium and chloride at the macula densa following dysfunction of proximal sodium reabsorption. Stimulation of the macula densa results in release of renin and local production of angiotensin II, thus increasing renal vascular resistance and lowering RBF. The low GFR may mask the absolute magnitude of the damage in tubule function; however, if one studies renal sodium reabsorption carefully over time in cases of ATN, this defect appears to improve more rapidly than that of GFR.[47]

Acute interstitial nephritis often is not recognized in horses; however, in a study conducted about 20 years ago, the condition was diagnosed in approximately one eighth of all human patients who needed renal biopsy for diagnosis of unexplained ARF.[48]

The disease is characterized by edema and diffuseor focal patches of interstitial inflammation. In human medicine, the number of drugs, toxins, and infectious agents known to induce this disease is growing. No reason exists to believe that similar agents are not capable of causing the same disease in equids. Inflammatory cells surround the tubules and can move between epithelial cells into tubule lumina. As a result, white blood cell casts are common. The leukocytes are also capable of disrupting the tubule basement membrane, making cell repair much less likely, and this is an important distinguishing feature between ATN and interstitial nephritis. With the former disease the basement membrane usually stays intact. Reduction of GFR and azotemia probably result from the interstitial edema, intratubular obstruction, and release of vasoactive agents.

Acute interstitial nephritis and ATN have similar fractional sodium clearance, U/P_{Cr}, and U/P_{osm} values; however, the urine sediment may be different for each disease. Sterile pyuria and microscopic hematuria commonly occur with interstitial nephritis, although red blood cell casts are rare. Mild proteinuria and eosinophiluria are also common. Eosinophiluria generally seems to be limited to renal interstitial disease, mainly drug-induced interstitial nephritis but also chronic pyelonephritis and systemic lupus erythematosus.[49,50] Eosinophiluria may or may not be accompanied by eosinophilia. Eosinophils in equine urine should be easy to observe using Wright's stain, given the alkaline nature of the fluid. Occasionally, fevers are believed to be associated with the development of interstitial nephritis. This clinical sign is relatively nonspecific and might be misleading if the animal is being treated with antibiotics for an infection. What one might see with close monitoring of the patient is an initial reduction or resolution of the fever after the onset of antimicrobial therapy followed by recurrence of the fever and development of azotemia. If this happens, acute interstitial nephritis should be on the rule-out list.

Acute glomerulonephritis usually is characterized by the nephrotic syndrome. Proteinuria is moderate to severe, and the urine usually is concentrated. Urine osmolality and U/P_{osm} are comparable with the values with prerenal ARF and are higher than those values normally seen in ATN. Urinary sodium concentration and fractional sodium clearance tend to be much lower than with other intrinsic renal and postrenal causes of ARF. The ratios of U/P_{UN} and U/P_{Cr} are often similar to those associated with prerenal failure and higher than those in ischemic, tubular, interstitial, or postrenal disease. Renal tubular secretion of creatinine is increased in glomerulonephritis, with the result that serum creatinine concentrations may not rise quickly and urea nitrogen-to-creatinine ratios stay high, as they frequently do with prerenal problems. Therefore although urinary indexes should make possible differentiation of acute glomerulonephritis from other parenchymal and postrenal 1227
diseases, they overlap much with those associated with prerenal ARF. One is going to differentiate ARF caused 1228
by acute glomerulonephritis from that caused by hypoperfusion based on the significant proteinuria and red cell numbers usually associated with the former disease. Red cell casts are more common with glomerulonephritis than with other intrinsic causes of ARF. Some causes of postrenal ARF also may manifest red cell casts, but the proteinuria is not normally as great in those situations.

17.4.5 Treatment

Initially, treatment of horses with ARF should focus on reversing the inciting or underlying cause and correcting fluid and electrolyte imbalances. Early identification of patients at risk and prevention of problems by rapid restoration and accurate maintenance of intravascular fluid volume, glomerular filtration, and urine

production, with fluids and possibly diuretics, is obviously preferable to treatment. The importance of prevention cannot be overstated. One should correct initial fluid deficits over the first 6 to 12 hours of treatment. Physiologic saline or a balanced electrolyte solution is the fluid of choice unless the patient is hypernatremic, as may be the case in prerenal ARF or acute glomerulonephritis. In the event of hypernatremia, a solutions of 0.45% sodium chloride and 2.5% dextrose is recommended. The addition of 50 to 100 g of dextrose per liter of fluid to saline or polyionic fluids helps address the caloric needs of anorectic horses. If fluid administration is begun early in the course of the disease or if the problem is not severe, diuresis should result. When this occurs, one should maintain intravenous fluid therapy at 40 to 80 ml/kg per day until serum creatinine concentration decreases dramatically. One then reduces the rate of fluid administration to 10 to 20 ml/kg per day until the creatinine concentration is normal or the horse is eating and drinking adequately.

In the event that the horse remains oliguric 10 to12 hours after starting fluid therapy, administration of dopamine in 5% dextrose slowly (3 to 5 μg/kg/min) may improve RBF and urine output. One should monitor blood pressure during dopamine infusion because the drug can induce significant hypertension. One should discontinue administration of dopamine if blood pressure starts to rise. One can attempt to restart the infusion at a lower rate when pressure has returned to normal. Blood pressure also may increase because of overhydration of oliguric patients who are receiving isotonic fluids. Regular monitoring of body weight, hematocrit, and serum total protein concentration, central venous pressure, and lung sounds is important if one is to avoid problems caused by overhydration.

The use of diuretic agents such as mannitol and loop diuretics to treat ARF is controversial. Furosemide and ethacrynic acid are the loop diuretics most commonly used. Furosemide is a particularly potent short-acting agent that acts by blocking the $Na^+/K^+/2Cl^-$ cotransporter in the ascending limb of the loop of Henle. In addition to promoting diuresis, cotransporter inhibition also may protect these tubule cells by reducing their metabolic rate and thus oxygen demand in the face of limited oxygen availability following hypoperfusion. To be effective, loop diuretics must gain access to the tubule lumina. Therefore loop diuretics may be of limited value in prerenal or ischemic problems, although the potential protective effect of furosemide on the especially vulnerable cells of the thick ascending limb may warrant its administration. Loop diuretic administration also may exacerbate or induce volume depletion in cases of ARF that are characterized by isosthenuria or polyuria (such as early ATN), thus making the condition worse and rendering the patient more susceptible to the effects of nephrotoxins such as gentamicin. Loop diuretics appear to be most beneficial when used in cases characterizedby tubule obstruction (e.g., pigmenturia) because the diuretic-induced increase in solute retention apparently helps flush these blockages and casts from the tubules.

Although loop diuretics have no direct effect on GFR, 20% mannitol (0.25 to 1.0 g/kg) given over 15 to20 minutes may help combat oliguria by increasing RBF and GFR. The increase occurs following reductions in plasma protein concentration and oncotic pressure. These changes in turn result from the systemic effects of the increase in intravascular osmolality induced by the mannitol. Mannitol also may induce synthesis of the vasodilator PGE_2 and release of atrial natriuretic peptide, which also would increase RBF and GFR. Once filtered, the agent also acts as an osmotic diuretic, thus decreasing urine solute concentration and boosting urine volume. Consequently, mannitol also can be effective in treating conditions characterized by tubular obstruction and swelling of tubule cells.

Hyperkalemia is uncommon in equine ARF, exceptin some postrenal cases. When present, hyperkalemia is usually mild and responds to administration of potassium-free intravenous fluids. When hyperkalemia (greater than 6.5 mEq/L) persists, correction of any associated acidosis with sodium bicarbonate and/or administrationof glucose (up to 10% solution) usually helps. In the worst or most refractory cases, insulin may be necessary.

Potassium supplementation (potassium chloride 20 to 40 mEq/L) may be necessary during the polyuric phase of recovery from ARF.

Calcium metabolism often is disrupted in cases of equine ARF, with hypocalcemia and hyperphosphatemia or hypercalcemia and hypophosphatemia having been reported variously. Hypercalcemia usually resolves witha 1228
switch to a grass or grass-hay diet and with time. Hypocalcemia probably results for a number of reasons, 1229
including skeletal resistance to parathyroid hormone during the early stages of ARF and deficiency of1,25-dihydroxycholecalciferol, which results from downregulation of renal 1,25-hydroxylase by the hyperphosphatemia, dysfunction of this enzyme following renal parenchymal damage, or both.[46,51] Hypoalbuminemia and enhanced deposition of calcium in injured tissues, as can occur in cases of rhabdomyolysis, are other factors to consider. Administration of a calcium salt as part of the intravenous fluid therapy protocol is usually sufficient to correct this.

Severe uremia often decreases red blood cell life span and induces platelet dysfunction. Consequently, anemia (possibly also caused by decreased production of erythropoietin) and bleeding tendencies may be associated with ARF. These conditions may require treatment with transfusions, synthetic erythropoietin, or conjugated estrogens.

Because aminoglycoside toxicity is one of the more commonly recognized causes of equine ARF,[4,9,52] continued aminoglycoside use in horses with ARF warrants specific attention. Gentamicin and amikacin are the aminoglycoside antibiotics most often used in equine practice, and their pharmacokinetics have been well studied in healthy animals. Large interindividual and age variations occur in the volume of distribution and clearance of these drugs in normal horses, and this variability is even greater in diseased horses. Therefore when a patient with ARF requires aminoglycoside antibiotics, monitoring serum trough concentrations of the drugto adjust the dosing interval is strongly indicated, for it provides the best protection against exacerbating renal damage. Renal dysfunction associated with aminoglycoside toxicity usually is indicated by an increase of at least 0.3 mg/dl in serum creatinine concentration. When this occurs, one should consider discontinuing therapy or increasing the dosing interval. One must remember that changes in urine sediment and development of enzymuria, mild proteinuria, glucosuria, and decreased urine-concentrating ability develop a number of days before serum creatinine concentration increases and are good indicators of the development of aminoglycoside nephrotoxicity. One usually can address the problem successfully by maintaining or increasing intravenous fluid therapy to guard against renal hypoperfusion and by appropriately adjusting the dosing interval with the antibiotic.

17.4.6 Prognosis

The prognosis for equine ARF depends on the underlying cause, the duration of renal failure, the response to initial treatment, and the development of secondary complications such as diarrhea, thrombophlebitis, and laminitis. Generally speaking, the duration of ARF before beginning therapy is the most important determinant of prognosis. Of the primary causes of ARF, severe ischemic failure and acute interstitial nephritis probably carry the worst prognosis in horses. ARF of any cause, however, should be associated with a reduced or poor prognosis in the event that early interruption of the pathophysiologic events leading to the ARF is not achieved or that the animal displays prolonged oliguria or anuria (longer than 12 hours) after institution of vigorous therapy. Most cases of postrenal ARF carry a favorable prognosis, provided the initiating disease is treated successfully. When discussing prognosis with horse owners, practitioners always must bear in mind that a successful outcome is not always associated with complete return of normal function. Many horseslive long after a bout of ARF but never fully regain theability to concentrate urine as well as they did beforethe disease;

or else they remain constantly polyuric. Acute tubular necrosis, following nephrotoxicity in particular, carries a favorable prognosis when tubule basement membranes remain intact.

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17.5 17.5—Chronic Renal Failure

Harold C. Schott, II

17.5.1 Predisposing Conditions

Chronic renal failure (CRF) is recognized infrequentlyin horses. For dogs and cats the prevalence of CRF has been reported to be 0.9% and 1.6%, respectively,[1] whereas the Veterinary Medical Data Base at Purdue University reported that only 515 of 442,535 horses admitted to participating veterinary teaching hospitals during the years 1964 through 1996 had CRF (prevalence of 0.12%). In actuality, this may be an underestimate, because when a diagnosis of CRF is established for a horse, the horse likely may be destroyed without presentation to a veterinary teaching hospital. As in dogsand cats, CRF appears to be a greater problem in older horses: the prevalence increased to 0.23% in horses older than 15 years. The 0.51% prevalence for intact males older than 15 years of age also suggests that stallionsmay be at greater risk.

Although the clinical syndrome of CRF is uncommon, one widely cited abattoir study revealed that 16% of 45 horses examined had glomerular lesions on light microscopic examination and 42% (22 of 53 horses examined) exhibited deposits of immunoglobulin or complement on immunofluorescence staining of tissue samples.[2] Although these findings suggest that as many as one third of horses may show microscopic evidence of renal disease, only one of the horses in this survey exhibited signs of CRF. This disparity can be attributed to a large renal reserve capacity, for clinical signs ofrenal failure do not become apparent until two thirds to three fourths of functional nephrons have been lost.[3] Although this rule of thumb is based on studies of partially

nephrectomized laboratory animals, supportfor similar renal reserve capacity in horses is found in several clinical reports of unilateral nephrectomy used successfully to manage disorders of the upper urinary tract.[4–8] In addition, after experimental unilateral nephrectomy in ponies[9] and horses (R. DeBowes, personal communication, 1991), renal function remained within normal ranges and the animals maintained body weight.

Disorders of the kidneys leading to CRF may be congenital or acquired. In horses younger than 5 years with a history that includes no event that might have been complicated by acute renal failure, one should suspect a congenital renal disorder: renal agenesis, hypoplasia, dysplasia, or polycystic kidney disease.[10–23] Although each of these congenital abnormalities occasionally is recognized, acquired disease consequent to glomerular or tubular injury is more often the cause of CRF in horses.[24–30] Acquired disease is usually insidious in onset, and renal injury may have been initiated years earlier. Thus identifying the cause of CRF is challenging because many horses have evidence of advanced glomerular and tubular disease, termed *end-stage kidney disease*, by the time clinical signs of CRF develop. Nevertheless, knowing the various causes of CRF affords a better overall understanding of CRF in horses.

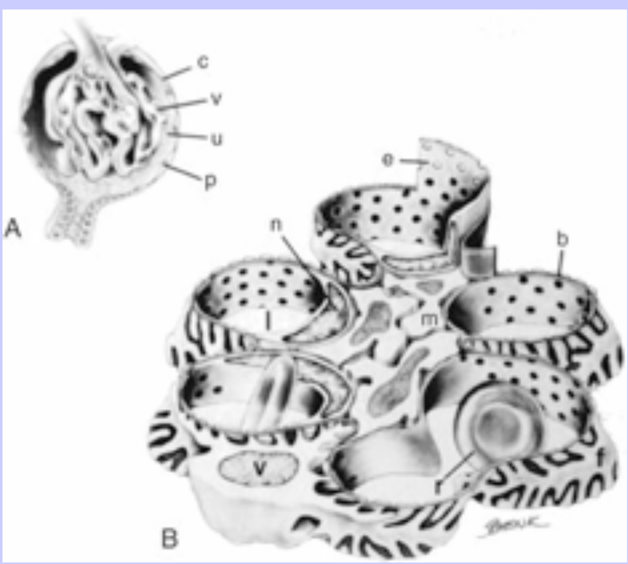
17.5.1.1 GLOMERULONEPHRITIS

Glomerular injury is a common precipitant of renal insufficiency and CRF in horses. Although immune-mediated glomerular injury is implicated most oftenin glomerulonephritis, glomerular integrity can be disrupted by a number of unrelated disease processes, including ischemia, toxic insults, and infection.[31] These mechanisms usually lead to significant vascular and tubulointerstitial changes in addition to glomerular injury. Thus the designation *glomerulonephritis* typically is reserved to denote renal disease of which immune-mediated glomerular damage is suspected to be the initiating factor in development of renal failure. Until the last decade, glomerulonephritis was considered a rare cause of CRF in domestic animals; interstitial nephritis was implicated more often.[32–34] Refinement of histologic examination with immunofluorescence staining techniques and electron microscopic examination of renal tissues has led to increased recognition of subclinical and clinically significant glomerulonephritis.[34]

A brief review of the subgross anatomy of the glomerulus sheds light on the pathophysiology of glomerulonephritis. The renal corpuscle, or glomerulus, is comprised of a tuft of glomerular capillaries surrounded by epithelial cells that line Bowman's capsule. The root that supports the pedicle of the glomerular capillary network is similar to the mesenteric root that supports the intestinal tract, and Bowman's capsule is analogous to the peritoneum (Figure 17.5-1, *A*). On a microscopic level, components of the glomerulus include capillary endothelial cells, mesangium (cells and matrix), glomerular basement membrane (GBM), and visceral epithelial cells. At the vascular pedicle, the latter become contiguous with parietal epithelial cells that line Bowman's space, much as mesothelial cells covering bowel serosa and mesentery become contiguous with the peritoneum at the mesenteric root.[31] Glomerular capillary endothelial cells are unique in that they are fenestrated with pores that represent the initial barrier for passage of blood components into the urinary space (Figure 17.5-1, *B*). The mesangium, which lies between endothelial and 1231
epithelial cells, is the support structure of the glomerular capillaries and is analogous to the mesenteric tissue 1232
supporting bowel. Mesangial cells are a component of the reticuloendothelial system and phagocytize macromolecular substances, among them are fragments of old GBM or larger molecules that pass through endothelial cell pores but cannot pass subsequently through the GBM. In addition, mesangial cells have contractile elements that allow them to participate in regulation of glomerular hemodynamics. Furthermore, these cells proliferate in response to glomerular injury and can release a number of cytokines that modulate the glomerular inflammatory response.[35] The GBM lies between endothelial and epithelial cells and

surrounds glomerular capillaries, except where mesangium is present (Figure 17.5-2, *A*). Returning to the abdominal cavity analogy, the GBM would lie beneath bowel serosa except in the area of mesenteric attachment, which would contain mesangial cells and matrix. The GBM consists of a central electron-dense layer, the lamina densa, and two thinner, more electron-lucent layers, the lamina rara externa and the lamina rara interna (Figure 17.5-2, *B*).[31,36] The main components of the GBM are collagen-like molecules and matrix glycoproteins that are produced principally by visceral epithelial cells. Normal GBM undergoes steady turnover with removal of debris by mesangial cells. The visceral epithelial cells, also called podocytes, cover the uriniferous duct side of the GBM and have many cytoplasmic extensions called foot processes that form extensive interdigitations with foot processes of adjacent epithelial cells. The narrow gap between foot processes—the filtration slit or slit pore—is bridged by a thin membrane, the slit pore diaphragm.[31,36]

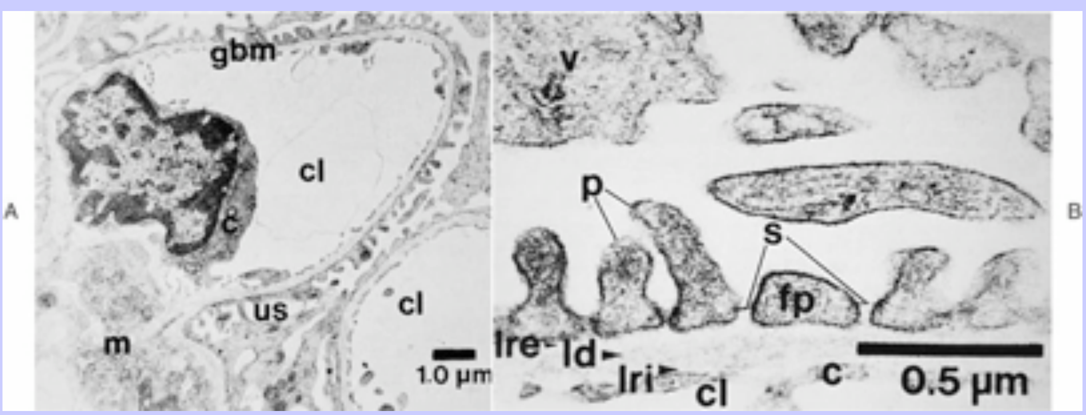
Figure 17.5-1 Subgross anatomy of a renal corpuscle. **A**, A renal corpuscle with a tuft of glomerular capillaries surrounded by Bowman's capsule *(c)* shows visceral *(v)* and parietal *(p)* epithelial cells separated by the urinary space *(u)*.**B**, Cross section of a portion of a glomerulus shows the nucleus *(n)* and fenestrations *(e)* of capillary endothelial cells, the glomerular basement membrane *(b)*, mesangial cells *(m)* separated by mesangial matrix, and the nucleus *(v)* of a visceral epithelial cell. A red blood cell *(r)* is in the lumen *(l)* of one of the glomerular capillaries. (From Osborne CA, Hammer RF, Stevens JB et al: The glomerulus in health and disease: a comparative review of domestic animals and man, *Adv Vet Sci Comp Med* 21:207, 1977.)



The filtration barrier of the glomerulus consists of fenestrated endothelial cells, GBM, and slit pores between epithelial cell foot processes. These structures constitute a size-selective and charge-selective filtration barrier. Although all components of this barrier are anionic (i.e., they repel anionic macromolecules), the GBM is thought to be the principal agent of the permeability characteristics of the filtration barrier. The GBM is rich in glycosaminoglycans, containing heparan sulfate and sialic acid residues. These strongly anionic molecules are responsible for its negative charge barrier, which limits filtration of anionic macromolecules, predominantly albumin.[36,37]

Glomerulonephritis is initiated by deposition of circulating immune complexes along the GBM and in the mesangium, leading to complement activation and leukocyte infiltration and adherence. Release of oxidants and proteinases by neutrophils and macrophages; production of eicosanoids, cytokines, and growth factors by macrophages and mesangial cells; and platelet aggregation and activation of coagulation factors lead to endothelial and epithelial cell swelling (with fusion of foot processes), formation of microthrombi in glomerular capillaries, and mesangial cell proliferation.[31,37,38] Immune complexes can be deposited in a subendothelial, intra-GBM, or subepithelial site, depending on their size and charge properties, but most tend to be found in the subendothelial space. On electron microscopic examination these immune complexes appear as electron-dense granular deposits (Figure 17.5-3, *A*), and staining with anti–immunoglobulin G and anticomplement (C3) antibodies reveals an irregular (granular or "lumpy bumpy") immunofluorescence staining pattern (Figure 17.5-3, *B*).[2,31,37] The GBM proliferates to surround the immune deposits, leading to irregular thickening ofthe filtration barrier. Despite widening of the filtration barrier, the size-selective and charge-selective filtration properties are compromised, and microscopic hematuria and proteinuria result. In rare instances, glomerulonephritis may be attributed to a true autoimmune disorder in which autoantibodies directed against GBM components (e.g., type IV collagen) are produced. Electron microscopic examination
in these cases also reveals GBM thickening with predominantly subepithelial electron-dense deposits (Figure 1232
17.5-4, *A*), and immunofluorescence staining shows a more regular or smooth, linear pattern of 1233
immunofluorescence (Figure 17.5-4, *B*).[31,39,40] Autoimmune glomerulonephritis, accompanied by proteinuria, also has been described as one of the manifestations of systemic lupus erythematosus in horses.[41] Another immune mechanism of glomerulonephritis in horses is production of mixed or monoclonal cryoglobulins and deposition of antibody-antibody immune complexes along the glomerular GBM.[42,43] Cryoglobulinemia is associated with a number of diseases in human beings[42,44] but has been described in only a few horses.[43,45] Deposition of antibody-antibody immune complexes along the GBM may be a more important precipitant of glomerulonephritis than was recognized previously because electron microscopic examination is required to demonstrate characteristic fibrillar or crystalline intracapillary and subendothelial deposits associated with the condition.[42] Regardless of what immune mechanism leads to glomerular injury, the end result is thickening of the filtration barrier, retarded glomerular filtration rate, and development of CRF in severe cases.

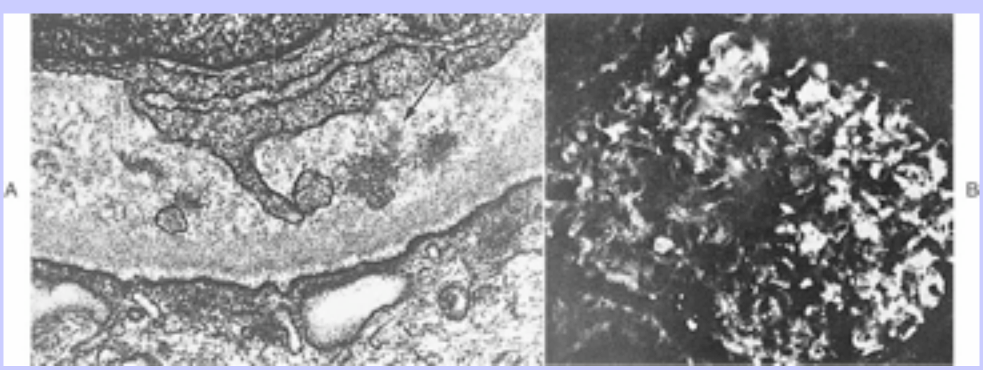
Figure 17.5-2 Electron micrograph of a normal glomerulus. **A**, Low-power magnification illustrates patent capillary lumens *(cl)* separated from the urinary space *(us)* by fenestrated endothelial cell cytoplasm *(c)*, the glomerular basement membrane *(gbm)*, and foot processes of visceral epithelial cells. Mesangial cells and matrix *(m)* are also apparent (bar = 1.0 μm). **B**, Higher-power magnification reveals the ultrastructural features of the filtration barrier including, from bottom to top, the capillary lumen *(cl)* and endothelial cell cytoplasm *(c);* the lamina rara interna *(lri)*, lamina densa *(ld)*, and lamina rara externa *(lre)* of the glomerular basement membrane; and cytoplasm *(v)*, foot processes *(fp)*, and slit diaphragms *(s)* of the visceral epithelial cells. Deposits of glomerular polyanion or glycosaminoglycans *(p)* are visible on the foot processes (bar = 0.5 μm). (From Osborne CA, Hammer RF, Stevens JB et al: The glomerulus in health and disease: a comparative review of domestic animals and man, *Adv Vet Sci Comp Med* 21:207, 1977.)



Although a number of terms are used to describe the specific morphologic changes associated with glomerular injury, glomerulonephritis is categorized most broadly histologically as proliferative or membranous.[31,34,37] Proliferative (or mesangioproliferative) glomerulonephritis describes glomerular injury associated with influx of inflammatory cells and proliferation of mesangial cells. The predominant histologic finding is increased cellularity in glomeruli (Figure 17.5-5, *A* and *B*). This lesion tends to be associated with the more acute stages of glomerulonephritis during which immune complexes are being deposited in a predominantly subendothelial site. *Membranous glomerulonephritis* describes glomerular injury

accompanied by significant thickening of the capillary wall and GBM, and with periodic acid–Schiff staining the predominant histologic finding is increased in the mesangial area and on the GBM (Figure 17.5-5, *C*). Methenamine silver stain further enhances visibility of the thickening of the GBM.[31,34] Membranous glomerulonephritis tends to be associated with more soluble immune complexes or autoantibodies that can pass through the GBM and localize in a predominantly subepithelial site, resulting in less infiltration of inflammatory cells. As would be expected, a spectrum of lesions is visible in naturally occurring glomerulonephritis, leading to varying histologic descriptions of the disease (membranoproliferative 1233
glomerulonephritis). As glomerular injury progresses, proliferation of the parietal epithelium also occurs, 1234
likely in response to filtration of macromolecules and cellular debris. Lesions associated with parietal cell proliferation can include layering of epithelial cells (termed *crescents*) on the inner aspect of Bowman's capsule (Figure 17.5-6, *A*), adhesion formation between the glomerular tuft and Bowman's capsule (Figure 17.5-6, *B*), and tuft collapse. *Glomerulosclerosis* describes the end stage of progressive, irreversible glomerular injury in which replacement of glomerular components with hyaline material is visible on histologic examination (Figure 17.5-6, *C*).[31]

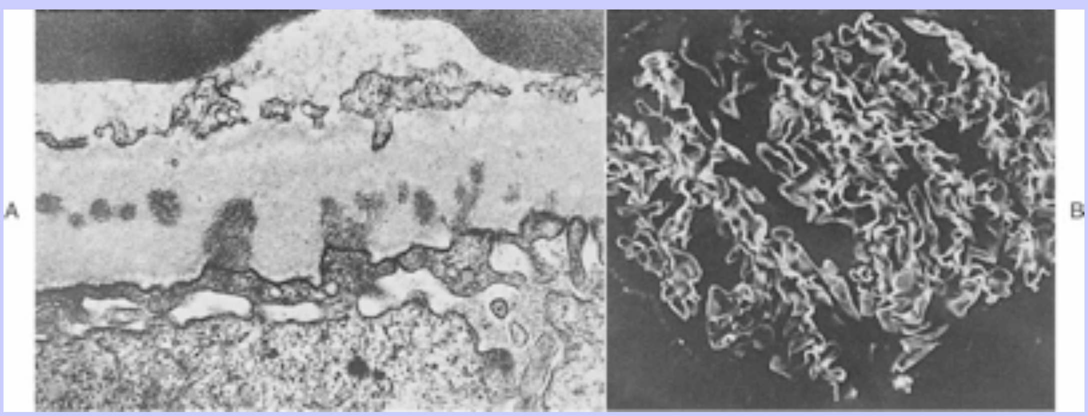
Figure 17.5-3 Immune-mediated glomerulonephritis in a horse 165 days after experimental infection with equine infectious anemia virus. **A**, An electron micrograph (×32,500) shows endothelial cell swelling *(top)*, thickening of the glomerular basement membrane with electron-dense immune deposits in a predominantly subendothelial location *(arrow)*, and fusion of foot processes of the visceral epithelial cells. **B**, Immunofluorescent staining with fluorescein-tagged antiequine immunoglobulin G antibody (×100) demonstrates granular or “lumpy bumpy” deposits of immunoglobulin G along the glomerular basement membrane and in the mesangium. (From Banks KL, Henson JB, McGuire TC: Immunologically mediated glomerulitis of horses. 1. Pathogenesis in persistent infection by equine infectious anemia virus, *Lab Invest* 26:701, 1972.)



Specific histologic categorization of glomerulonephritis provides etiologic and prognostic information for human beings with renal failure attributable to glomerular disease.[35,44] Subcategorization of glomerulopathies also has been done in a prospective study of naturally occurring chronic renal disease in canines. Although classifications—including focal glomerulonephritis, mesangioproliferative glomerulonephritis, endocapillary proliferative glomerulonephritis, crescentic glomerulonephritis, and sclerosing glomerulonephritis—could be made, histologic findings were not prognostically useful.[46] This study also revealed that glomerular disease was responsible for 52% of canine CRF; however, an inciting disease (vegetative endocarditis) could be identified in only one of 31 dogs with glomerulonephritis.

Glomerulonephritis also appears to be an important cause of CRF in horses.[47] Of 60 reported cases of CRF attributable to acquired disease, glomerulonephritis was identified as the inciting cause of CRF in 32 (53%) (Table 17.5-1).[24–26,38,42,43,48–60] Glomerulonephritis also may be the initiating disease process in cases of end-stage kidney disease (ESKD) for which gross and histopathologic changes are so extensive that a primary mechanism of renal injury cannot be identified. A number of systemic inflammatory and infectious disease processes in horses may be accompanied by glomerulonephritis, but progression to CRF appears to be a rare sequela. For example, experimental *Leptospira pomona* infection produced subacute glomerulonephritis characterized by hypercellularity and edema of capillary tufts,[61] but leptospirosis appears to be a rare cause of clinical renal disease in horses.[62–64] Similarly, experimental infection with equine infectious anemia virus produced histologic and immunofluorescent evidence of glomerulonephritis in 75% and 87% of infected horses, respectively.[39] Immunoglobulins with anti–equine infectious anemia activity were eluted from glomeruli collected from experimentally infected horses, but none of the horses showed clinical signs of renal disease. Poststreptococcal glomerulonephritis is a well-recognized cause of renal disease in human beings,[35,65] and Roberts and Kelly speculated that glomerulonephritis in a horse with 1234
chronic pleuritis and purpura hemorrhagica likely resulted from circulating immune complexes involving 1235
streptococcal antigens.[66] Recently, Divers, Timoney, Lewis, et al. provided support for this hypothesis by eluting group-C streptococcal antigens from immune complex deposits in glomeruli collected from a horse with CRF.[60] Finally, an occasional case of equine glomerulonephritis may result from true autoimmune disease, and in one instance, anti-GBM antibody was eluted from glomeruli with linear GBM immunofluorescent staining pattern isolated from a horse.[2] Little information is available about histopathologic subcategories of glomerulonephritis in horses, although several reports have attempted to make comparisons with glomerular lesions that have been better characterizedin other species.[42,58,67] No reports have attempted to correlate histologic changes with the degree of renal failure in horses. Thus assessment of the severity ofrenal disease associated with glomerulonephritis in horses currently is based more on clinical findings (e.g., body condition, magnitude of azotemia) than on histologic changes in a renal biopsy sample.

Figure 17.5-4 Spontaneous immune-mediated glomerulonephritis in a horse. **A**, An electron micrograph (×22,750) shows a red blood cell in the capillary lumen *(top)*, relatively normal fenestrated endothelial cytoplasm, thickening of the glomerular basement membrane with electron-dense immune deposits in a predominantly subepithelial location, and fusion of foot processes of the visceral epithelial cells. **B**, Immunofluorescent staining with fluorescein-tagged antiequine immunoglobulin G antibody (×160) demonstrates smooth, linear deposits of immunoglobulin G. (From Banks KL, Henson JB, McGuire TC: Immunologically mediated glomerulitis of horses. 2. Antiglomerular basement membrane antibody and other mechanisms of spontaneous disease, *Lab Invest* 26:708, 1972.)



17.5.1.2

CHRONIC INTERSTITIAL NEPHRITIS

Tubulointerstitial disease usually results from acute tubular necrosis following ischemia, endotoxemia, sepsis, or exposure to nephrotoxic compounds. Hypovolemia associated with acute blood loss, colic, diarrhea, endotoxemia, or sepsis can lead to renal hypoperfusion and ischemic damage.[68] Severe localized infection (e.g., pleuritis, peritonitis) or septicemia also may be accompanied by tubule damage. Aminoglycoside antibiotics, nonsteroidal antiinflammatory drugs (NSAIDs), vitamin D, vitamin K_3, acorns, and heavy metals such as mercury are potentially nephrotoxic.[69] Intravascular hemolysisor rhabdomyolysis also can lead to acute tubular damage following the nephrotoxic effects of hemoglobin and myoglobin (see Chapter 17.4). In horses tubulointerstitial disease culminating in CRF also can be causedby ascending urinary tract infection resulting in pyelonephritis[24,26,56,70–75] or bilateral obstructive disease caused by ureteroliths or nephroliths.[21,26,76–81] In other cases a cause of the tubule disease may not be identified.[82,83] Finally,

although this has not yet been described in horses, immune mechanisms, including anti–tubular basement membrane disease, can lead to chronic interstitial nephritis (CIN) in human beings.[84]

Chronic interstitial nephritis is defined most strictly by clinical signs of renal disease associated with histologic changes of tubular damage and an interstitial inflammatory cell infiltrate (Figure 17.5-7).
Inflammatory cells include lymphocytes, monocytes, and occasionally plasma cells. Neutrophils are 1235
uncommon; however, eosinophilic infiltrates suggest drug reactions in human patients.[84] Major glomerular 1237
and vascular lesions arenot apparent. The hallmark that distinguishes CINfrom acute tubular and interstitial disease is interstitial fibrosis.

Figure 17.5-5 Histologic changes in equine glomerulonephritis. **A**, Photomicrograph of a normal glomerulus. Bowman's capsule is lined by flattened parietal epithelial cells *(p)*. Visceral epithelial cells *(v)* are adjacent to the glomerular basement membrane, which is of uniform thickness. Mesangial cells *(m)* are surrounded entirely by glomerular capillaries (×100, periodic acid–Schiff stain). **B**, Photomicrograph demonstrates proliferative glomerulonephritis in a horse after experimental infection with equine infectious anemia virus. A combination of neutrophil infiltration *(arrows)* and mesangial cell proliferation is apparent (×160, hematoxylin-eosin stain). **C**, Photomicrograph demonstrates membranous glomerulonephritis in a horse after experimental infection with equine infectious anemia virus. The glomerular basement membranes are thickened *(A)* and mesangial areas contain periodic acid–Schiff–positive material *(B)* (×160, periodic acid–Schiff stain). (**A** from Osborne CA, Hammer RF, Stevens JB et al: The glomerulus in health and disease: a comparative review of domestic animals and man, *Adv Vet Sci Comp Med* 21:207, 1977; **B** and **C** from Banks KL, Henson JB, McGuire TC: Immunologically mediated glomerulitis of horses. 1. Pathogenesis in persistent infection by equine infectious anemia virus, *Lab Invest* 26:701, 1972.)

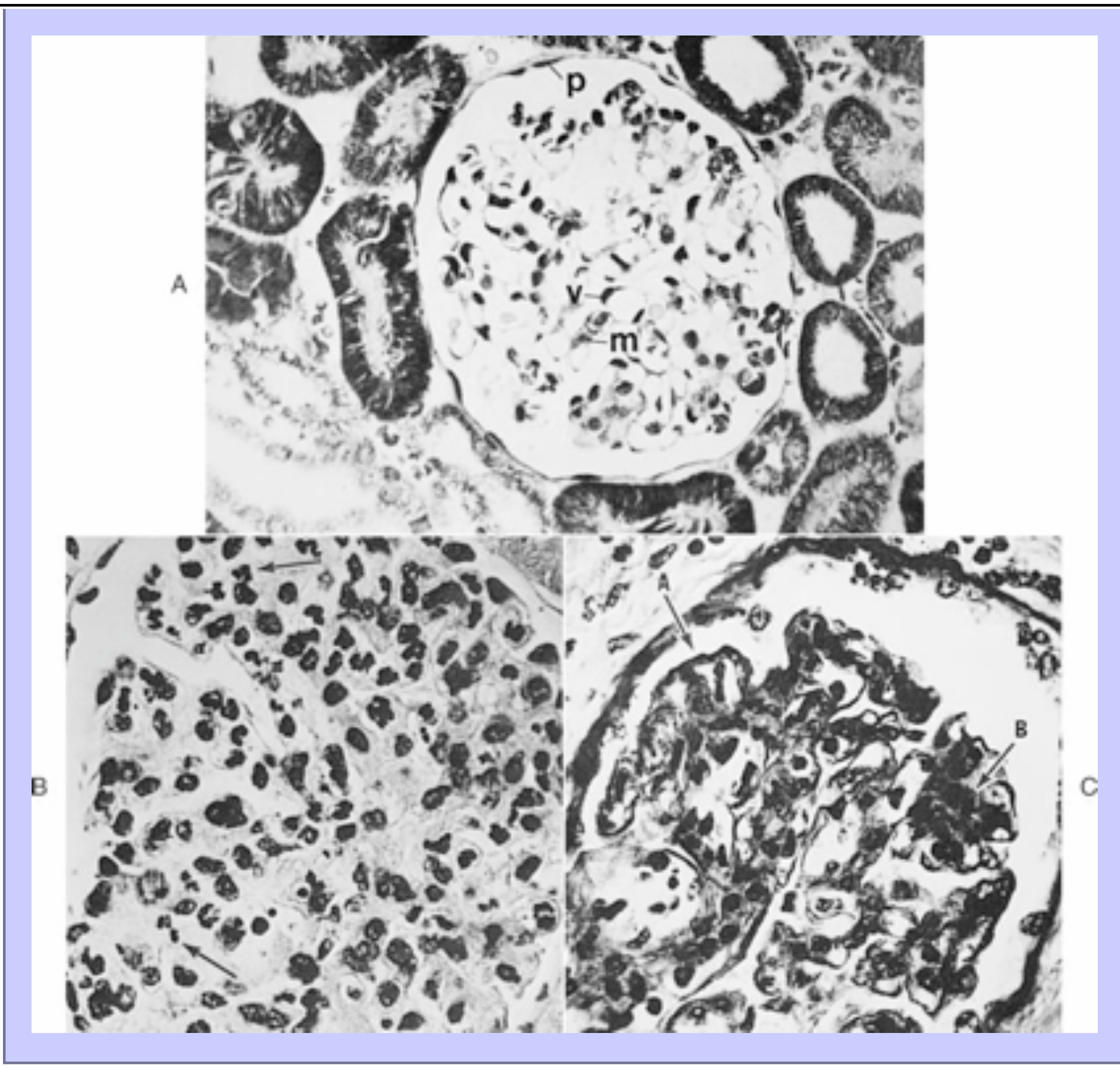

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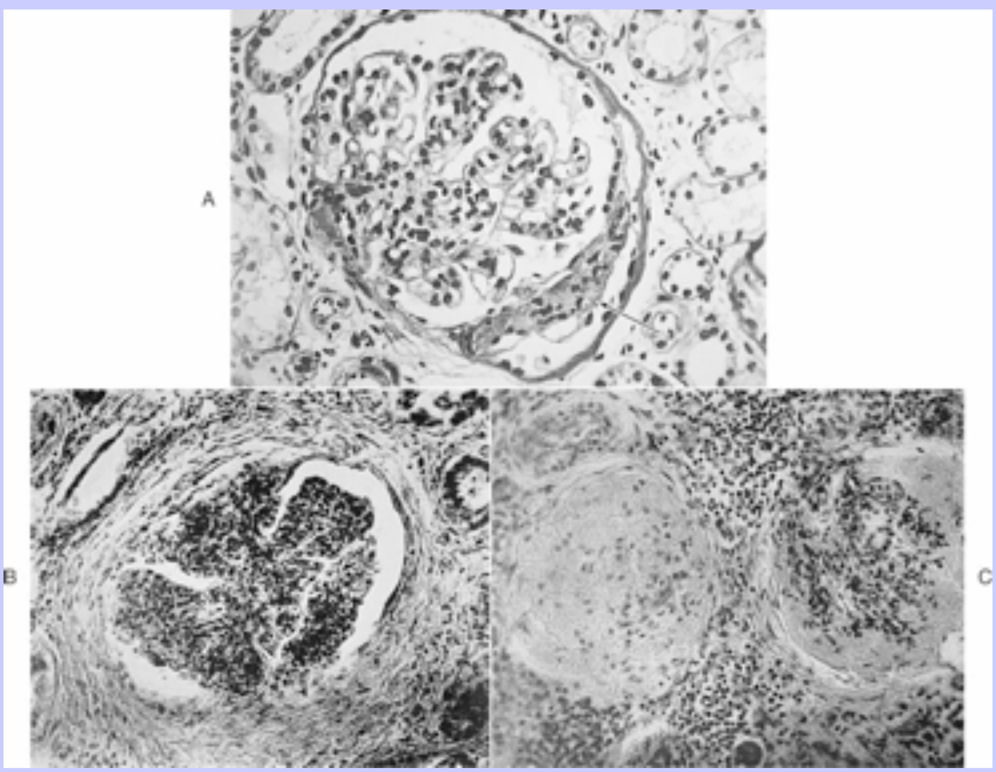
Figure 17.5-6 Progressive histologic changes in equine glomerulonephritis. **A**, Photomicrograph of a renal biopsy specimen illustrates membranoproliferative glomerulonephritis (an increase in cell numbers and thickening of the glomerular basement membrane) and parietal epithelial cell proliferation resulting in crescent formation (*arrow*, ×100, periodic acid–Schiff stain). **B**, Photomicrograph demonstrates progressive glomerulonephritis resulting in adhesion formation between the capillary tuft and proliferating parietal epithelial cells. **C**, Photomicrograph of end-stage glomerulosclerosis shows replacement of glomerular components with hyaline material (more complete in glomerulus on the left). (**A** from Osborne CA, Hammer RF, Stevens JB et al: The glomerulus in health and disease: a comparative review of domestic animals and man, *Adv Vet Sci Comp Med* 21:207, 1977; **B** and **C** from Fincher MG, Olafson P: Chronic diffuse glomerulonephritis in a horse, *Cornell Vet* 24:356, 1934.)



Although CIN has a fairly strict histologic definition, a number of disease processes can lead to tubular damage. For all practical purposes, CIN is a catchall term for extraglomerular causes of CRF in horses. As a consequence, gross findings in horses with CIN can vary dramatically. For example, analgesic nephropathy (NSAID toxicity, of which phenylbutazone has the greatest nephrotoxic potential[85]) can produce papillary necrosis[86,87] manifested by hematuria[88] in the early stages of disease, whereas chronic disease may be associated with nephrolithiasis and hydronephrosis. The area of papillary necrosis serves as a nidus for stone formation, and subsequent obstructive disease leads to hydronephrosis.[78,81] Similarly, upper urinary tract infection can lead to minor or major changes in the architecture of the kidneys and to variable histologic changes. 1237

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TABLE 17.5-1 Causes of Chronic Renal Failure in 75 Horses (Excluding Reports of Congenital Renal Failure and Experimental Induction of Chronic Renal Failure)

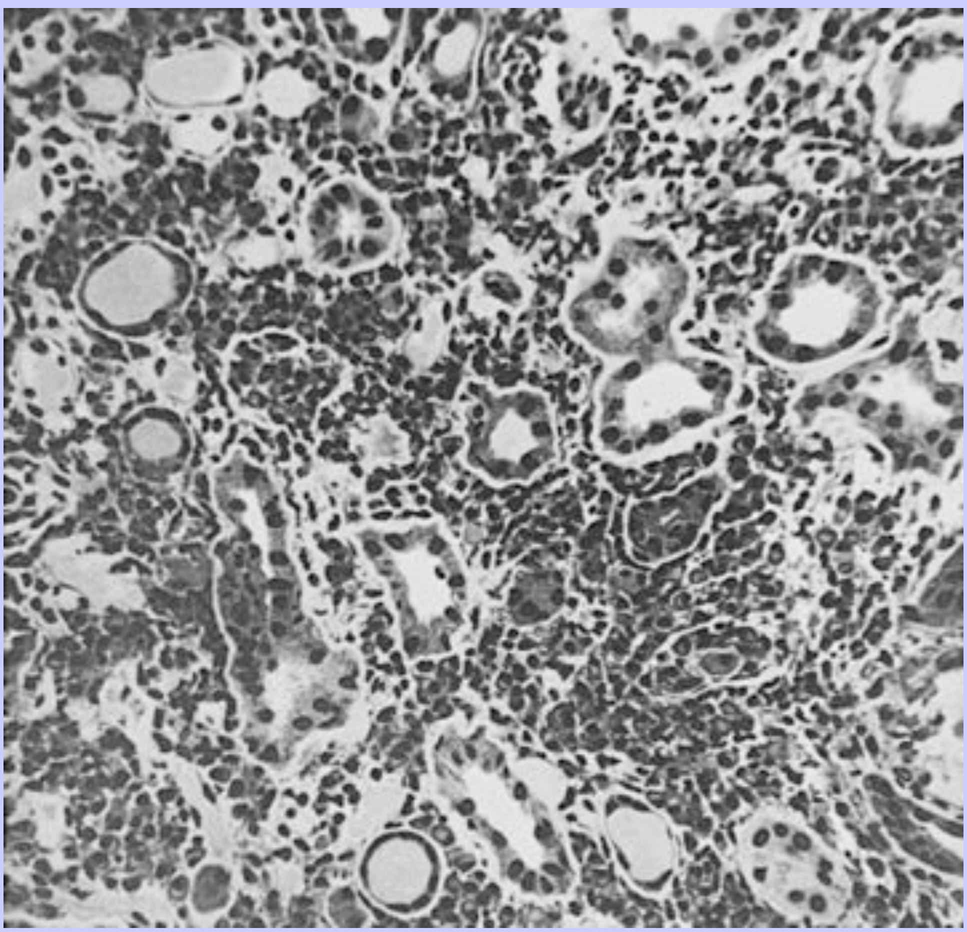
DISORDERS	NUMBER OF CASES	REFERENCES (NUMBER OF CASES)
CONGENITAL DISORDERS (15 OF 75 [20%])		
Renal agenesis/contralateral obstruction and hydronephrosis	1	10 (1)
Renal hypoplasia	4	12 (3); 23 (1)
Renal dysplasia	6	13 (1); 14 (1); 15 (2); 20 (1); 21 (1)
Polycystic kidney disease	4	17 (1); 18 (1); 19 (1); 22 (1)
ACQUIRED DISORDERS (60 OF 75 [80%])[*]		
Glomerulonephritis	32	24 (1); 25 (2); 26 (1); 38 (1); 42 (7); 43 (1); 48 (1); 49 (1); 50 (1); 51 (1); 52 (1); 53 (1); 54 (1); 55 (1); 56 (5); 57 (2); 58 (1); 59 (2); 60 (1)
Chronic interstitial nephritis	2	82 (1); 83 (1)
With obstructive nephrolithiasis and/or ureterolithiasis	11	76 (1); 77 (1); 78 (7); 79 (1); 80 (1)
With pyelonephritis	8	24 (1); 26 (1); 56 (1); 71 (1); 72 (1); 73 (1); 74 (1); 75 (1)
With papillary necrosis	2	26 (1); 86 (1)
End-stage kidney disease[†]	5	83 (1); 89 (1); 90 (1); 91 (1); 92 (1)

* In many reports of acquired renal disease leading to chronic renal failure, histopathologic changes involve glomeruli and interstitium. Categorization in this table is based on author's conclusions in these reports. When severe lesions involved glomeruli and interstitium, a categorization of end-stage kidney disease was made.

† End-stage kidney disease and reports of oxalate nephropathy.

Using CIN as a broad category for nonglomerulonephritis causes of CRF in horses, this group of tubulointerstitial diseases was responsible for 38% (23 of 60) of the previously reported cases of CRF in horses (see Table 17.5-1). Although in theory CIN would be expected to be accompanied by greater evidence of tubular dysfunction (e.g., enzymuria, glucosuria, and increased fractional clearance of sodium), abnormal measures of tubule function have not been detected regularly. Similarly, microscopic hematuria and proteinuria, the hallmarks of glomerular disease, would not be expected.

Figure 17.5-7 Histologic changes in interstitial nephritis. The interstitium contains dense infiltrates of lymphocytes and plasma cells superimposed on moderate interstitial fibrosis and tubular atrophy (×275, hematoxylin-eosin stain). (From Bennett WM, Elzinga LW, Porter GA: Tubulointerstitial disease and toxic nephropathy. In Brenner BM, Rector FC, editors: *The kidney*, ed 4, vol 2, Philadelphia, 1991, WB Saunders.)



17.5.1.3 END-STAGE KIDNEY DISEASE

End-stage kidney disease describes the severe gross and histologic changes in kidneys collected from animals in the final stages of CRF. Grossly, the kidneys typically are pale, shrunken, and firm, and they may have an irregular surface and an adherent capsule. Histologically, severe glomerulosclerosis occurs along with hyalinization and extensive interstitial fibrosis. The end-stage lesions make determining the initiating cause of renal disease virtually impossible. In several cases with a pathologic description consistent with ESKD the underlying cause of renal injury could not be established.[83,89–92]

17.5.1.4 CHRONIC RENAL FAILURE OF OTHER CAUSES

Several early cases of CRF in horses were attributed to oxalate poisoning because oxalate crystals were observed in renal tubules.[82,89] Horses appear to be more resistant than other domestic species to oxalate-induced renal damage,[93] however, and experimental administration of various forms of oxalate (in large doses) produced hypocalcemia and gastrointestinal signs rather than renal failure.[94] In fact, the early reports of oxalate nephropathy in horses failed to demonstrate that the affected horses had been exposed to oxalates. [82,89] Furthermore, long-term ingestion of plants containing oxalate produces fibrous osteodystrophy 1238
(oxalates bind calcium in the intestinal tract, decreasing intestinal calcium absorption), but renal damage in 1239
affected horses has been minimal.[95] Formation of oxalate crystals in diseased equine kidneys now is recognized as a secondary change likely related to stasis of urine in damaged renal tubules.[54]

Amyloidosis is an unusual cause of CRF in horses.[93,96] Amyloid deposits associated with systemic disease typically are composed of aggregates of the amino-terminal fragment of serum amyloid A protein, an acute phase protein.[97–100] Concentrations of serum amyloid A increase with many inflammatory disease processes, and chronic elevations can result in amyloid deposition (AA type) in a variety of tissues, a condition termed *systemic reactive amyloidosis*.[101,102] Incidence and tissue localization of amyloid exhibit considerable species variation. Dogs appears to be affected most often, but the fact that the disease is familial in Abyssinian cats implies a genetic basis for the disease.[102] Amyloid deposits accumulate as stacks of protein in a β-pleated sheet conformation and are identified in tissue samples by their extracellular location and homogeneous, eosinophilic appearance when stained with hematoxylin and eosin. They are birefringent when viewed under polarized light, and staining with alkaline Congo red solution imparts a characteristic green color.[101,102] Amyloid deposition in the kidney is most common in dogs and cattle, and renal amyloidosis is a significant cause of CRF in dogs.[102] In horses, localized amyloidosis of the upper airway or skin is more common than systemic amyloidosis.[101] In the localized form, amyloid deposits are composed of immunoglobulin light chains (AL type). Although reports describe systemic reactive amyloidosis consequent to heavy parasite infestation in horses, renal involvement was minimal or not apparent.[103,104] Systemic amyloidosis has been recognized most often in horses hyperimmunized for antiserum production, and hepatic and splenic involvement may be more common than renal involvement in these horses.[93,96,98]

A final acquired cause of CRF, renal neoplasia, is discussed in greater detail elsewhere in this chapter. Although horses with renal neoplasia may have weight loss, the tumors are usually unilateral and development of CRF is uncommon.

17.5.2 Uremic Toxins and theUremic Syndrome

Regardless of the underlying cause of nephron loss, the ensuing renal insufficiency leads to developmentof azotemia and its clinical manifestation, the uremic syndrome.[97–99,105,107] The uremic syndrome is a multisystemic disorder that develops as a result of the effects of uremic toxins on cell metabolism and function. Although the uremic syndrome originally was attributed to the effects of increased blood urea nitrogen concentration (BUN), a number of nitrogenous compounds now are known to accumulate in CRF and contribute to alterations of cell metabolism and function. In fact, the correlation between the severity of the uremic syndrome and the magnitude of azotemia is poor. For example, urea administered to human beings with normal renal function results simply in fluid shifts, osmotic diuresis, and increased thirst.[106] Urea toxicity also has been studied in nephrectomized dogs and in human patients with CRF sustained by dialysis. Increasing the urea concentrations of the dialysate to maintain BUN at artificially higher values has produced variable results. For example, BUN values up to about 150 mg/dl were associated with few clinical signs, whereas lethargy, weakness, anorexia, vomiting, and a bleeding diathesis (caused by platelet dysfunction) were producedwhen BUN was increased to values exceeding 175 to 200 mg/dl. Excess circulating urea also can degrade spontaneously to ammonia, carbonate, or cyanate. Cyanate can react with the N-terminal amino groups on a number of proteins and by altering tertiary structure can interfere with enzyme activity and structural integrity of cell membranes.[107] Thus accumulation of urea is likely responsible for some of the signs of uremic syndrome.

Creatinine and other guanidino compounds also accumulate in renal failure. These compounds arestrong organic bases that contain an amidino group(N-C=NH).[107] With CRF, urinary excretion of creatinine and other guanidino compounds actually may increase (because of tubular secretion) but not enough to prevent increases in blood concentrations. For example, the author has found that in naturally occurring cases of equine CRF (author's unpublished data), endogenous creatinine clearance, when measured simultaneously with inulin clearance, overestimates glomerular filtration rate (GFR) by 50% to 100%. These data indicate that tubular secretion of creatinine, which Finco and Groves were unable to document in healthy ponies,[108] is initiated by or is quantitatively much greater with CRF. Although metabolic pathways have not been elucidated fully, guanidino compounds appear to be produced predominantly in the liver, and their concentration in blood and tissues increases with decreased renal function or increased dietary protein intake.[106] The relationship between guanidino compounds and the syndrome of uremia is also unclear. For example, administration of methylguanidine to healthy dogs resulted in weight loss, neurologic signs, and anemia, but only when blood concentrations were an order of magnitude greater than those in spontaneous cases of CRF.[107] In contrast, administration of another guanidino compound, guanidinopropionic acid, led to hemolysis by depleting 1239
erythrocyte glutathione concentrations (supporting a role for this uremic toxin in the anemia of CRF). 1240

Products of intestinal bacterial metabolism—including secondary methylamines (from metabolism of choline and lecithin), aromatic amines (from metabolism of tyrosine and phenylalanine), polyamines (from metabolism of lysine and ornithine), and tryptophan breakdown products (indole, skatole, indoleacetic acid, and others)—can contribute to the clinical signs associated with the uremic syndrome. Some of these compounds have been studied thoroughly (e.g., the inhibitory effect of the polyamine spermine on erythropoiesis), whereas others are ill understood but likely contribute to altered neuromuscular and neurologic function.[105–107]

Another group of larger uremic toxins have been termed *middle molecules*.[106,107] These compounds are higher-molecular-weight compounds (500 to 3000 d) that are removed more readily from uremic patients by peritoneal

dialysis than by hemodialysis. These compounds are not well characterized, and their existence is supported more by the difference in clinical response to peritoneal dialysis and hemodialysis in uremic human patients awaiting renal transplantation.

In addition to nitrogenous compounds, abnormal metabolism of hormones and trace minerals also accompanies the decline in renal function associated with acute and chronic renal failure. Secondary hyperparathyroidism (leading to osteodystrophy) and insulin insensitivity are well-recognized endocrine contributions tothe uremic syndrome in human and small animal patients.[106,107,109,110] Endocrine dysfunction can be attributed to several factors: (1) decreased productionof renal hormones (erythropoietin, vitamin D_3); (2) decreased hormone clearance prolonging plasma half-life (parathormone, gastrin); (3) decreased hormone production (testosterone); (4) tissue insensitivity (insulin, parathormone); and (5) hypersecretion to reestablish homeostasis (parathormone).[107] Regarding trace minerals, uremia may be accompanied by aluminum toxicity and zinc deficiency. Aluminum may contribute to some of the neurologic signs associated with azotemia (especially with acute renal failure), whereas zinc has been implicated in testicular atrophy and abnormal taste in uremic human patients.[106,107]

Clearly, as so many potential uremic toxins exert their effects as renal function declines, a single compound or even a few compounds likely will not ever be identified as the primary cause of the uremic syndrome in patients with CRF. Furthermore, these compounds together are more likely to impair basic cell function in a number of tissues, and the signs of uremia are more likely to reflect multisystemic organ dysfunction. Methylation of a number of membrane proteins recently has received considerable attention as one of the common mechanisms of cell dysfunction.[111] What is truly remarkable, however, is the tremendous adaptive capacity of the failing kidneys to maintain sodium and water balance within narrow ranges until GFR declines to 20% of the normal value or less.[112]

17.5.3 Clinical Signs

Horses with CRF present relatively late in the disease course, when their owners note lethargy, anorexia, and weight loss. A history of months to years of polydipsia and polyuria in some cases supports renal disease of long duration. In other animals, preexisting disease (colic, colitis, pleuropneumonia) or prolonged medication (aminoglycoside antibiotics or NSAIDs) may provide important information about the initiation and duration of renal failure. In most cases, however, the onset is insidious and identifying a precipitating event or gauging the duration of renal disease is not possible.

Chronic weight loss is the most common presenting complaint for horses with CRF.[24–30] Partial anorexia, ventral edema, polydipsia and polyuria, rough hair coat, lethargy, and poor athletic performance are other owner concerns. In addition, horses with advanced CRF may have a characteristic odor that likely reflects the combined effects of uremic halitosis and increased urea excretion in sweat. For the horses presented in Table 17.5-1, weight loss, ventral and peripheral edema, and polydipsia and polyuria, respectively, were reported in 53 of 63 (84%), 24 of 56 (43%), and 21 of 48 (44%) of the cases. Lethargy and weight loss can be attributed to several factors. An increase in the concentration of nitrogenous wastes in blood can have a direct central appetite-suppressant effect that can lead to partial or complete anorexia.[106,107] Next, as azotemia develops, excess urea diffuses across gastrointestinal epithelium and is metabolized to ammonia and carbon dioxide by bacterial urease. In the oral cavity, excess ammonia can lead to excessive dental tartar formation (Figure 17.5-8), gingivitis, and oral ulcers. In the gastrointestinal tract, excess urea and ammonia can lead to ulceration and mild to moderate protein-losing enteropathy, and severely uremic animals may produce soft feces.[1,106,107]

The prolonged half-life of gastrin (eliminated through the kidneys) may contribute further to ulcer formation because of increased gastric acid secretion.[107] Finally, as the combined effects of uremic toxins render the affected patient catabolic, body mass declines as body reserves are tapped to meet basal energy requirements.[106,107]

Mild ventral edema with CRF may be attributable to three factors: decreased oncotic pressure, increased vascular permeability, and increased hydrostatic pressure. Because albumin accounts for approximately 75% of plasma colloid oncotic pressure, decreases in albumin concentration (less than about 2.0 g/dl) can decrease 1240
plasma oncotic pressure despite a normal total plasma protein concentration.[113,114] The effects of uremic toxins 1241
on endothelial cell membranes can alter vascular permeability, which contributes to edema.[106,107] Chronic renal insufficiency can lead to renal hypoxia and hypoperfusion, which stimulate renal juxtaglomerular cells to release renin. Activation of the renin-angiotensin system tends to elevate blood (and capillary hydrostatic) pressure and contributes to edema. Activation of the renin-angiotensin system also leads to increased sodium reabsorption in the proximal (direct effect of angiotensin II) and distal (effect of aldosterone) tubules.[114] Sodium retention leads to expansion of circulating volume, which is another factor in edema formation. Alterations in blood pressure in horses with CRF have not been evaluated routinely as they have in small animal and human patients, nor have increased circulating concentrations of angiotensin II or aldosterone been documented. As a result the nephrotic syndrome (characterized by edema, hypoalbuminemia, and heavy proteinuria) is not as well documented in horses as in small animals and human beings with CRF. However, horses with CRF appear less at risk for the significant pleural effusion or ascites that can accompany the nephrotic syndrome in small animals.[1]

Figure 17.5-8 Excessive dental tartar on the canine tooth and lower corner incisor of a horse with chronic renal failure.

Polydipsia and polyuria are variable findings in horses with CRF. The magnitude of polydipsia and polyuria theoretically is related to the degree of tubulointerstitial damage; however, the degree of polyuria does not appear to correlate with the magnitude of azotemia in clinical cases. Typically, polyuria is not as severe with CRF as with diabetes insipidus or psychogenic water drinking and may not be noticed by an owner (see Chapter 17.9). The wide variation in water intake in normal horsesand common use of automatic waterers and large stock tanks also make observation of polydipsia more difficult. The mechanisms of polyuria with CRF can include(1) increased tubular flow rate in surviving nephrons,(2) decreased medullary hypertonicity, and (3) impaired response of collecting ducts to vasopressin (acquired nephrogenic diabetes insipidus). Although these mechanisms may contribute to the polyuria of CRF, which of them may be most important is not known.[1,115]

An early complaint for horses with CRF is poor performance, which may be related to mild anemia (packed cell volume 25% to 30%) and lethargy. Although the anemia of CRF has been attributed to several factors—including blood loss, decreased erythrocyte survival time, nutritional deficiencies, and decreased erythropoeitin activity—the latter clearly has emerged as the principal cause of anemia in human beings and small animals with CRF.[1,116,117] In fact, administration of recombinant human erythropoietin (rhEPO) to patients awaiting kidney transplant has been one of the most significant advances in management of human CRF because it has eliminated the need for blood transfusions, has improved exercise capacity, and has decreased morbidity associated with the uremic syndrome.[116,117] Administration of rhEPO has also benefited anemic dogs and cats with CRF, although both species may neediron supplementation to support erythropoiesis.[1,116] The response often has been only temporary because many small animal patients develop refractory anemia following production of anti-rhEPO antibodies within a few weeks to months after initiation of treatment.[1] Plasma concentrations of erythropoeitin have been determined in normal horses,[118] and rhEPO administration (15 IU/kg intravenously, three doses per week for 3 weeks) has been reported to increase the hematocrit in splenectomized horses[119]; however, no reports describe rhEPO administration to horses with CRF. Furthermore, moderate to severe anemia has been reported anecdotally to develop after repeated rhEPO administration to racehorses,[120,121] suggesting that anti-rhEPO antibodies also would limit the effectiveness of the treatment for CRF in horses.

17.5.4 Diagnosis and Laboratory Evaluation

One establishes a diagnosis of CRF when persistent isosthenuria (specific gravity of 1.008 to 1.014) accompanies azotemia and typical clinical signs.[25–30,122] Rectal palpation of the left kidney may be normal or may reveal a kidney that is small or firm with an irregular surface. Less often, the kidneys and ureters may be enlarged if obstructed by uroliths or if infection or neoplasia is present. The ratio of BUN to serum creatinine
concentration (BUN/Cr ratio) in horses with CRF is typically greater than 10:1 (Table 17.5-2); that for horses 1241
with prerenal azotemia or acute renal failure is often less than 10:1.[26] This difference can be attributed in part to 1242
different volumes of distribution for urea (all body fluids) and creatinine (primarily the extracellular fluid space). As a consequence, with acute reductions in renal blood flow the increase in serum creatinine concentration is usually greater, on a relative or percentage basis, than the increase in BUN. The point deserves emphasis, however, that BUN is influenced by dietary protein intake so that BUN/Cr values do not always distinguish acute renal failure from CRF. In fact, one also can use the BUN/Cr ratio to assess adequacy of dietary protein intake in the management of horses with CRF because a value greater than 15:1 can reflect excessive protein intake.[28]

TABLE 17.5-2 Abnormal Laboratory Values Reported for Horses With Chronic Renal Failure

PARAMETER	NUMBER[*]	PERCENT
BUN/Cr >10	29/34	85
BUN/Cr >15	17/34	50
Anemia (packed cell volume <30%)	12/30	40
Hypoalbuminemia (<2.5 g/dl)	12/14	86
Hypoalbuminemia (<2.0 g/dl)	7/14	50
Hyponatremia (Na^+ <135 mEq/L)	26/40	65
Hyperkalemia (K^+ >5.0 mEq/L)	23/41	56
Hypochloremia (Cl^- <95 mEq/L)	19/41	46
Hypercalcemia (Ca^{2+} >13.5 mg/dl)	26/39	67
Hypophosphatemia (P < 1.5 mg/dl)	17/36	47
Acidosis (pH <7.35)	3/5	60

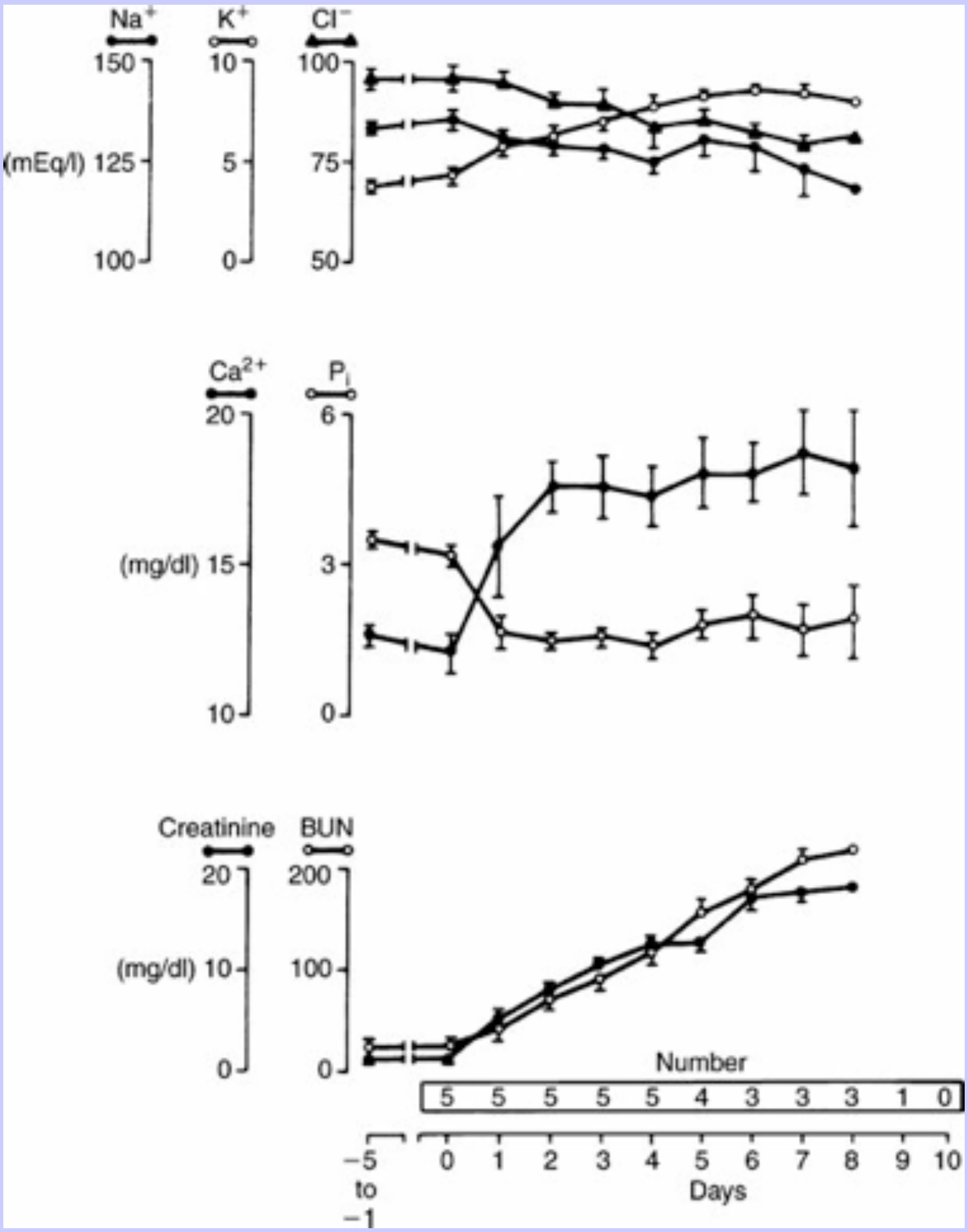
* Number of reports with this laboratory finding/total number of reports in which this laboratory parameter was reported.

In addition to azotemia, further abnormal laboratory data accompanying CRF can include mild anemia, hypoalbuminemia, hyponatremia, hyperkalemia, hypochloremia, hypercalcemia, hypophosphatemia, and metabolic acidosis (see Table 17.5-2). A nonregenerative anemia is related largely to a deficient supply of the renally secreted glycoprotein erythropoietin; however, reduced erythrocyte life span may be another significant contributor to anemia. Normally, equine erythrocytes have a life span of 140 to 155 days.[121] With uremia, erythrocyte life span is shortened because excessive nitrogenous waste products alter the integrity of erythrocyte membranes and the function of ion channels, which regulate erythrocyte volume.[108] These less resilient erythrocytes are more likely to be removed from the circulation by the reticuloendothelial system.

The electrolyte alterations associated with CRF reflect loss of tubule function. Because sodium, chloride, bicarbonate, and phosphate are conserved by renal tubules, excessive urinary loss of these electrolytes accompanies CRF. Although fractional electrolyte clearance values (see Chapter 17.3) may remain within normal ranges or increase only slightly in horses with CRF, significant daily urinary loss of electrolytes still may occur. As an example, if a horse with CRF and serum creatinine and sodium concentrations of 5.0 mg/dl and 130 mEq/L, respectively, is producing 20 L of urine daily with respective creatinine and sodium concentrations of 50 mg/dl and 12.5 mEq/L, the fractional clearance of sodiumis 1.0% and daily urinary sodium loss 250 mEq. An increase in urinary sodium concentration to 25 mEq/L following another decrease in tubular reabsorption would result in an increase in fractional sodium clearance to 2% but would represent an additional 250 mEq of daily sodium loss in the urine. The latter value would approach 1% to 2% of the exchangeable sodium in the body and would require an additional 15 g of salt intake daily to accommodate this loss. Hypercalcemia and hypophosphatemia (Williams-Smith syndrome) are fairly common findings in horses with CRF (see Table 17.5-2), and the degree of hypercalcemia appears to vary with the amount of calcium in the

diet.[24,53,56,123] In human beings with ESKD, hypercalcemia is an occasional finding attributed to hyperparathyroidism, vitamin D supplementation, or use of calcium-containing dialysate solutions. Furthermore, the osteodystrophy of CRF is associated with aluminum deposition in bone, which has been speculated to reduce skeletal buffering capacity for increases in serum calcium concentration and thereby contributes to hypercalcemia.[124] Because the equine kidney is an important route of calcium excretion (via calcium carbonate crystals), impaired tubular function in the face of continued intestinal absorption is the most common explanation for calcium accumulation in blood. This explanation is supported by the rapid development of hypercalcemia after experimental bilateral nephrectomy in ponies fed alfalfa hay (Figure 17.5-9).[9] In addition, parathormone concentrations have been found to be below the lower limit of detection in horses with CRF, indicating that hyperparathyroidism does not play a role in development of hypercalcemia.[22,83] However, parathormone clearance by the kidney also is reduced with CRF and may be associated with a change in the regulatory set-point for serum calcium concentration in uremic human beings.[124] Whether hypercalcemia in horses with CRF leads to exacerbation of renal disease or tissue mineralization remains unknown. Nevertheless, one can demonstrate the effect of dietary calcium by changing the type of hay fed to horses with CRF.Horses with serum calcium concentrations exceeding20 mg/dl on a diet of mostly alfalfa can have theirserum calcium concentrations returned to the normal range within a few of days after changing the diet to grass hay.[19,26] Similarly, nephrectomized ponies fedgrass hay did not become hypercalcemic.[26] The 1242
1243
cause of hypophosphatemia in horses with CRF also remains undocumented, although it has been explained by the law of mass action, which leads to a decrease in serum phosphate concentration in association with hypercalcemia.[123,124] A similar response is not observable in horses that eat *Cestrum diurnum* (leading to a syndrome of hypervitaminosis D): they develop hypercalcemia without hypophosphatemia.[125] Another possibility that has been suggested is that hypophosphatemia may result from long-standing anorexia associated with CRF.[83] Regardless of the cause, clinical problems associated with hypophosphatemia have not yet been recognized in horses with CRF.

Figure 17.5-9 Changes in serum electrolyte concentrations, blood urea nitrogen concentration *(BUN)*, and serum creatinine concentration following bilateral nephrectomy in Shetland ponies. (From Tennant B, Lowe JE, Tasker JB: Hypercalcemia and hypophosphatemia in ponies following bilateral nephrectomy, *Proc Soc Exp Biol Med* 167:365, 1981.)



A degree of metabolic acidosis accompanies CRF in human beings and small animals and is attributed to decreased ability of failing kidneys to excrete hydrogen ions and regenerate bicarbonate.[1,107] Normally, acid-base balance is maintained by reabsorption of filtered bicarbonate and excretion of hydrogen ions along with ammonia and phosphate. As renal function declines in the early stages of renal failure, hydrogen ion excretion via renal ammoniagenesis and ammonium excretion increases. As renal failure progresses, compromised renal ammoniagenesis and decreased medullary recycling of ammonia caused by structural renal damage likely contribute to impaired ammonium excretion. Because hepatic glutamine synthesis is required for renal ammoniagenesis, the earlier increase in ammonium excretion may contribute to protein malnutrition in patients 1243
with CRF.[1] Metabolic acidosis also contributes to a number of the clinical signs of the uremic syndrome and 1244
may exacerbate some of the electrolyte alterations (e.g., hyperkalemia) of CRF. Metabolic acidosis has been reported in a limited number of horses with CRF (see Table 17.5-2); however, in the author's experience and that of others,[25,83] most horses with CRF have normal acid-base status or are alkalotic until the terminal stages of the disease, when metabolic acidosis typically develops. Metabolic alkalosis has been attributed to enhanced bicarbonate reabsorption and production, in association with hypochloremia and increased renal ammoniagenesis, respectively. In some instances, hypochloremic metabolic alkalosis may be accompanied by paradoxical aciduria.[83] The mechanism of paradoxical aciduria is likely similar to that of hypochloremic metabolic alkalosis in ruminants with abomasal outflow obstruction.[126] Briefly, after all chloride has been reabsorbed from the glomerular filtrate, further sodium reabsorption occurs by exchange with (excretion of) potassium or hydrogen ions. Thus paradoxical aciduria is most likely to occur with concomitant hypokalemia or whole-body potassium depletion.

Horses with CRF also can develop hypercholesterolemia and hyperlipidemia (hypertriglyceridemia), and occasionally an animal has grossly lipemic plasma (hyperlipemia).[26,28,122] In fact, Naylor, Kronfeld, and Acland reported a positive correlation between serum triglyceride and creatinine concentrations in a group of azotemic horses.[127] With azotemia, hyperlipidemia can develop as a result of increased synthesis, decreased degradation, or increased mobilization of triglycerides from fat stores.[128] Decreased lipoprotein lipase activity has received the most attention in horses, likely because heparin treatment(40 IU/kg subcutaneously every 8 hours) has been recommended to stimulate lipoprotein lipase in an attempt to clear the serum.[28,129] Hypercholesterolemia and hyperlip-idemia increase the risk of atherosclerotic cardiovascular disease in human beings with CRF.[128] Furthermore, these conditions stimulate mesangial cell proliferation and matrix production in diseased glomeruli and thus accelerate progression to glomerulosclerosis.[130]

The failing kidneys have a tremendous adaptive capacity to maintain tubular function until GFR is low.[112] In the author's experience, tubular dysfunction resulting in significant sodium or phosphorous wasting (manifested by increased fractional clearance values), glucosuria, or enzymuria is more common with acute renal failure than with CRF. When present, however, abnormal tubule function rarely leads to values for fractional sodium clearance in excess of 5%.[79,92,131] In contrast, loss of concentrating ability resulting in isosthenuria is a consistent feature of CRF that usually develops before azotemia. The associated polyuria may or may not be observed by the client, but the horse usually maintains water balance well through polydipsia. Polyuria typically results in urine that is clear and essentially devoid of crystals and mucus. Sediment examination is generally unremarkable, but increased numbers of red or white blood cells may occur with nephrolithiasis or ureterolithiasis or with pyelonephritis, respectively. Gross hematuria further supports lithiasis or neoplasia. One should include a quantitative urine culture in the minimum database of all horses with CRF because bacteriuria may not always be accompanied by pyuria.

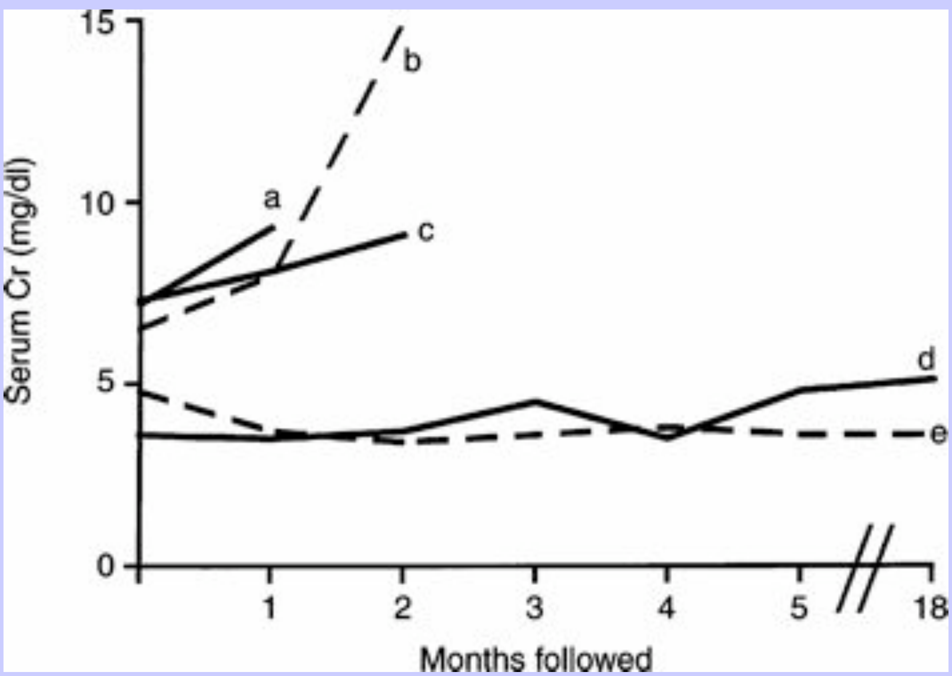
Alterations in the integrity of the highly anionic glomerular filtration barrier also can lead to loss of protein, predominantly albumin, in the urine. Few quantitative data are available on urinary protein loss in horses with CRF because in most previous reports proteinuria was assessed using urine reagent strips. Reagent strip results of +++ or ++++ correlate with protein concentrations of 100 to 300 and 1000 to 2000 mg/dl, respectively, depending on which reagent strip one uses. In a proteinuric horse that is producing 20 L of urine daily, these values would yield a wide range of urinary protein loss: 20 to 400 g daily. The latter value would approach 25% of the total protein content of plasma and so is not realistic. In human beings with CRF, urinary protein loss exceeding 3.5 g per day (50 mg/kg per day for a 70-kg person) is classified as nephrotic-range proteinuria, and some patients with heavy proteinuria may lose in excess of 15 g per day (more than 200 mg/kg per day).[132] The upper limit of acceptable urinary protein excretion in dogs is 20 mg/kg per day.[133] Using these values, estimates for the upper acceptable limits for urinary protein loss and nephrotic-range proteinuria in a 500-kg horse would be 10 g and in excess of 25 g per day, respectively. These values agree well with a mean value of 3.2 mg/kg (1.6 g per day) and a range of 3.6 to 22.3 mg/kg (1.8 to 11.2 g) per day in normal mares reported by the Schott, Hodgson, and Bayly[134] and by Kohn and Strasser,[135] respectively. Another method to document proteinuria is to determine the ratio of urinary protein to urinary creatinine (in milligrams per deciliter). This technique is more practical because it avoids a timed urine collection period. Although a normal range has not yet been reported for horses, values in excess of 1.0:1 and 3.5:1 are considered above normal and indicate nephrotic-range proteinuria in dogs[133] and human beings,[132] respectively. Thus a urine protein/creatinine ratio greater than 2.0:1 likely supports significant proteinuria in a horse with CRF. Finally, a horse with CRF and heavy proteinuria (more than 200 mg/kg per day) could excrete as much as 100 g of protein daily (5% to 7% of total plasma protein). Proteinuria of this magnitude can increase urine specific gravity to 1.020 or greater and certainly would be great enough to lead to a decline in serum albumin (and total protein) concentration, despite 1244
increased hepatic albumin production. In some horses with CRF and a normal total plasma protein 1245
concentration, increased globulin concentration offsets mild hypoalbuminemia, whereas in other cases hyperglobulinemia actually can result in an increase in total plasma protein concentration.

One can use ultrasonography to determine kidney size in horses with CRF and to evaluate for the presence of cysts or nephroliths. Horses with ESKD typically have small kidneys that are more echogenic than normal (becauseof sclerosis and possible tissue mineralization).[136–140] Cystoscopy also can be a useful diagnostic aid to assess urine production qualitatively in each kidney and is particularly useful when ultrasonographic imaging fails to identify a kidney.[138] Renal scintigraphy is another imaging option to detect functional renal tissue that may not be apparent by ultrasonography.[141] One can perform renal biopsy using ultrasonographic guidance to document renal disease. Unfortunately, because most horses are presented for evaluation in the later stages of disease, biopsy results typically reveal glomerular, tubular, and interstitial lesions consistent with ESKD. Rarely do the lesions provide information about the inciting cause of the renal disease unless immunofluorescence testing and electron microscopy are pursued. These require placing samples in special fixatives, in addition to the sample placed in formalin for routine histopathologic examination. In some cases, renal biopsy results supporting pyelonephritis or a congenital anomaly (dysplasia) as the cause of CRF are useful in developing a therapeutic plan or providing a prognosis.

One can assess the severity of CRF in affected horses using available sophisticated techniques. The magnitude of azotemia is the most readily available and practical parameter but is an insensitive one.[132] Azotemia becomes apparent only after 75% or more of renal function is lost. Furthermore, the degree of azotemia can vary with nonrenal factors such as diet, body mass, and hydration. In general, creatinine concentration is a more reliable measure than BUN, and doubling of creatinine roughly correlates to a 50% decline in GFR (see Figure 17.3-1). Serum creatinine concentrations in the range of 5 to 10 mg/dl indicate a significant decline in renal

function, and values exceeding 15 mg/dl are consistent with a grave prognosis. In contrast, horses with a creatinine concentration below 5 mg/dl may exhibit few clinical signs and can be managed for months or years (Figure 17.5-10). Plotting the inverse of serum creatinine concentration (1/Cr) over time also has been used to monitor progression of CRF in human beings[142,143] and in one horse[19] in an attempt to predict the end point of the disease process (Figure 17.5-11). Unfortunately, these plots are subject to considerable variation (because of changes in tubular secretion of creatinine) and have not proved to be of any more value than monitoring creatinine over time.[142,143]

Figure 17.5-10 Serum creatinine concentrations *(Cr)* in five horses with chronic renal failure. The three horses with an initial creatinine concentration greater than 5 mg/dl (*a* to *c*) had rapid progression of renal disease over a 1- to 2-month period, necessitating euthanasia, whereas the two horses with an initial creatinine concentration less than 5 mg/dl (*d* and *e*) were maintained with supportive care for longer than 18 months.



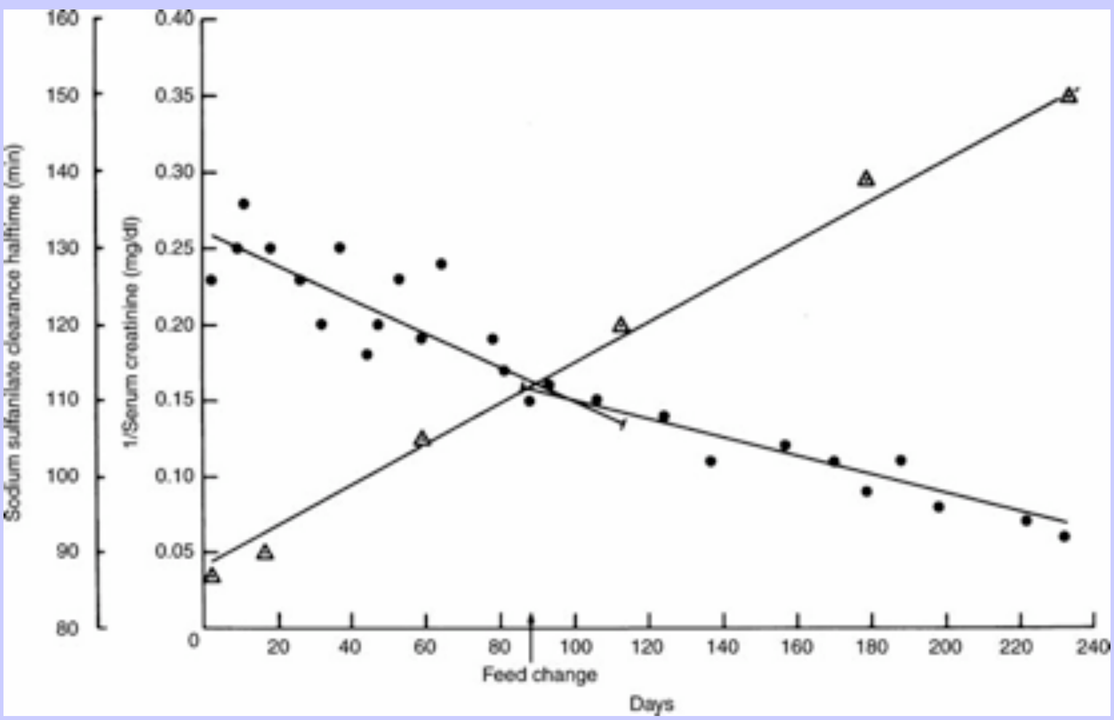
Measurement of GFR provides the most accurate quantitative assessment of renal function but is pursued rarely because it is more time consuming and technically demanding than measurement of serum creatinine concentration. Although a number of methods are available to measure GFR,[144] measurement of endogenous creatinine clearance (Cl_{Cr}) or the plasma disappearance of exogenous creatinine, sulfanilate,[19,145] or 99-metastable technetium tagged to diethylenetriaminepentaacetic acid[146] are the most practical methods in a clinical setting. The former requires timed urine collections, whereas the latter may require special assays or

nuclear medicine capabilities (see Chapter 17.3). Horses in the earlier stages of CRF can develop tubular creatinine secretion, which could lead to overestimation of GFR measured by the Cl_{Cr} technique. As renal disease progresses, however, creatinine excretion decreases faster than GFR declines because of loss of compensatory secretion. Despite these limitations, repeated measurement of Cl_{Cr} in a single animal can be useful for monitoring progression of CRF over time (Figure 17.5-12).

17.5.5 Disease Progression

One of the hallmarks of CRF is the progressive natureof the disease,[147–149] and progression appears to be largely independent of the inciting cause because of the final common pathways of renal injury. The response to a decrease in renal functional mass is a compensatory increase in filtration (termed *single-nephron GFR*) and 1245
tubular function (e.g., creatinine secretion, ammoniagenesis) by remaining nephrons. The increase in single- 1246
nephron GFR results from increased glomerular capillary blood flow and hydrostatic pressure leading to glomerular hyperfiltration. Hyperfiltration is associated with increased permeability of the GBM and proteinuria. Furthermore, increased filtration of macromolecules leads to activation and proliferation of mesangial and epithelial cells and eventually progression to glomerulosclerosis.[113,147–150] In addition to causing proteinuria, increased filtration of protein is accompanied by increased proximal tubular protein reabsorption. The latter leads to upregulation of genes encoding vasoactive and inflammatory mediators by tubular and interstitial cells and contributes substantially to injury of the renal interstitium.[149]

Figure 17.5-11 The inverse of the serum creatinine concentration *(filled circles)* and the whole-blood sodium sulfanilate clearance half-time in a horse with chronic renal failure associated with polycystic kidney disease. The slope of the line describing the reciprocals of serum creatinine concentration from day 1 to day 98 was significantly different (*P* <0.05) from that from day 99 to 235 and the slope change was associated with a change from alfalfa to grass hay. (From Bertone JJ, Traub-Dargatz JL, Fettman MJ et al: Monitoring the progression of renal failure in a horse with polycystic kidney disease: use of the reciprocal of serum creatinine concentration and sodium sulfanilate clearance half-time, *J Am Vet Med Assoc* 191:565, 1987.)

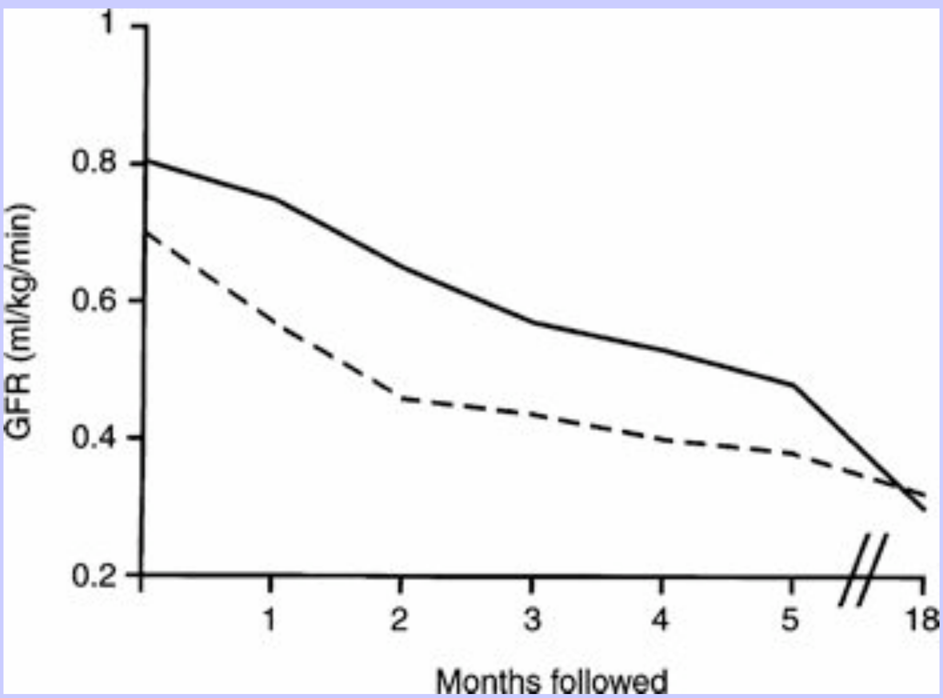


A number of studies investigating the mechanismsof glomerular hypertension have demonstrated that activation of the renin-angiotensin system and angiotensin II production are of considerable importance, for angiotensin II is a potent constrictor of glomerular efferent arterioles.[151–153] Activation of the intrarenal renin-angiotensin system can produce significant glomerular hypertension without producing increases in systemic angiotensin II concentration or blood pressure. In fact, administration of specific angiotensin II receptor antagonists or angiotensin-converting enzyme inhibitors has been demonstrated to decrease glomerular capillary hydrostatic

pressure and the magnitude of proteinuria in experimental studies of renal disease.[151–154] Angiotensin-converting enzyme inhibitors have helped to control hypertension and proteinuria in small animals,[1,155] but no reports describe use of angiotensin-converting enzyme inhibitors in horses with CRF.

The role of dietary protein in the progression of CRF has been the subject of a number of investigations in human beings and small animals with a variety of renal diseases.[156–161] A well-established fact is that one can ameliorate clinical signs of uremia by decreasing dietary protein content; however, whether decreasing dietary 1246
protein slows progression of renal disease remains controversial. Increased dietary protein has been 1247
demonstrated to increase activation of the renin-angiotensin system; therefore decreasing dietary protein could be protective for the reasons discussed in the preceding paragraph. Next, a decrease in protein intake leads to production of smaller amounts of urea and other nitrogenous wastes. In theory, the workload on the kidneys is similarly decreased. Because all work (aerobic adenosine triphosphate production and use) is associated with a degree of free radical production, greater protein intake would generate more free radicals. Free radicals appear to be especially injurious to diseased kidneys because their scavenging mechanisms are compromised. Increased dietary protein also requires increased ammoniagenesis by renal tubules to excrete the associated proton load; however, ammoniagenesis can lead to noninflammatory, nonimmune complement activation in proximal tubule epithelial cells. One can ameliorate this proinflammatory consequence of ammoniagenesis by supplementation with sodium bicarbonate. In summary, excessive dietary protein leads to a greater degree of uremia and has the potential to exacerbate renal damage in CRF by several mechanisms.

Figure 17.5-12 Glomerular filtration rate *(GFR)* as measured by endogenous creatinine clearance *(solid line)* and inulin clearance *(dashed line)* in a mare (see horse *e* in Figure 17.5-10) with chronic renal failure. Glomerular filtration rate declined steadily over the 18-month period despite minimal change in serum creatinine concentration. Compared with inulin clearance, endogenous creatinine clearance overestimated glomerular filtration rate until the terminal stage of the disease process. This difference likely reflects tubule secretion of creatinine in the earlier stages of chronic renal failure, which subsequently diminished during the final weeks of the disease course.



In contrast, protein-calorie malnutrition is associated strongly with increased morbidity and mortality in human CRF patients and can be attributed to increased protein catabolism when dietary protein intake is marginal to low. In human beings a number of serum components, including prealbumin, cholesterol, and insulin-like growth factor; plasma and muscle amino acid profiles; and body composition measurements have been studied as potential indexes of nutritional status; however, serum albumin concentration is the most practical and most extensively studied nutritional index. In addition to heavy proteinuria, hypoalbuminemia also can be attributed to protein-calorie malnutrition. Furthermore, a BUN/Cr ratio less than 10:1 may be another indication of inadequate protein intake. Thus current dietary recommendations call for dietary protein and caloric intake at levels that meet or slightly exceed predicted requirements, which should render nitrogen balance neutral.[162]

Finally, a critically important but often overlooked aspect of nutritional management of CRF patients is provision of a palatable diet.[1,162] Feeding smaller meals more frequently and varying the diet help to increase food intake in CRF patients of all species.

Experimental studies of CRF in horses are limited to the reports by Roberts and Seawright and colleagues that describe the effects of prolonged daily dosing of horses with mercurial compounds.[163–166] In these four reports, each involving a single horse, the subjects were destroyed between 85 and 191 days after mercury administration began. Anorexia, weight loss, and nonpruritic urticaria were the major clinical signs. The renal lesion was CIN, which was accompanied by a decrease in concentrating ability and an increase in water consumption. Additional tubular dysfunction included glucosuria in all four horses and a variable degree of proteinuria. Interestingly, the clinical signs and tubular dysfunction preceded the development of azotemia in all horses, and excretion of sodium or chloride did not increase. The final report focused principally on the effects on renal function, and azotemia did not develop until the final week of life, and GFR (assessed by endogenous Cl_{Cr}) decreased to 35% of the highest measured value onthe day before euthanasia.[166] Aciduria and increased phosphate excretion, in addition to glucosuria and proteinuria, also were detected during the final week of life.

17.5.6 Treatment

At the time of presentation most horses with CRF exhibit obvious weight loss and other clinical signs. Because of the progressive and irreversible nature of the renal disease, the long-term prognosis is grave. Specific corrective treatment for CRF (renal transplantation) is not available for horses, and maintenance by peritoneal dialysis or hemodialysis would only be practical for valuable breeding animals. Pyelonephritis could be considered an exception because antibiotic treatment in theory could lead to resolution of infection and 1247
improved renal function. Unfortunately, significant renal damage usually has occurred by the time the diagnosis 1248
is established in most cases of bilateral pyelonephritis, so that the prognosis for a return to normal renal function is guarded to poor. In contrast, the short-term prognosis may be more favorable. Some horses with CRF may maintain serum creatinine concentration of less than 5 mg/dl for months with minimal deterioration. To predict which patients will deteriorate more rapidly is difficult, but the recent history and initial ability to counteract weight loss with improved management are useful indicators. Laboratory analysis of blood samples at 2- to 4-week intervals to follow the degree of azotemia and serum electrolyte alterations may be useful in monitoring disease progression. In general, animals that are eating well and maintain reasonable body condition carry the best short-term prognosis and may still be able to perform some limited work. Their use as breeding animals may be reduced because azotemia and eventual weight loss would reduce the chance for normal conception and gestation. The goal in each case is provide appropriate supportive care and to monitor the horse closely so as to provide humane euthanasia before the patient develops uremic decompensation.

As previously discussed, once significant renal disease is established, irreversible decline in GFR and progression of renal failure generally ensue.[147–150] Thus management of the equine patient afflicted with CRF involves palliative efforts to minimize further loss of renal function. The goals are to prevent complicating conditions (e.g., by providing plenty of water), to discontinue administration of nephrotoxic agents, and to provide a palatable diet to encourage appetite and minimize further weight loss.[26–30,167] Intravenous fluid therapy to promote diuresis, usually with 0.9% sodium chloride solution, is of much greater benefit in cases of acute, reversible renal failure but also may benefit patients that suffer a sudden exacerbation of CRF. One must administer intravenous fluid therapy cautiously because horses with CRF can develop significant peripheral or pulmonary edema.

Supportive care also can include supplementation of sodium bicarbonate (50 to 150 g per day) when serum bicarbonate concentration is consistently less than20 mEq/L.[167] One may need to add supplemental bicarbonate to a bran mash. If sodium bicarbonate supplementation aggravates ventral edema, one should limit or discontinue supplementation. Edema is not usually a significant problem, and the horse should tolerate mild edema (rather than being treated with diuretics that could be ineffective or lead to further electrolyte wastage) unless the edema interferes with ambulation. In the previous edition of this text, the author also recommended supplementation with sodium chloride to replace potential loss of these electrolytes in urine in horses with CRF. However, a recent study[168] found that supplementation with sodium chloride (used in an attempt to increase water intake) in cats with preexisting renal disease led to progression of renal disease that remained after salt supplementation was discontinued. Thus one should approach salt supplementation in horses with CRF with caution and should consider it only for patients with hyponatremia and hypochloremia.

Substituting high-calcium and high-protein feed sources such as alfalfa hay with good-quality grass hay and carbohydrates (corn and oats) may help control hypercalcemia and the magnitude of azotemia. Ideally, the hay and grain should contain an adequate but not excessive amount of protein (less than 10% crude protein), which should maintain the BUN/Cr ratio in a target range between 10:1 and 15:1.[26] Providing unlimited access to fresh water and encouraging adequate energy intake by feeding a variety of palatable feeds are important. In fact, if appetite for grass hay deteriorates, offering less ideal feeds such as alfalfa hay or increased amounts of concentrate to meet energy requirements and reduce the rate of weight loss is preferable. Often horses continue to graze at pasture when their appetite for hay is diminished. Administration of B vitamins or anabolic steroids for their touted appetite-stimulating effects may benefit some animals. Although dietary fatis calorie dense, one must approach supplementation judiciously in patients with hyperlipidemia and hypercholesterolemia.

Administration of corticosteroids or NSAIDs can limit the intrarenal inflammatory response associated with renal failure and also may attenuate renal injury. For example, administration of meclofenamate limited proteinuria in a group of human patients with severe manifestations of the nephrotic syndrome[169]; however, nonspecific blockade of prostaglandin production by corticosteroids and most available NSAIDs has the adverse effect of decreasing production of important renal vasodilating agents (prostaglandin E_2 and prostacyclin). Production of these prostanoids increases during periods of renal vasoconstriction or ischemia to maintain intrarenal blood flow, particularly to the renal medulla. With excessive or long-term NSAID administration, renal papillary necrosis develops following medullary ischemia.[78,79,86,87] Thus the negative effects of corticosteroids and NSAIDs on renal blood flow outweigh possible benefits, and they are not recommended routinely for the management of CRF in horses.

The progressive renal injury that occurs in CRF is associated with continued damage to glomerular and tubular
membranes mediated by ongoing activation of the inflammatory cascade. In theory, treatment with antioxidant 1248
medications and free radical scavengers could be of benefit, but experimental data in horses do not bear this 1249
out. Similarly, interest in the role of dietary fatty acids as precursors of eicosanoids has been considerable. Specifically, dietary supplementation with sources rich in ω-3 fatty acids (linolenic acid) compared with ω-6 fatty acids (linoleic acid), appear to decrease generation of more damaging fatty acid metabolites during activation of the inflammatory cascade. In horses, dietary supplementation with ω-3 fatty acids (in the form of linseed oil) has been effective at ameliorating the effects of endotoxin in studies in vitro[170–172] and supplementation with fish oil (another rich source of ω-3 fatty acids) slowed the progression of renal failure in laboratory animals.[173,174] Unfortunately, the effects of endotoxin in vivo were not ameliorated by feeding linseed oil in preliminary equine studies,[175] and the possible benefits of feeding ω-3 fatty acids to horses with CRF are not known at this time.

Recently, control of hypertension and reduction of proteinuria have been recognized as the most successful interventions to limit progression of renal disease in human beings with CRF.[149] Thus monitoring the blood pressure and the level of proteinuria (urine protein/urine creatinine ratio) in horses with CRF seems tobe prudent. Treatment with angiotensin-converting enzyme inhibitors could be beneficial in horses with either of these problems but has not yet been pursued because of the expense of the available medications. Attention also has been directed to use of more specific antiinflammatory or immunosuppressant medications to limit renal injury in immune-mediated glomerulonephritis. For example, inhibition of thromboxane synthetase activity (thromboxane A_2 is a potent vasoconstricting agent and platelet activator) was demonstrated to limit renal histologic and functional changes in a canine model of immune-mediated glomerulonephritis.[176] Similarly, cyclosporine was used as an adjunct treatment in a prospective study of naturally occurring canine glomerulonephritis. Unfortunately, renal function declined in cyclosporine-treated dogs, as it did in control dogs. The lack of any beneficial effect, along with the adverse reactions to cyclosporine, led to the conclusion that cyclosporine was of no use for treating CRF.[177] As these studies demonstrate, specific manipulation of the inflammatory or immune response can limit renal injury when one can administer medications before or early in the course of renal disease; however, with long-standing, naturally occurring disease, such treatments are much less likely to retard progression of renal failure significantly. Finally, other investigators are focusing on developing therapeutic strategies that may modulate or limit renal fibrosis. Their studies of the effects of cytokines, lymphokines, and proteoglycans on matrix synthesis and degradation by mesangial cells and on fibroblast activation in damaged glomeruli may lead to novel treatment options in the future.[178,179]

17.5.7

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17.6

17.6—Urinary Tract Infections

Harold C. Schott, II

In human beings, bacterial urinary tract infections (UTIs) are among the most common infections.[1] In contrast, bacterial UTIs appear to be uncommon in horses.[2–7] As in other species, ascending UTIs are more common,

although septic nephritis may be an occasional consequence of septicemia, especially in neonatal foals.[8] Mares are at higher risk for UTIs than geldings or stallions because of their shorter urethra.

Development of a UTI requires initial urethral colonization with pathogenic bacteria, entry of pathogens into the bladder, and subsequent multiplication in the bladder.[1,9] Urethral colonization involves adherence to uroepithelial cells, typically by fecal bacteria that possess fimbrial adhesins (pili) that bind to specific glycolipid receptors on uroepithelial cells. Not surprisingly, pathogenic *Escherichia coli* are rich in these specific surface adhesins, whereas nonpathogenic *E. coli* have few specific surface adhesins. Further characterization of human pathogenic *E. coli* strains by their somatic (O), flagellar (H), and capsular (K) antigens has revealed that a small number of *E. coli* strains are responsible for a large percentage of UTIs.[1,10,11] Normal vulvar and preputial flora protect against urethral colonization by pathogenic bacteria, but any anatomic defect leading to turbulent urine flow compromises maintenance of normal flora and may increase the likelihood of colonization by pathogens.[12,13] Although broodmares have not been proved to be at greater risk, intercourse is a well-established risk factor for development of UTIs in women. In addition, human prostatic secretions contain a heat-stable cationic protein that has potent antibacterial activity.[1] Thus stallions may be at lower risk than geldings for UTIs.

Once a pathogen has colonized the distal urethra, rapid proliferation between micturitions allows invasion of the proximal urethra and bladder, which do not have a protective flora. Host defenses in the bladder include immunoglobulins in urine and a mucopolysaccharide layer rich in glycosaminoglycans covering the uroepithelial surface.[10,11] Production of protective glycosaminoglycans is under hormonal control by estrogen and progesterone in rabbits.[14] Thus lack of these hormonal effects has been suggested as an explanation for the increased risk for UTIs in prepubertal and postmenopausal women and in spayed dogs.[9] Furthermore, women with recurrent UTIs have been speculatedto have decreased concentrations of secretory immunoglobulin A in their urine.[15] Although continued urine production dilutes proliferating bacteria, once pathogens have gained access to the bladder, the rate of replication far outweighs any dilution effect and allows the UTI to become established.[1] Although antibiotic therapy is highly effective in eliminating most UTIs, recurrent UTIs can be a challenge to manage. In addition to thorough evaluation to eliminate predisposing factors in these patients, one may consider additional approaches to prevention. For example, fimbrial vaccines have been shown to be effective against experimental UTIs in monkeys.[10]

17.6.1 Urethritis

Bacterial urethritis has been described as a cause of hemospermia in stallions[16,17]; however, with the exception of traumatic, parasitic (habronemiasis), or neoplastic conditions of the penis or urethra that interfere with urine flow, the author is unaware of documented cases of primary bacterial urethritis resulting in dysuria.[18,19] Furthermore, hemospermia likely attributable to urethritis in a number of previous cases probably resulted from 1253
proximal urethral defects, which have become easier to identify with high-resolution videoendoscopy (see 1254
Chapter 17.8).[20] Bacterial infections of accessory sex glands or the prepuce also may cause dysuria. Accessory sex gland infections generally are limited to intact males and are more likely to cause infertility or hemospermia than dysuria.[21,22] Preputial infections can occur following trauma, presence of a foreign body, habronemiasis, or neoplasia, and affected horses typically have a malodorous, swollen sheath. Examination of the sheath and penis, along with biopsy of abnormal tissue, allows diagnosis of the primary problem. Occasionally, an older gelding may develop recurrent sheath swelling or infection that cannot be attributed to a primary disease process. The pathogenesis of this problem is not known, although fat accumulation, poor hygiene, and inactivity may be contributing factors. Treatment involves repeated sheath cleaning, application of topical

antiinflammatory and antibacterial ointments, and when involvement is more severe, systemic antibiotic administration.

17.6.2 Cystitis

Bacterial cystitis is usually a secondary problem that may accompany alterations in urine flow caused by urolithiasis, bladder neoplasia, bladder paralysis, an anatomic defect of the bladder or urethra, or instrumentation of the urinary tract (e.g., catheterization and endoscopy).[2–7,12,13,23–27] Dysuria may be manifested by pollakiuria, stranguria, hematuria, or pyuria. One may observe scalding and accumulation of urine crystals on the perineum of affected mares or on the front of the hindlimbs of affected male horses. One should not confuse these findings with normal estrus activity in the mare. Although nosocomial UTIs are a well-documented problem in hospitalized human[1] and small animal patients,[28] this complication has not been well-recognized in equine patients except for ill neonatal foals. Diagnostic evaluation includes physical and rectal examinations and collection of a urine sample for urinalysis and quantitative bacterial culture. In the absence of uroliths or other bladder masses, transrectal palpation of the bladder is usually within normal limits; however, endoscopic and ultrasonographic examination of the bladder may be helpful in assessing mucosal damage and wall thickening in horses with cystitis.[29,30] Because normal equine urine is rich in mucus and crystals, gross examination may be unrewarding, but sediment examination may reveal increased numbers of white blood cells (more than 10 leukocytes per high-power field) and presence of bacteria in some cases of cystitis. Normal sediment examination results do not rule out UTI. A definitive diagnosis requires quantitative culture results exceeding 10,000 colony-forming units (CFUs) per milliliter in a urine sample collected by midstream catch or urethral catheterization.[5,7,31] For best results, one should evaluate urine sediment within 30 to 60 minutes of collection and should cool samples for culture during transport because bacterial numbers may increase in samples left at room temperature. Organisms that may be recovered on culture include *E. coli, Proteus, Klebsiella, Enterobacter, Streptococcus*, or *Staphylococcus* species, *Pseudomonas aeruginosa*, and rarely *Corynebacterium renale*.[2–7,25] Isolation of more than one organism is common. *Salmonella* spp. occasionally have been isolated from the urine of apparently healthy horses, and *Candida* infections of the lower urinary tract also have been documented in sick neonatal foals receiving broad-spectrum antibiotics.[4,5]

Successful treatment of bacterial cystitis requires correction of predisposing problems such as urolithiasis and administration of systemic antibiotics. Ideally, selection of an antibiotic is based on the results of susceptibility testing of isolated organisms, and the initial recommended course of treatment traditionally has been at least 1 week.[2–7] A trimethoprim-sulfonamide combination, ampicillin, penicillin, and an aminoglycoside, or ceftiofur can be initial choices. In human beings with uncomplicated cystitis, single-dose antimicrobial therapy, which is less costly and is associated with fewer adverse effects, has a success rate comparable with that of longer-term conventional therapy (75% or greater). Furthermore, relapse following single-dose therapy is not accompanied by more severe clinical signs or more extensive urinary tract involvement[1]; however, if clinical signs recur after treatment is discontinued, one should repeat urine culture along with additional diagnostic evaluation to determine a cause for altered urine flow or bacterial persistence.

Treatment of recurrent UTI usually requires long-term medication (4 to 6 weeks) and ease of administration and cost become additional considerations in antibiotic selection. Trimethoprim-sulfonamide combinations and the penicillins are excreted via the kidneys and concentrated in urine. Although results of in vitro susceptibility testing of isolated pathogens may reveal resistance, these drugs may have effective antimicrobial activity against the causative organisms because of the high concentrations achieved in urine.[7] Metabolism of the

antibiotic is another consideration. For example, sulfamethoxazole is metabolized mostly to inactive products before urinary excretion, whereas sulfadiazine is excreted mostly unchanged in urine.[32]

Additional treatments for recurrent UTI can include supplementation with 50 to 75 g of loose salt to the diet[7] or provision of warm water during cold weather in an attempt to increase water intake and urine production.
Administration of the urine-acidifying agent ammonium chloride (20 to 40 mg/kg per day by mouth) also has 1254
been recommended in cases of cystitis and urolithiasis.[23] Use of ammonium chloride at this dose, however, has 1255
not produced a consistent decrease in urine pH. Recently, use of larger oral doses of ammonium chloride (60 to 520 mg/kg per day),[33–35] methionine (1 g/kg every 24 hours), vitamin C (1 to 2 g/kg per day),[36] or ammonium sulfate (175 mg/kg per day)[37] were more successful in reducing urine pH to less than 6.0 in a limited number of horses; however, at these doses medications were usually unpalatable and had to be administered by dose syringe or stomach tube. Adding graninto the diet is another simple way to decrease urine pH, although the decline is modest and urine pH typically remains greater than 7.0.[36] A final treatment aid is bladder lavage. This procedure most benefits horses with accumulations of large amounts of crystalloid material in the bladder, a condition that has been termed *sabulous urolithiasis*.[37] Although one can add a number of antiseptics to the sterile polyionic lavage fluid, the most important consideration is adequate volume to flush the crystalline debris completely from the bladder. Concurrent cystoscopy is a useful tool to assess the efficacy of bladder lavage.

One report of experimental induction of cystitis in equids exists.[38] After chemical irritation of the bladder mucosa, 2.5×10^{13} CFUs of *Proteus mirabilis* were instilled into the bladders of nine female ponies. Three days later all ponies demonstrated stranguria, andculture results yielded 20,000 to 100,000 CFUs/ml of *P. mirabilis*. Sediment examination revealed increased numbers of white blood cells (more than 10 per high-power field) in seven of nine ponies, and bacteria were observed in all samples (although in low numbers of 1to 3 per high-power field). In two untreated ponies, the cystitis resolved spontaneously between 2 and 4 weeks after inoculation; however, resolution was complete within 3 to 6 days in ponies treated with a trimethoprim-sulfadiazine combination.

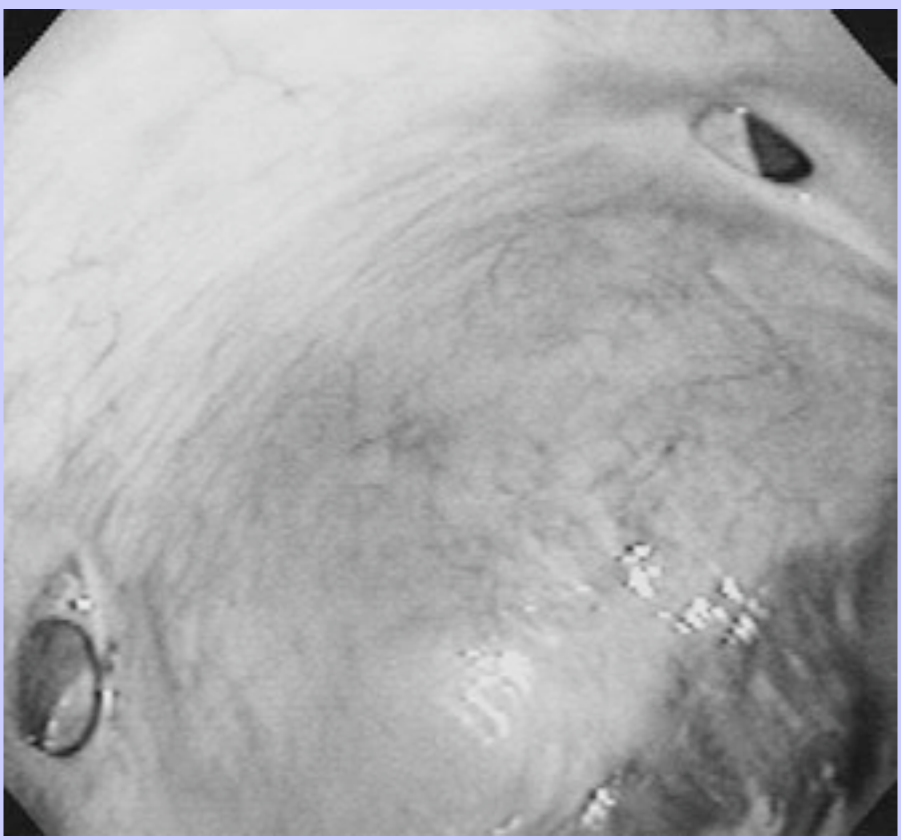
Epizootics of cystitis also have been reported in the southwestern United States[25,39] and in Australia.[40] In the former reports a syndrome of ataxia and urinary incontinence was associated with ingestion of Sudan grass and Johnson grass (hybrids of *Sorghum* species). Both problems were attributed to sublethal intoxication with hydrocyanic acid in the plants, which resulted in demyelination of the lower spinal cord and bladder paralysis. Pyelonephritis was often the cause of death in affected horses.[39] Another outbreak of cystitis, manifested by hematuria more than incontinence, occurred in the Northern Territories and Western Australia in 1963.[40] The kidneys and ureters were not affected, and ataxia was not observed in these horses. The problem, which resulted in loss of more than 200 horses, began shortly after the end of the wet season. Affected horses were on range pasture and, although this was not proved, a fungal toxin was suspected because sporidesmin (a toxin produced by *Pithomyces chartarum*) was known to cause bladder lesions in sheep and cattle. An environmental cause was substantiated further when no additional cases developed in 1964, after a “dry” wet season.

17.6.3 Pyelonephritis

Upper UTIs involving the kidneys and ureters are rarein horses.[3–5,7] The course of the distal segment of the ureters in the dorsal bladder wall creates a physical barrier or valve to prevent vesicoureteral reflux (VUR), a prerequisite for ascending pyelonephritis. Vesicoureteral reflux is more common in children because the

intramural portion of the distal ureter lengthens with growth. Furthermore, congenital VUR often occurs in families, which suggests a genetic tendency for the problem, which has been associated with developmental anomalies of the intramural insertion of one or both ureters.[1] More obvious problems that interfere with this barrier and increase the risk for VUR and associated upper UTIs include ectopic ureter or bladder distention, which may occur with bladder paralysis or urethral obstruction. Over time, VUR leads to progressive ureteral dilation and renal scarring (Figure 17.6-1). This effect explains the common finding of ureteral dilation in young horses with ectopic ureters and provides further support for unilateral nephrectomy, rather than 1255
reimplantation of the ureter, as the treatment of choice for ectopic ureter.[41,42] In addition to VUR, intrarenal 1256
reflux also is required to initiate renal damage predisposing the parenchyma to ascending infection. The renal papillae contain collections of papillary duct openings. These are typically conical structures that protrude into the renal pelvis. Similar to the protective nature of the intramural ureteral segment against VUR, this morphology protects against intrarenal reflux; however, in human infants and young pigs a second type of large, concave, "refluxing" papillae also have been described in the areas of the renal pelvis most often affected with renal scarring.[1] Thus VUR and intrarenal reflux are important in development of pyelonephritis.

Figure 17.6-1 Endoscopic image of the bladder of a pony mare with recurrent cystitis and pyelonephritis. The ureteral openings were distended easily by insufflation of the bladder with air. Ureteral dilation resulted from long-term vesiculoureteral reflux.



The role of recurrent lower UTI in the development of pyelonephritis is less clear. For example, pyelonephritic scarring (without infection) can develop following high-pressure urinary tract obstruction (urethrolithiasis) and can predispose to future upper UTI. In contrast, many cases of recurrent cystitis never proceed to involve the upper urinary tract.[1] Because the kidneys are densely vascular organs, septic nephritis may develop in association with septicemia in neonatal or adult horses.[8] Unless renal involvement is extensive, the upper UTI may go undetected but could lead to development of nephrolithiasis or chronic renal failure months to years later. As in the bladder, defense mechanisms within the normal kidney act to minimize bacterial colonization and proliferation. Efficacy of renal clearance varies with the species of bacteria entering the kidney. In addition, obstruction to urine flow (unilateral ureterolithiasis or nephrolithiasis) increases, rather than decreases, the risk of bacterial proliferation in the obstructed kidney.

Pyelonephritis in horses has been described in association with urolithiasis, recurrent cystitis, and bladder paralysis.[3–5,7,24,39] Other causes have included accidental amputation of the penis during castration,[43] foreign bodies in the bladder,[44] and lower urinary tract neoplasia.[26,45] With pyelonephritis, dysuria is manifested by hematuria or pyuria rather than stranguria and pollakiuria (as for cystitis). In addition, horses with upper UTIs generally have other clinical signs, including fever, weight loss, anorexia, or depression.[3–5,7,44–52] Upper UTIs also can be accompanied by nephrolithiasis or ureterolithiasis or both.[52] In such cases, whether lithiasis or infection develops first or whether both are consequences of VUR, intrarenal reflux, and renal parenchymal damageis unclear. In an occasional case, small uroliths may travel down the ureter and lead to recurrent urethral obstruction with renal colic as the presenting complaint.

As for cystitis, diagnostic evaluation includes physical and rectal examinations, urinalysis, and a quantitative urine culture. Careful palpation may allow detection of an enlarged ureter or kidney, although the kidney also may become shrunken in long-standing cases. In addition to the organisms causing cystitis, one also can isolate *Actinobacillus equuli, Streptococcus equi, Rhodococcus equi,* or *Salmonella* spp. from horses with hematogenous septic nephritis.[5,8] In horses with upper UTIs, one should perform a complete blood count and serum biochemistry profile to assess the systemic inflammatory response and renal function. Finally, cystoscopy (including watching for urine flow from each ureteral opening) and ultrasonographic imaging of the bladder, ureters, and kidneys are helpful adjunct diagnostic procedures.[29,30] Ureteral catheterization (by passing polyethylene tubing via the biopsy channel of the endoscope or by using a No. 8 to 10 French polypropylene catheter, which can be passed blind in mares) may allow collection of urine samples from each ureter to distinguish unilateral from bilateral disease.[53]

Treatment for upper UTIs includes a prolonged course of appropriate systemic antibiotics (selected based on susceptibility testing results on isolated pathogens). In select cases with unilateral disease, one may consider surgical removal of the affected kidney and ureter.[47,54,55] Prerequisites for a nephrectomy include documentation of unilateral disease by normal laboratory results for renal function (absence of azotemia), recovery of insignificant numbers of bacteria (fewer than 10,000 CFUs/ml) from urine collected from the ureter leading to the nonaffected kidney, and ultrasonographic evidence of abnormal structure (e.g., fluid-filled structures and nephrolithiasis) in the affected kidney. Alternatively, poor response to several weeks of appropriate antimicrobial therapy or recurrence of clinical signs of pyelonephritis are additional indications for nephrectomy. Unfortunately, successful treatment of bilateral pyelonephritisis rare, but the poor prognosis likely is related to failure to establish the diagnosis until late in the diseasecourse.

17.6.4 Parasitic Infections

One occasionally finds parasitic lesions associated with the nematodes *Strongylus vulgaris, Halicephalobus* (previously *Micronema*) *deletrix*, and *Dioctophyme renale* in equine kidneys.[56] Although larval migration of *S. vulgaris* in the renal artery and parenchyma is considered aberrant,[57] larval migration was found in more than 20% of horses in one abattoir survey. Passage through renal tissue may result in infarction or subcapsular or pelvic hemorrhage when parasites localize in these sites.[58] Although rare, *H. deletrix* infection is often life threatening because of central nervous system involvement leading to a variety of neurologic deficits that generally require euthanasia.[59–63] *Halicephalobus deletrix* has been suggested to be the most important cause 1256
of verminous meningoencephalomyelitis in horses.[64] Only the female parasite has been identified in equine 1257
tissues, typicallyin highly vascular organs. One usually finds large, granulomatous lesions that are full of the rhabditiform nematodes in the kidneys. The life cycle of *H. deletrix* is unknown, but the apparent saprophyte appears to have worldwide distribution. The finding of gingival lesions and oral granulomata in some horses suggests that ingestion is the likely route of infection. Attempts to find nematode larvae or eggs in urine have been unsuccessful, and whether the horse is an accidental host or is important for the life cycle of the parasite is unclear.[59] The free-living form is found in decaying organic debris (e.g., tree stumps) and also has been described to affect human beings.[63] Antemortem diagnosis has not been made, and cerebrospinal fluid cytologic changes in affected horses cannot differentiate between nematodiasis and protozoal encephalomyelitis.[62] Renal involvement is typically inapparent, although one affected horse demonstrated a 2-week course of stranguria and polyuria before onset of neurologic deficits.[60] *D. renale* is a large, bright red nematode, and the female may reach 100 cm in length. The typical hosts are carnivorous species, but the parasite occasionally affects horses that ingest the intermediate host (annelid worm) while grazing or drinking from natural water sources.[65] Once localized in the kidney, the parasite may live 1 to 3 years; eggs are shed in the urine. The parasite completely destroys the renal parenchyma, and death of the parasite leads to shrinking of the host kidney into a fibrous mass. Occasionally, hydronephrosis or renal hemorrhage may be a serious complication of parasitic infection.[66,67]

In contrast to the nematodes, infection with the coccidian parasite *Klossiella equi* appears common, yet no reports describe clinical disease associated with this coccidial infection.[68–73] Although disorders accompanied by immunosuppression have been suggested to increase its likelihood, *K. equi* infection is still considered an incidental finding in affected horses. The life cycle has not been elucidated fully, but one proposal is that ingested sporocysts (or sporozoites) enter the bloodstream and undergo schizogony in endothelial cells of the glomeruli. Merozoites are released into the urinary space and undergo one or more additional rounds of schizogony in tubular epithelial cells. Eventually, a population of merozoites develops into microgametes and macrogametes. Little evidence of an inflammatory response to parasite replication in renal tissues is apparent. Sporogony follows with the subsequent release of sporocysts into the urine.[69,73] Although *K. equi* has not been associated with clinical disease, it warrants mention that the organism has been found worldwide in horses, ponies, donkeys, burros, and zebras and a recent postmortem survey of47 horses in Australia revealed that 6 (12.8%) were infected.[73]

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17.7 17.7—Obstructive Diseaseof the Urinary Tract

Harold C. Schott, II

Most cases of obstructive urinary tract disease in horses are caused by urolithiasis. Urinary tract displacement, trauma, and neoplasia are other causes.[1–4] Urinary tract obstruction can result in a variety of clinical signs depending on the site and degree of obstruction. Incomplete obstruction can result in dysuria, incontinence, and mild abdominal pain, whereas complete obstruction usually results in moderate to severe pain termed *renal colic*. Another complication of complete obstruction is rupture of the bladder or urethra. Signs of pain subside after rupture but are replaced by depression and inappetance, which accompany postrenal acute renal failure. In some cases of disruption of the urinary tract, one also may observe progressive abdominal distention and enlargement of the penis and prepuce.

17.7.1 Epidemiology of Urolithiasis

The epidemiology of urolithiasis varies with species.[5–8] Lower urinary tract stones predominate in veterinary species, whereas upper urinary tract stones are more common in human beings. Historically, lower urinary tract stones were a more substantial problem in human beings as well, and they remain the more common form of urolithiasis in underdeveloped countries. The shift in prevalence from lower to upper urinary tract stones appears to have accompanied industrialization, but the reasons for the shift are not entirely clear.[8]

From 1970 to 1989, urolithiasis was responsible for 0.11% of equine admissions to 22 veterinary teaching hospitals and accounted for 7.8% of the diagnoses of urinary tract disease.[5] Male horses, especially geldings, are predisposed to urolithiasis (75% of all cases), but a breed predisposition has not been described. This sex predilection has been attributed to the shorter, distensible urethra of the mare, which likely permits voiding of small calculi.[6] Urolithiasis is typically an adult disease and the mean age of affected horses is about 10 years.[5] Nevertheless, young horses can be affected, and the author has seen bilateral nephrolithiasis in a weaning foal (likely a consequence of neonatal septicemia) and dysuria in a3-month-old colt caused by multiple cystoliths that formed on sutures used for repair of a ruptured bladder as a neonate. Uroliths are most common in the urinary bladder (60%), although they also may develop in the kidneys (12%), ureters (4%), and urethra (24%).[5] Interestingly, as many as 10% of affected horses have had uroliths in multiple locations.[5] Uroliths can vary greatly in size. In one mare a cystolith weighing more than 6 kg was detected as an apparently incidental finding in a horse destroyed for a limb fracture.[9]

17.7.2 Pathophysiology of Urolithiasis

In general, two steps are required for calculus formation: (1) nucleation and (2) crystal growth.[10–13] Factors that contribute to precipitation of urinary crystals and nucleation include supersaturation of urine; prolonged urine retention; genetic tendencies to excrete larger amounts of calcium (hypercalciuria), uric acid (hyperuricosuria), or oxalates (hyperoxaluria); and inhibitors and promoters of crystal growth. For two or more ions in a solution to precipitate into a crystal, the product of their individual ion activities must exceed the equilibrium solubility product (K_{sp}). A supersaturated solution is one in which the ion activity product exceeds the K_{sp}. A mildly supersaturated solution is termed *metastable*, for crystals tend to precipitate and dissolve at similar rates, so that crystal growth does not occur and the solution remains clear. Once the ion activity product exceeds a critical value (formation product ratio), however, precipitation outpaces dissolution and rapid crystal growth occurs and the solution becomes cloudy.[10–13] Normal human urine typically is supersaturated with one or more ion activity products; however, the formation product ratios are considerably higher in urine (10 times greater than K_{sp}) than they are in an aqueous solution because of the presence of inhibitors of crystal growth.[10] This activity explains why observation of crystals in urine sediment examination is common, yet calculus formation is uncommon. Furthermore, although K_{sp} values are constant for each type of crystal, they vary with temperature and pH. Typically, cooling promotes crystal formation (as when samples are refrigerated), whereas effects of pH vary with the type of calculus (acidification leads to dissolution of calcium crystals but promotes crystallization of urate crystals).[11] Next, any problem resulting in urine retention or incomplete bladder emptying increases the chance of crystal growth. Although not described for horses, genetic variations in ion excretion rates are well-documented risk factors for human and canine urolithiasis. For example, hypercalciuria is inherited as an autosomal dominant trait in human beings and is responsible for 30% to 40% of nephroliths.[10] Similarly, dogs with cystine urolithiasis have an inherited defect in renal tubular transport of cystine, whereas Dalmatians are afflicted with urate stones because of a defect in uric acid metabolism in the breed.[7]

Normal urine is rich in a number of inhibitors of crystal growth including pyrophosphate, citrate, magnesium 1259
ions, glycosaminoglycans, and several glycoproteins including nephrocalcin.[10,11,13] The degree of inhibitory 1260
activity varies with crystal type; for example, pyrophosphate is responsible for 50% of the inhibitory activity against calcium phosphate stone formation in human urine but has a much less inhibiting effect on calcium oxalate stone formation.[10] Although poorly documented, inhibitors of crystal growth in equine urine, including its high mucous content, likely play an important protective role against calculus formation, which would seem

especially true in light of the substantial urinary excretion of calcium carbonate crystals by normal horses. Similar to the risk associated with increased ion excretion, one should not be surprised that defects in inhibitor activity also have been documented in syndromes of human urolithiasis.[10,11] Other urine components may act as promoters of crystal growth. These components principally include organic matrix components of calculi: matrix substance A, uromucoid, and a number of serum proteins.[11,12] Finally, some urine components may have inhibitor and promoter activity. For example, Tamm-Horsfall glycoprotein, a protein secreted in the ascending limb of the loop of Henle that forms the backbone of urine casts, has been shown to promote struvite crystal formation in feline urine.[14] In contrast, the glycoprotein also inhibits calcium oxalate crystal aggregation, and a group of human patients with calcium oxalate urolithiasis recently were demonstrated to have an abnormality in Tamm-Horsfall mucoprotein.[15]

Because normal urine of most species typically is supersaturated and is in balance between crystal precipitation and dissolution, spontaneous nucleation rarely initiates calculus growth. Rather nucleation generally requires stasis of urine flow, increasing the chance of contact between crystalloid material and uroepithelium (as occurs in areas of the renal pelvis) or a damaged uroepithelial surface.[11–14] The latter results in local activation of inflammatory and clotting pathways, producing a nidus for local crystal adherence.[16] In addition, desquamated epithelial cells, leukocytes, or necrotic debris may provide a nidus for crystal growth at more distal sites in the urinary tract. Tissue damage from a variety of causes is likely the most important factor for the development of uroliths in horses. For example, after urinary tract instrumentation (e.g., catheterization and endoscopy), areas of traumatized uroepithelium are covered rapidly with a fine layer of crystalline material. This material usually resolves spontaneously unless infection develops. Similarly, nephroliths may form in the renal medulla or pelvis following papillary necrosis accompanying phenylbutazone toxicity. Once crystal growth begins around a nidus, equine urine has the disadvantage of being highly alkaline, favoring crystallization of most urolith components, especially calcium carbonate.

Horses develop two basic forms of uroliths, and both are composed primarily of calcium carbonate.[6,17] More than 90% are yellow-green, spiculated stones that easily fragment (Figure 17.7-1, *A*). Less commonly, uroliths are gray-white, smooth stones that are more resistant to fragmentation (Figure 17.7-1, *B*). The latter stones often contain phosphate in addition to calcium carbonate. The crystalline composition of normal equine urine sediment (calcium carbonate crystals predominate, although calcium oxalate and phosphate crystals also occur) and uroliths is similar: calcium carbonate in the form of calcite (a hexagonal crystal form of $CaCO_3$) is most common, followed by vaterite (a metastable, hexagonal crystal form in which $CaCO_3$ is replaced partially by magnesium or to a lesser extent by manganese, strontium, and sulfur). Other less common components include aragonite (an orthorhombic crystal form of $CaCO_3$), weddellite (calcium oxalate dihydrate), struvite (magnesium ammonium phosphate hexahydrate), hydroxyapatite, and uric acid (Table 17.7-1).[17–22] Neumann, Ruby, Ling, et al.[17] recently examined the cut surface of a number of equine uroliths by scanning electron microscopy and described a pattern of irregular, concentric bands around the core (Figure 17.7-2, *A*) that were separated by small spherules of crystalline material (Figure 17.7-2, *B*). This pattern suggested that calculus growth occurs by accretion of preexisting microscopic spherules (the crystals already present in normal equine urine) on the surface of the growing urolith rather than by de novo crystal formation at the surface of the urolith. Furthermore, banding was speculated to represent growth through incorporation of organic matrix on the surface of the urolith at times when fewer spherules were present in urine. The gaps between adjacent spherules result in porosity to the urolith. Because precipitation and dissolution occur simultaneously during growth of urinary calculi, porosity allows exposure of inner aspects of the urolith to urine, which can lead to dissolution as urine composition changes. Neumann, Ruby, Ling, et al. described two types of porosity observed by electron microscopy: primary porosity, consisting of the original pores or gaps between spherules,

and secondary porosity, which developed following dissolution of inner areas of the uroliths. Greater dissolution or secondary porosity developed preferentially in areas of the urolith with higher magnesium content (vaterite). More extensive developmentof secondary porosity theoretically leads to increased urolith fragility, which has the therapeutic benefit of increasing the chance that the urolith can be crushed or fragmented before removal.

The role of urinary tract infection (UTI) in the development of urolithiasis appears to vary with species.[7,10,12] 1260
Struvite urolithiasis in human beings and dogs appears to be almost exclusively a consequence of UTI, whereas 1261
most struvite uroliths in cats and sheep are not associated with infection.[12] In addition to contributing to uroepithelial injury and nidus formation, UTI with urease-positive bacteria (*Proteus* species and coagulase-positive staphylococci are most common) allows splitting of urea into two ammonia molecules, which are hydrolyzed rapidly to ammonium ions (a component of struvite crystals).[10,12,13] In a review of 68 horses with urolithiasis, Laverty, Pascoe, Ling, et al. reported positive urine culture results in only 2 of 19 horses in which urine culture was performed[5]; however, culture of material from the centers of 30 calculi yielded positive results from 19 (63%), and a variety of different bacterial species were isolated. Only 1 of 28 calculi examined 1261
in this study contained struvite. The significance of finding bacteria in the center of equine calcium carbonate 1262
uroliths is not known, especially for isolates other than coagulase-positive staphylococci and *Proteus* species, and culture of an appropriately collected sample of urine always is preferred over culture of a calculus.[5,23] Nephroliths and ureteroliths also are found in some cases of pyelonephritis.[24–27] Laing, Raisis, Rawlinson, et al. described a 2-year-old colt with bilateral nephrolithiasis and chronic renal failure. They recovered a *Proteus* species from urine, and the nephroliths were composed principally of calcium carbonate but also contained lesser amounts of struvite.[27] In contrast, Ehnen, Divers, Gillette, et al. found evidence of infection in only one of eight horses with nephrolithiasis or ureterolithiasis or both and chronic renal failure.[28] In the author's experience, the presence of stones in the upper urinary tract or presence of multiple uroliths warrants concurrent evaluation for UTI, for the author has seen two horses with recurrent urinary tract obstruction with urethroliths that were determined ultimately to be sequelae of unilateral pyelonephritis. Holt and Pearson described a similar case in which a renal calculus and abscess were found 5 months after removal of a cystic calculus.[29]

Figure 17.7-1 Equine cystic calculi. **A**, The more common flattened, spherical type of bladder calculus usually is spiculated. **B**, The less common form of gray, smooth-surfaced calculus may be more irregular in shape. (**B** from DeBowes RM: Surgical management of urolithiasis, *Vet Clin North Am Equine Pract* 4:461, 1988.)

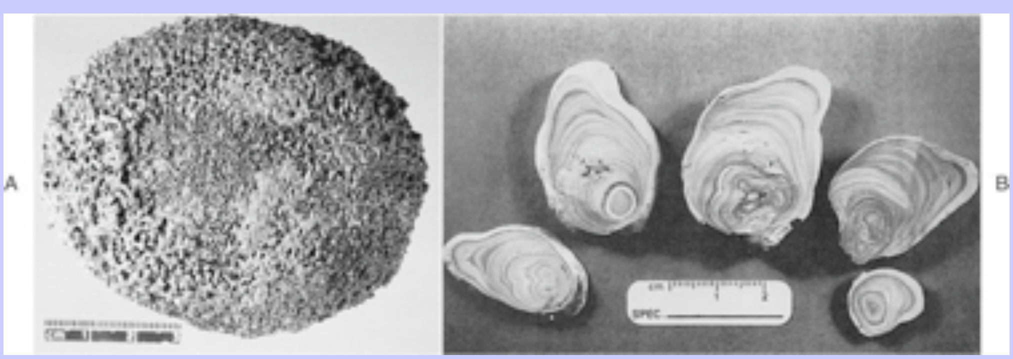


Figure 17.7-2 Scanning electron microscopic appearance of the cut surface of equine cystic calculi. **A**, Lower-power micrograph reveals the intricate pattern of concentric banding around the core (bar = 500 μm). **B**, Higher-power micrograph reveals the ultrastructural features, including bands *(1)*, spherules *(2)*, and primary porosity in black *(3)* (bar = 50 μm). (From Neumann RD, Ruby AL, Ling GV et al: Ultrastructure and mineral composition of urinary calculi from horses, *Am J Vet Res* 55:1357, 1994.)

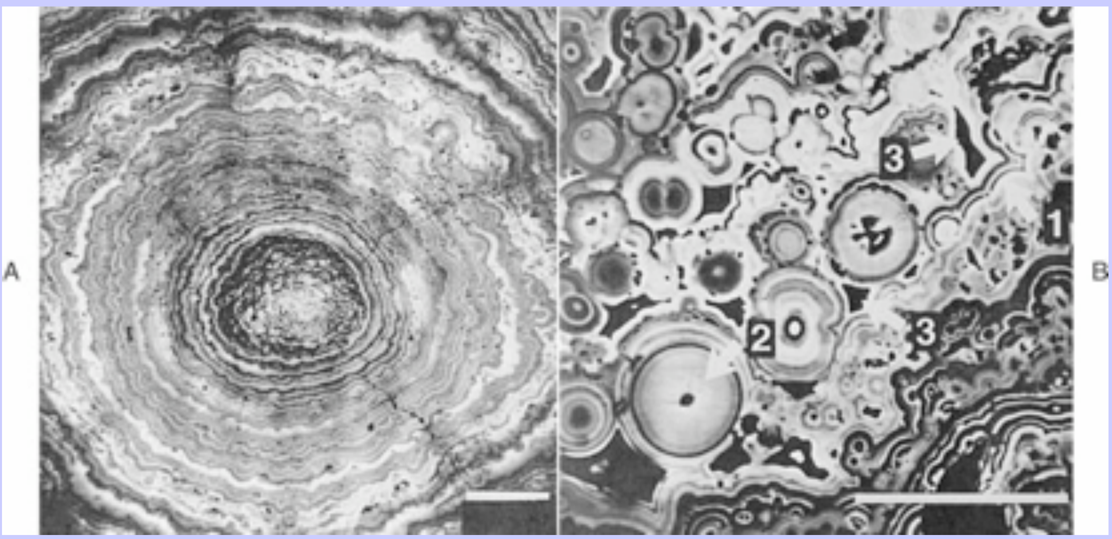


TABLE 17.7-1 Published Results of X-Ray Crystallographic Analysis of Equine Urinary Calculi

TYPE OF CALCULUS	REFERENCE			
	18	19	20	17
Total number of calculi	4	157	18	17
Calcite	2	58	2	11
Calcite/vaterite	1	11	9	5
Calcite/aragonite	1	—	—	1
Calcite/weddellite	—	63	4	—
Calcite/whewellite	—	2	—	—
Calcite/hydroxyapatite	—	8	—	—
Calcite/octacalcium phosphate	—	5	—	—
Calcite/struvite	—	3	—	—
Calcite/gypsum	—	2	—	—
Calcite/vaterite/weddellite	—	2	2	—
Calcite/whewellite/weddellite	—	3	—	—
Calcite/vaterite/sodium acid urate	—	—	1	—

17.7.3 Nephrolithiasis and Ureterolithiasis

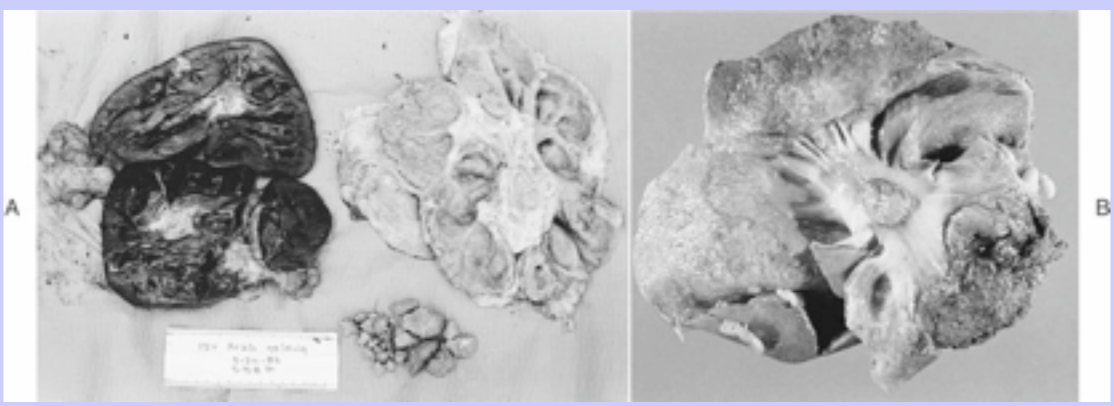
Renal or ureteral calculi rarely were described as a cause of equine urolithiasis before the last decade[30]; however, a number of recent reports[5,26–28,31–35] have described nephrolithiasis and ureterolithiasis in horses. In a review of 68 horses with urolithiasis by Laverty, Pascoe, Ling, et al., 16% had uroliths in the kidneys and ureters and a few horses with cystic calculi also had calculi in the upper urinary tract.[5] Interestingly, 9 of 15 horses with nephroliths in this review were stallions; 3 were geldings, and 3 were mares. Whether a true increase in prevalence of nephrolithiasis and ureterolithiasis has occurred or whether these conditions have become easier to document with the simultaneous development of ultrasonographic imaging as a diagnostic tool for equine medicine is not clear. An undocumented speculation is that young racehorses may be at greater risk of developing renal calculi because of the common use of nonsteroidal antiinflammatory drugs (and risk for development of papillary necrosis) in these athletes.[28] The important point is that one should not overlook upper urinary tract lithiasis in horses.

Nephroliths may develop around a nidus associated with a variety of renal diseases, including polycystic kidney disease (see Figure 17.1-6), pyelonephritis (Figure 17.7-3, *A*), papillary necrosis (Figure 17.7-3, *B*), or neoplasia. At present, data on upper urinary tract stones in horses are insufficient to know whether they develop spontaneously (in the absence of tissue damage) as in human beings or whether they differ significantlyin mineral composition from cystic calculi. Although nephrolithiasis and ureterolithiasis are painful conditions in human beings, horses with nephroliths or ureteroliths often remain asymptomatic until bilateral obstructive

disease leads to development of acute or chronic renal failure. Upper urinary tract stones also may be an incidental finding at necropsy.[30] When clinical signs are present, nonspecific presenting complaints consistent with uremia (poor performance, lethargy, inappetance, and weight loss) are more common than signs of obstructive disease (colic, stranguria, hematuria). Occasionally in the horse, a stone or nidus may pass down the ureter and lead to urethral obstruction and signs of acute obstructive disease. Rectal palpation may reveal an enlarged kidney or ureter (or bladder with urethral obstruction), and ureteral calculi may be palpable in an enlarged ureter. Because normal ureters are not palpable on rectal examination, one should palpate the entire course of the ureters (retroperitoneally along the dorsal abdominal wall to the dorsolateral aspects of the pelvic canal to their insertion at the dorsal bladder neck) because an enlarged ureter is easy to overlook.

One usually diagnoses renal and ureteral calculi during rectal or ultrasonographic examination (Figure 17.7-4). Although ultrasonographic imaging may provide information on the presence, number, and location of calculi, one can miss stones smaller than 1 cm in diameter despite complete examination. Other ultrasonographic findings to support upper tract lithiasis include dilation of the renal pelvis or proximal ureter and, in long-
standing cases, hydronephrosis.[36,37] Although azotemia generally accompanies bilateral disease, horses with 1262
unilateral disease often maintain normal renal function. For reasons detailed previously, one should perform a 1263
quantitative urine culture in all horses with nephrolithiasis or ureterolithiasis to assess possible intercurrent UTI.

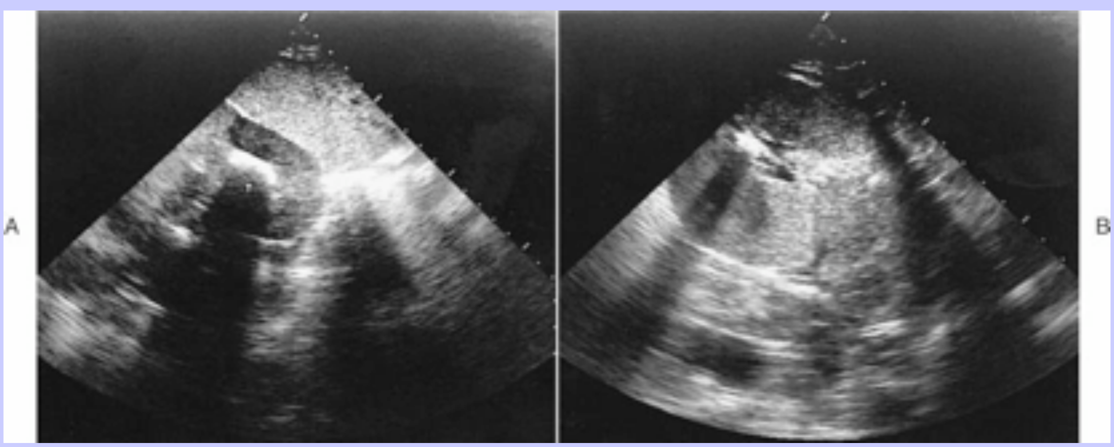
Figure 17.7-3 **A**, Multiple nephroliths developed in association with unilateral pyelonephritis in a gelding that was presented for repeated urethral obstruction. **B**, A small nephrolith lodged in the renal pelvis resulted in ureteral obstruction and development of hydronephrosis in a Standardbred gelding that had a 4-year history of phenylbutazone therapy.



Because most horses with nephrolithiasis or ureterolithiasis are in chronic renal failure by the time the diagnosis is established,[28,38,39] few cases are amenable to treatment. Thus reports of successful managementof horses with renal and ureteral calculi are few. Removal of the calculus, limited to horses with unilateral disease or mild azotemia, has been the only effective means of treatment.[31,33,35] In the absence of azotemia, nephrectomy is the preferred technique for management of unilateral renal calculi.[33] Furthermore, removal of the affected kidney and ureter should eliminate any associated upper urinary tract infection or chance of recurrence. The approach

involves a dorsal flank incision, rib resection, and blunt retroperitoneal dissection to expose the kidney.[40–42] In one horse with mild azotemia, nephrotomy (via an approach similar to nephrectomy) was performed successfully to remove obstructing calculi in the renal pelvis and proximal ureter. Unfortunately, little 1263
improvement in azotemia occurred, and the horse was destroyed a few weeks later.[28] Ureteral calculi also have 1264
been removed by ureterolithectomy via ventral celiotomy and paralumbar approaches.[31,34] A basket stone dislodger (Dormia Stone Dislodger, V Mueller Co., McGow Park, Illinois) introduced through a vestibulourethral approach and guided by rectal palpation also has been used for removal of distal ureteral calculi in the mare.[31] Medical management (antibiotics, grass hay, and salt to promote diuresis) of bilateral ureterolithiasis was attempted in a 3-year-old Thoroughbred filly with incomplete ureteral obstruction and mild azotemia.[34] After 4 weeks, deterioration of clinical signs and more severe azotemia prompted ureterolithectomy to remove stones from the left ureter. Percutaneous nephrostomy was used successfully for placement of a catheter in the right renal pelvis (to establish percutaneous urine flow) for short-term management of azotemia in the postoperative period. Unfortunately, the filly was destroyed after developing cecal impaction 6 days later, and necropsy examination revealed a shrunken, nonfunctional left kidney and a previously undetected nephrolith in the right kidney. This case demonstrated the feasibility and potential benefits of accessing the renal pelvis of the horse via percutaneous nephrostomy.[43,44]

Figure 17.7-4 Ultrasonographic images of the left **(A)** and right **(B)** kidneys of a 10-month-old Arabian filly that developed bilateral nephrolithiasis and chronic renal failure as sequelae of neonatal septicemia. The nephroliths are highly echogenic and cast acoustic shadows in both kidneys.



Rodger, Carlson, Moran, et al. recently described the successful use of electrohydraulic lithotripsy through a ureteroscope to disintegrate a single, unilateral ureterolith in a horse with evidence of bilateral renal disease.[35] Electrohydraulic lithotripsy is a means of converting electric energy into mechanical energy that can be directed to fragment the urolith.[35,45,46] Basically, the device produces an electric discharge arc (a spark) between two electrodes at the tip of the instrument. The heat associated with the discharge causes a small amount of the liquid medium (urine) to burst into gas bubbles, and the associated shock wave fractures the urolith. One must keep the end of the instrument adjacent to the urolith yet away from the mucosa, which could be disrupted by

the same shock waves that destroy the calculus. Although the technique has not been highly successful for treatment of canine uroliths,[47] equine uroliths may be more amenable to its use because they are commonly porous (and fragile). Although electrohydraulic lithotripsy has been effective in treating selected equine cystoliths[45,46] and one ureterolith,[35] the expense of the equipment and availability of other surgical options likely will limit its use to selected cases that are not amenable to routine surgical treatments. A more recent development in upper tract stone removal for human beings and dogs is extracorporeal shock wave lithotripsy.[44] This noninvasive technology uses a reflector to focus the energy from a shock wave generated outside the body on a nephrolith in situ and has proved efficacious in treating human nephrolithiasis. Although extracorporeal shock wave lithotripsy and laser technology have not yet been attempted in horses, they provide future treatment options for equine nephrolithiasis and ureterolithiasis.

17.7.4 Cystic Calculi

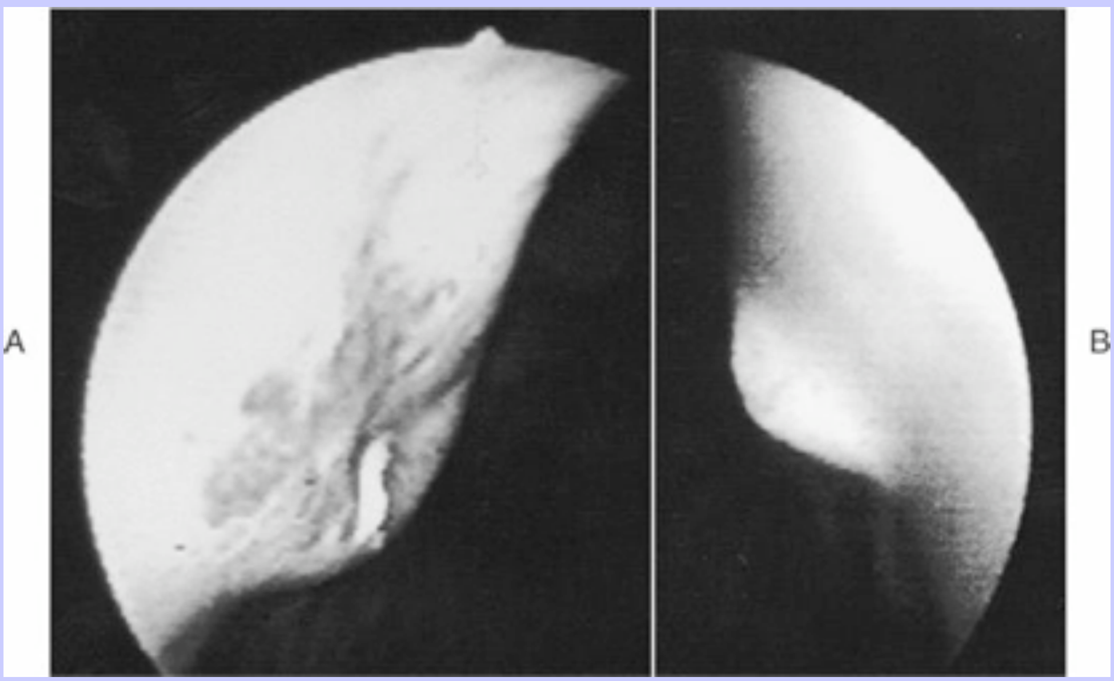
Cystic calculi are the most commonly recognized form of equine uroliths.[1–6,29] Cystoliths typically are flattened, spherical stones with a spiculated or smooth surface. Dysuria resulting from cystoliths may include hematuria, stranguria, pollakiuria, pyuria, or incontinence. Hematuria may be more apparent after exercise. An affected male horse may demonstrate stranguria by repeatedly dropping its penis and posturing to urinate but voiding little or no urine. An affected mare also may posture repeatedly to urinate and demonstrate winking, and these signs could be confused with estrus activity. Less common signs include an irritable attitude, recurrent colic, and loss of condition; one burro was presented for recurrent rectal prolapse.[48]

One usually diagnoses cystic calculi by palpation of the bladder per rectum. Bladder uroliths are usuallylarge enough to be detected easily; however, if the bladder is distended, one may need to empty it by passing a catheter to facilitate palpation of the stone. Bladder catheterization also allows assessment of urethral patency and collection of samples for urinalysis and quantitative culture. One should perform a complete blood count and serum biochemical profile to document whether anemia, inflammation, or azotemia has developed. Cystoscopic examination is helpful in assessing the severity of damage to the bladder mucosa and any asymmetry in appearance or function of the ureteral openings (Figure 17.7-5).[49,50] Because one may find caluculi in multiple sites in the urinary tract, thorough evaluation of the upper urinary tract is warranted for all cases of cystic urolithiasis.

In contrast to upper urinary tract lithiasis, many reports[45,46,48,51–66] and several reviews[*] detail the clinical signs and surgical options for management of cystic calculi. The size of the calculus, gender of the horse, and surgeon's preference play a role in treatment selection. The preferred technique in males, especially for larger stones, is laparocystotomy through a ventral midline or paramedian incision with the horse in dorsal recumbency under general anesthesia. For removal of smaller cystoliths, one can perform a perineal urethrotomy in the standing male horse with the use of local or epidural anesthesia. One catheterizes the urethra to facilitate identification of the urethra and makes an incision at the level of the ischial arch. After incising the 1264
urethra, one uses forceps to grasp and remove the calculus and lavages the bladder to remove remaining debris. 1265
One may attempt removal of larger calculi via this approach by using a lithotrite to crush the urolith into smaller fragments. One can close the urethral incision, but it usually is allowed to heal by second intention. Although one can perform this approach at less expense and avoid the risks of general anesthesia, the risk of complications is greater. Complications include urethral trauma and stricture formation,[29,69,70] urethrolith formation at the surgical site,[5,71] development of a urethral diverticulum,[71] and persistent urine passage through a fistula at the surgical site.[70,72] The distensible urethra of the mare allows retrieval of cystic calculi via this route in many cases. Using sedation and epidural anesthesia, one can remove the stone intact with forceps or by

direct grasping if the surgeon has small hands. One may crush a spiculated urolith with a lithotrite to ease removal. One can manipulate spiculated stones or urolith fragments further into a sterile plastic bag or palpation sleeve to minimize trauma to the urethral mucosa during removal. If necessary, one also can enlarge the urethral lumen by performing sphincterotomy in the dorsal aspect of the urethra.[73] A pararectal (Gökels) approach for a dorsal cystotomy[40,66] and electrohydraulic lithotripsy,[45,46] as described for ureterolithiasis, also have been used in male and female horses to treat cystic calculi.

Figure 17.7-5 View of the abnormal left **(A)** and normal right **(B)** ureteral openings of a horse with unilateral pyelonephritis and recurrent cystic calculi. The diagnosis was confirmed by culture of urine samples collected from each ureter.

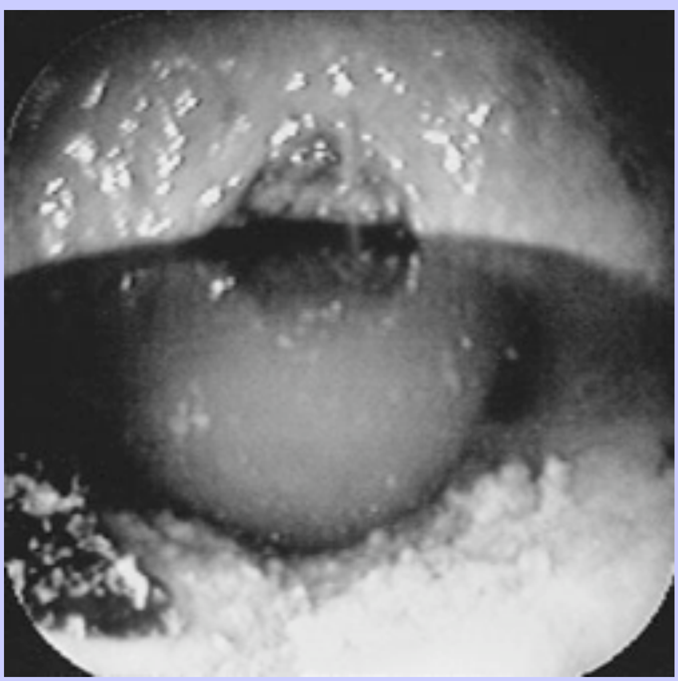


Following surgical removal of cystoliths, one administers systemic antibiotics and an antiinflammatory agent for a minimum of 1 week. As for cystitis, one shouldbase antibiotic selection on susceptibility testing of recovered isolates. If culture results are negative, a sulfonamide-trimethoprim combination is an appropriate selection. An early report by Lowe described excellent long-term results—and no recurrence—after removal of cystic calculi by laparocystotomy in four horses.[71] Similar low rates of recurrence have been echoed in several reviews of equine urolithiasis.[6,29,40,67] In contrast to these favorable reports (most of which do not provide supporting data), Laverty, Pascoe, Ling, et al.[5] reported that clinical signs of urolithiasis recurred in 12 of 29 horses (41%) for which follow-up data were available. The interval between episodes of recurrence was 1 to 32 months (mean 13 months). As initially described in 1965 by Lowe,[71] Laverty, Pascoe, Ling, et al.[5] also found greater recurrence of cystic calculi after treatment by perineal urethrotomy (7 of 15 horses) compared with laparocystotomy. Other complications of cystic calculi unrelated to the surgical approach have included vesicoureteral reflux and renal failure[74] and concurrent urolithiasis at other sites.[5] Although a more common

complication of urethral obstruction,[69] bladder rupture also may occur following cystic urolithiasis. The author also has seen a case of squamous cell carcinoma of the bladder that was associated with a large cystic calculus and development of uroperitoneum following bladder rupture (Figure 17.7-6).

Postoperative recommendations that may help prevent recurrence include control of UTI and use of urinary acidifiers, although the benefits of the latter are not well-documented (see Cystitis in Chapter 17.6).[6,64,67,68,75] Other considerations for decreasing recurrence include dietary modifications to decrease calcium excretionand 1265
to promote diuresis. Changing the diet from a high-calcium hay such as alfalfa to grass or oat hay would 1266
decrease dietary calcium and thus should decrease urinary calcium excretion because fecal calcium excretion is relatively constant in horses.[76] This dietary change should decrease total calcium excretion and may decrease urinary nitrogen excretion and daily urine volume.[77] The latter changes could enhance supersaturation of urine. In theory, the addition of 50 to 75 g of loose salt daily to the concentrate portion of the diet should promote diuresis; however, in a study of ponies fed sodium chloride as 1%, 3%, or 5% of the total dry matter of the diet (1% approximates 75 g of sodium chloride for a 500-kg horse), no differences in water intake, urine production, or calcium excretion were observed.[78] Another factor that affects urine pH and urine calcium excretion is dietary cation-anion balance (DCAB = [Na + K] – [Cl + S]). A lower DCAB has been associated with a decrease in urine pH and an increase in urinary calcium excretion.[79–81] Increasing the amount of grain in the diet, changingto lower-quality hay, or adding one or more minerals to the diet (e.g., ammonium chloride, calcium chloride, or ammonium sulfate) usually lowers DCAB. Not surprisingly, supplements that decrease DCAB are familiar as urinary acidifying agents. Because a diet low in calcium and DCAB could result in a negative calcium balance, a possible long-term effect could be decreased skeletal calcium content.

Figure 17.7-6 A large urolith is present in the bladder below the surface of the pool of urine. The cystolith was accompanied by bacterial cystitis and squamous cell carcinoma of the bladder.



Despite the success of dietary management (low-protein, phosphorous, and magnesium) for medical dissolution of canine[82] and feline[83] uroliths, dietary management is unlikely to replace surgical treatment of cystic urolithiasis in horses. This problem can be attributed to the fact that dietary management for small animals has been directed at struvite urolithiasis and that such stones are not common in horses. Nevertheless, one should not overlook dietary management as one of the postoperative recommendations for urolithiasis, for it could decrease the risk of recurrence. At the least, legume hays and dietary supplements containing calcium should be avoided, and the diet could be supplemented with 50 to 75 g of loose salt daily. Remillard, Modranksy, Welker, et al. successfully used these dietary manipulations effectively, along with administration of ammonium sulfate as a urine-acidifying agent, to manage one recurrent case of equine urolithiasis.[84]

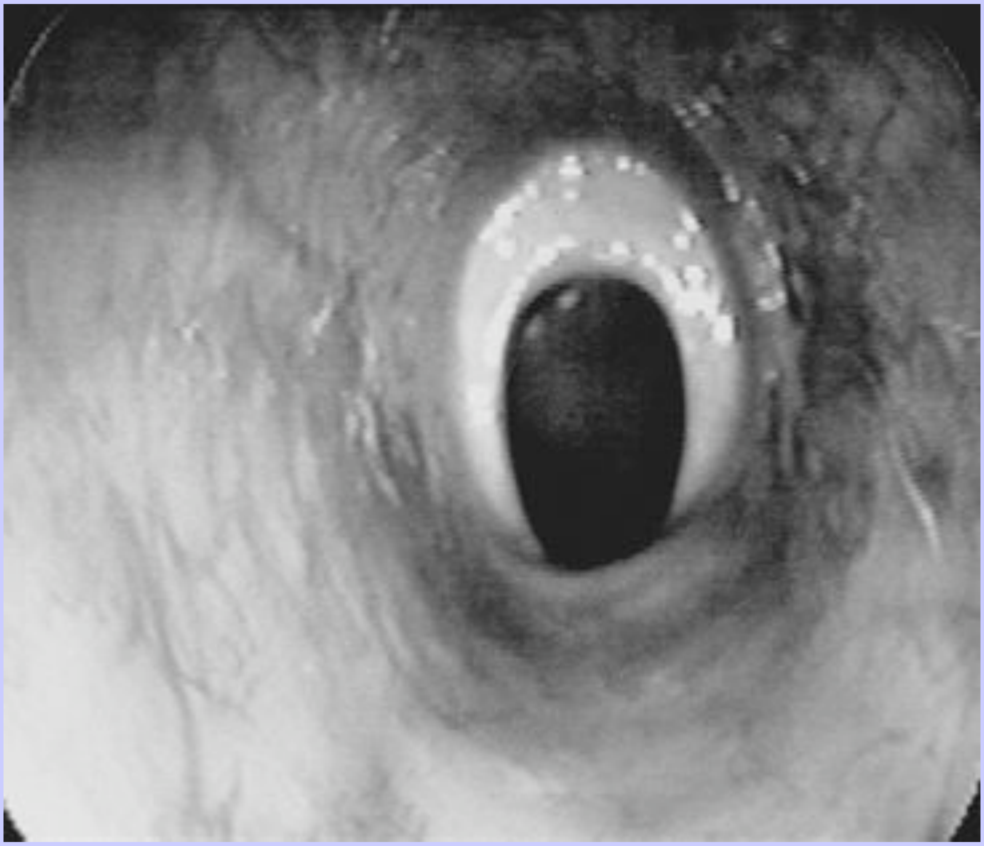
* References 5, 6, 29, 40, 67, 68.

17.7.5 Urethral Calculi

Urethral calculi are primarily a problem of malehorses,[1–6,69,71,85,86] although they have been detected ina few mares.[5] In the absence of predisposing urethral damage or stricture formation, urethroliths are usually small cystoliths passed into the urethra. Thus most urethroliths initially lodge where the urethra narrows as it passes over the ischial arch. They may pass slowly farther down the urethra, until complete obstruction produces signs of renal colic. One should consider an obstructing urethral calculus when male horses show colic signs and frequently posture to urinate. Occasionally, one may see blood on the end of the urethra. Palpation of the penis may reveal repeated urethral contractions or a firm mass in the urethra. Rectal palpation reveals a distended bladder that is turgid, unlike the flaccid bladder distention of bladder paralysis. If the bladder ruptures, colic signs are supplanted by progressive depression and anorexia following the development of postrenal acute renal failure.[5,69,85,86] One confirms the diagnosis by passage of a urinary catheter that is then obstructed by the urolith or by endoscopic examination of the urethra. One can confirm suspected bladder rupture by measuring a twofold or greater increase in peritoneal fluid creatinine concentration compared with serum creatinine concentration.

One can remove calculi lodged at the ischial arch through a perineal urethrotomy. Passage of a catheter into the bladder, if that is not achieved before surgery, is necessary to ensure a patent urinary tract after stone removal. The urethrotomy is allowed to heal by second intention, and temporary use of an indwelling bladder catheter usually is not necessary. One often can remove calculi lodged in the distal urethra from a sedated horse by gentle transurethral crushing of the urolith with a hand or forceps. For a calculus lodged distal to the ischial 1266
arch and unpalpable in the distal portion of the penis, general anesthesia and positioning the horse in dorsal 1267
recumbency are generally necessary for surgical removal of the stone. One may close the urethra or allow it to heal by second intention. Follow-up endoscopic examination of the urethra allows assessment of urethral healing and possible stricture formation (Figure 17.7-7). Further treatment includes administration of antibiotics and antiinflammatory agents until dysuria resolves. Although initial treatment of urinary tract obstruction caused by a urethrolith is straightforward, the prognosis for affected horses should remain guarded because a number of potential complications of perineal urethrotomy (described previously) may occur. Furthermore, a substantial number of horses have had poor outcomes because of associated bladder rupture and peritonitis[5,69,85] or upper urinary tract lithiasis or pyelonephritis.[5,29]

Figure 17.7-7 Stricture formation in the urethra at the level of the ischial arch formed as a complication of a perineal urethrotomy.



17.7.6
Sabulous Urolithiasis

Another form of equine urolithiasis termed *sabulous urolithiasis* also has been described in a limited number of horses.[29,87] Sabulous (Greek for *sand*) urolithiasis refers to the accumulation of large amounts of crystalloid urine sediment in the ventral aspect of the bladder. This condition is a secondary problem resulting from bladder paralysis or other physical or neurologic disorders interfering with complete bladder emptying.[88–90] Affected horses usually are presented for evaluation of urinary incontinence or hindlimb weakness and ataxia, and one may detect accumulation of urine sediment in a distended bladder during rectal palpation. Symptomatic treatment includes repeated bladder lavage, medications that promote bladder emptying, and broad-spectrum antibiotics, but the condition carries a poor prognosis unless the primary problem resulting in bladder paralysis can be resolved (see Chapter 17.12).

17.7.7
Bladder Displacement

Displacement of the urinary bladder is a rare cause of obstruction and dysuria.[91–95] In mares, two types of bladder displacement can occur: (1) extrusion through a tear in the floor of the vagina or (2) true prolapse with

eversion of the bladder.[96] Urethral obstruction also may occur with vaginal or uterine prolapse. In male horses, scrotal herniation of the bladder has been described, but this type of bladder displacement is rare.[97] Bladder displacements typically result from repeated abdominal contractions or straining. Thus they most often are associated with parturition and to a lesser extent with colic. Perineal lacerations following trauma or foaling may lead to extrusion, whereas excessive straining without laceration leads to prolapse/eversion. Because the bladder turns inside out with the latter problem, one establishes the diagnosis by recognizing the appearance of the bladder mucosa and ureteral openings. Eversion does not always result in obstruction.

With urethral obstruction, one should pass a catheter into the bladder before correction of the displacement. In the absence of obstruction, one corrects extrusions during repair of the perineal or vaginal laceration. One should institute a course of broad-spectrum antibiotics and an antiinflammatory agent because pelvic abscess and peritonitis are potential complications. Manual reduction of bladder eversions may be successful in some cases, but more often than not one may need to perform urethral sphincterotomy to replace the bladder.[95] In some cases, reduction via laparotomy may be necessary because the everted bladder may be filled with the pelvic flexure, complicating manual reduction.[94] A purse-string suture placed in the area of the external urethral sphincter may be of benefit to prevent recurrence of the prolapse, and medical treatment should include broad-spectrum antibiotics and an antiinflammatory agent because UTI is a potential complication.

17.7.8 Penile Trauma

Urinary tract obstruction is an occasional complication of penile trauma or paraphimosis, and one should consider the patency of the urethra in all cases of penile injury. Causes may include blunt trauma, breeding injuries, use of stallion rings, sedation with phenothiazine tranquilizers, or laceration during castration.[98–101] In 1267
addition to preputial swelling, injury may result in a penile hematoma or possible paraphimosis.[102,103] In one 1268
report, hematoma formation in the corpus spongiosum penis of a Quarter Horse stallion resulted in complete obstruction and bladder rupture.[104] In addition to ensuring patency of the urinary tract, treatment includes administration of antibiotics and antiinflammatory agents until most of the swelling resolves. One may close lacerations of the urethra or leave them to heal by second intention depending on location and condition ofthe wound. Because stricture formation is a possible complication, some wounds may be treated better by phallectomy than by urethral repair.[100]

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17.8 17.8—Hematuria

Harold C. Schott, II

Hematuria can be a presenting complaint for a variety of disorders of the urinary tract, including vascular malformation, urinary tract infection, urolithiasis, and neoplasia. In addition to these problems, which are discussed elsewhere in this chapter, several other specific causes of hematuria occur. These causes range from microscopic hematuria accompanying exercise to more severe conditions that can result in life-threatening urinary tract hemorrhage. In addition, normal equine urine may contain pyrocatechine, an oxidizing agent that can cause urine to turn red to brown after exposure to air, snow, or bedding (especially wood shavings).[1]

Although values have not been determined in horses, normal human urine contains about 5000 (range 2000 to 10,000) red blood cells per milliliter.[2] This range of red blood cell excretion should yield negative results on reagent strip analysis and a report of not more than 5 red blood cells per high-power field on sediment examination. Increases in red blood cell excretion may lead to microscopic or macroscopic hematuria. Microscopic hematuria, which implies an increase in red blood cell excretion that is not visible grossly, usually is associated with increases in the range of 10,000 to 2.5 million red blood cells per milliliter of urine. On sediment examination at least 10 red blood cells per high-power field should be apparent. Reagent strip analysis results can range from trace to +++. One must realize that reagent strip results, which use the peroxidase-like activity of hemoglobin and myoglobin to oxidize a chromogen in the test pad, do not differentiate between hemoglobin and myoglobin.[3] Thus positive results are not specificfor hematuria and may be more appropriately termed *pigmenturia*. Despite this limitation, one can use reagent strips to differentiate hematuria from hemoglobinuria or myoglobinuria when the color change is limited to scattered spots on the test pad. This pattern implies that intact red blood cells were adsorbed onto the pad, underwent lysis, and produced a localized color change caused by hemoglobin activity on the chromogenic substrates. Ability to differentiate hematuria from excretion of the heme pigments is limited to a threshold of 250,000 to 300,000 red blood cells per milliliter of urine, unless urine samples are diluted with normal saline. Other limitations of reagent strip analysis include false-positive reactions when urine samples are contaminated with oxidizing agents (e.g., disinfectants) or false-negative reactions when urine samples contain vitamin C or have been preserved with formalin.[3]

Macroscopic or gross hematuria indicates red blood cell excretion in excess of 2.5 million to 5 million red blood cells per milliliter of urine (or about 0.5 ml of blood per liter of urine).[2–5] One can differentiate macroscopic hematuria from other causes of pigmenturia by centrifuging a sample of urine to produce a red cell pellet and yellow supernatant urine. Quantification of erythrocyte numbers in macroscopic hematuria is of little clinical value. In contrast, urinary red blood cell numbers may provide diagnostic and prognostic information in cases of

microscopic hematuria in human beings.[2] However, variations in urine concentration complicate accurate counts. In concentrated urine (specific gravity greater than 1.020), red blood cells tend to become crenated because of the osmotic shift of water out of the cells. In urine with a specific gravity less than 1.010, osmotic swelling and dilution of hemoglobin lead to "ghost cell" formation.[2,6] Furthermore, many red blood cells lyse in dilute urine (especially alkaline urine) so that red blood cell excretion is vastly underestimated. Reagent strip analysis can be useful in dilute urine samples to detect hemoglobin released from lysed erythrocytes.[5]

Microscopic examination of urine sediment in cases of hematuria is helpful in distinguishing glomerular from nonglomerular bleeding. The hallmark of glomerular bleeding is a substantial variation in red blood cell size, shape, and hemoglobin content (termed *dysmorphism*), whereas bleeding from other sites produces a more uniform population of urinary erythrocytes.[2,5,7] Dysmorphism is attributed to membrane deformation, which occurs as erythrocytes traverse the glomerular filtration barrier.[7] Urinary red blood cells in normal persons are 1270
typically dysmorphic, indicating glomerular origin, but the excretion rate is low.[2] Thus one must interpret urinary 1271
red blood cell morphologic characteristics along with urinary red blood cell numbers to determine significance.[7,8] The volume of dysmorphic cells tends to be lower than that of erythrocytes of nonglomerular origin, so that measurement of mean corpuscular volume also has been used to separate glomerular from nonglomerular bleeding.[9] The presence of red blood cells or hemoglobin casts is also pathognomonic for glomerular bleeding.[2,5,7] These casts form as urinary red blood cells and hemoglobin from the proximal portion of the nephron (glomerulus) combine with Tamm-Horsfall mucoprotein secreted in the ascending limb of the loop of Henle. Because urinary red blood cells and casts deteriorate rapidly in urine samples, other methods of detecting glomerular hematuria such as immunocytochemical staining for Tamm-Horsfall glycoprotein have been developed but have not gained widespread use.[10]

Noting the timing of hematuria is usually a more practical means of initially localizing the site of urinary tract hemorrhage.[6] Hematuria throughout urination is consistent with hemorrhage from the kidneys, ureters, or bladder, whereas hematuria at the beginning of urination often is associated with lesions in the distal urethra. Hematuria at the end of urination usually results from hemorrhage from the proximal urethra or bladder neck. A thorough diagnostic evaluation, including physical examination, rectal palpation, analyses of blood and urine, endoscopy of the lower tract, and ultrasonography, usually is rewarding in establishing the source and cause of urinary tract hemorrhage.[1]

Urinary tract infection, although uncommon in horses, may result in hematuria. With infection of the upper urinary tract, partial anorexia, weight loss, and fever may be additional presenting complaints, whereas horses with cystitis generally manifest stranguria and pollakiuria; however, hematuria has been the presenting complaint in several reports of cystitis and pyelonephritis.[11–15] The presence of uroliths at any level of the urinary tract may cause mucosal irritation and hemorrhage, resulting in hematuria.[16–18] Typically, affected horses also show signs of renal colic or painful urination (stranguria or pollakiuria), especially with uroliths in the bladderor urethra. Finally, neoplasia of the kidneys, ureters, bladder, or urethra also may result in hematuria as the presenting complaint.[19–22] These conditions are discussed in detail in other sections in this chapter.

17.8.1 Drug Toxicity

Nephrotoxicity, particularly following administrationof nonsteroidal antiinflammatory drugs (especially phenylbutazone), may result in microscopic or gross hematuria.[23–27] Historical or current use of nephrotoxic

medications supports this diagnosis, and discontinuation of the nephrotoxic agent and supportive care are the appropriate treatments.

17.8.2 Urethral Defects

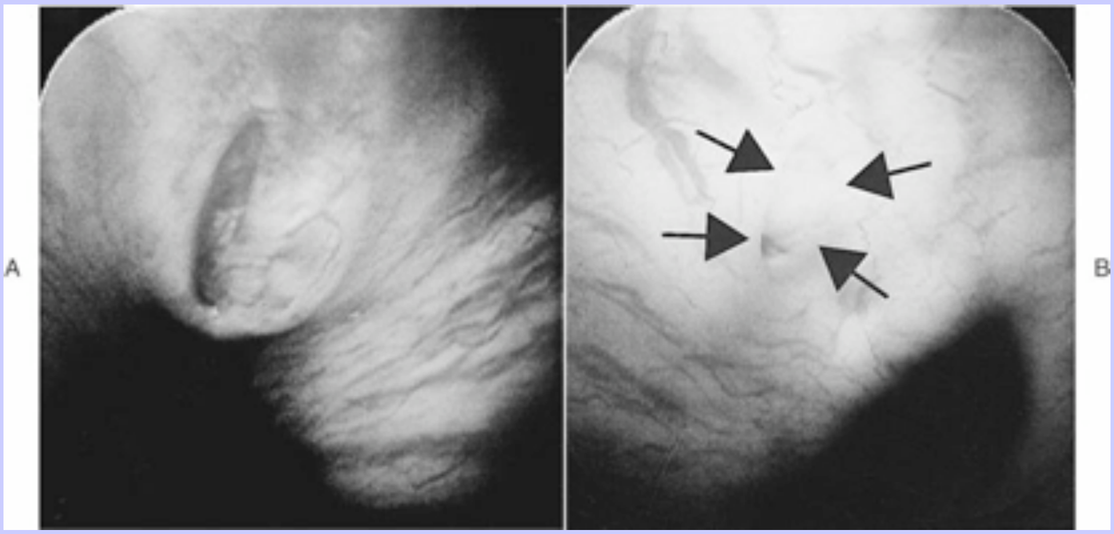
Although a recognized cause of hemospermia in stallions, rents or tears of the proximal urethra at the level of the ischial arch are a more recently described cause of hematuria in geldings.[1,28,29] Because the defects are difficult to detect without high-resolution videoendoscopic equipment, the lesions likely may have been missed in previous reports of urethral bleeding.[30,31] Consequently, hematuria has been attributed to urethritis or hemorrhage from "varicosities" of the urethral vasculature.[28,31] Because the vasculature underlying the urethral mucosa becomes prominent when the urethra is distended with air during endoscopic examination, especially in the proximal urethra (to the point that one can see blood flowing in the submucosal vasculature), one easily would suspect that hemorrhage could arise from apparent urethritis or urethral varicosity.

Urethral rents or tears typically result in hematuriaat the end of urination in association with urethral contraction.[1,28,29] Affected horses generally void a normal volume of urine that is not discolored. At the end of urination, a series of urethral contractions results in squirts of bright red blood. Occasionally, the horse may pass a smaller amount of darker blood at the start of urination. In most instances the condition does not appear painful or result in pollakiuria. Interestingly, most affected stallions with hemospermia and geldings with hematuria have been Quarter Horses or Quarter Horse crosses that have been free of other complaints.[29,31] Treatment with antibiotics for suspected cystitis or urethritis routinely has been unsuccessful, although hematuria has resolved spontaneously in approximately half of the cases seen by the author.

Examination of affected horses is often unremarkable. In comparison, horses with hematuria caused by neoplasms involving the distal urethra or penis usually are presented with additional complaints such as pollakiuria, a foul odor to the sheath, or presence of a mass in the sheath or on the penis.[32] With urethral rents, laboratory analysis of blood reveals normal renal function, although mild anemia can be an occasional finding. Urine samples collected midstream or by bladder catheterization appear grossly normal. Urinalysis may have normal results or the number of red blood cells may be increased on sediment examination, a finding that also would result in a positive reagent strip result for blood. Bacterial culture of urine yields negative results.

One makes the diagnosis via endoscopic examination of the urethra, during which one typically sees a lesion 1271
along the dorsocaudal aspect of the urethra at the level of the ischial arch (Figure 17.8-1, *A*). With hematuria of 1272
several weeks' duration, the lesion appears as a fistula communicating with the vasculature of the corpus spongiosum penis (Figure 17.8-1, *B*). External palpation of the urethra in this area is usually unremarkable but can help localize the lesion because external digital palpation is visible via the endoscope as movements of the urethra.

Figure 17.8-1 Endoscopic images of urethral defects or tears at the level of the ischial arch. **A**, A more acute lesion (hematuria of 2 weeks' duration) is surrounded by a raised rim of tissue. **B**, A chronic lesion (hematuria of 6 months' duration) is flat to recessed *(between arrows)*. Evidence of inflammation around both lesions is minimal.



Although the pathophysiology of this condition remains unclear, the defect has been speculated to result from a "blowout" of the corpus spongiosum penis (cavernous vascular tissue surrounding the urethra) into the urethral lumen (Figure 17.8-2).[29] Contraction ofthe bulbospongiosus muscle during ejaculation causes a dramatic increase in pressure in the corpus spongiosum penis, which is essentially a closed vascular space during ejaculation. The bulbospongiosus muscle also undergoes a series of contractions to empty the urethra of urine at the end of urination; thus the defect into the urethra may develop by a similar mechanism in geldings. Once created, the lesion is maintained by bleeding at the end of each urination, and the surrounding mucosa heals by formation of a fistula into the overlying vascular tissue. An explanation for the consistent location along the dorsocaudal aspect of the urethra at the level of the ischial arch has not been documented but may be related to the anatomy of the musculature supporting the base of the penis and an enlargement of the corpus spongiosum penis in this area. Furthermore, a narrowing of the lumen of the urethra at the distal extent of the ampullar portion of the urethra occurs that also may contribute to the location of the defects. An anatomic predisposition in Quarter Horses has not been documented but could be speculated to be based on an apparently increased risk in this breed. In addition, some of the affected horses have asymmetry to the musculature under the tail in this area, supporting a possible developmental defect (Figure 17.8-3).

Because hematuria may resolve spontaneously, no treatment may be required initially. If hematuria persists longer than a month or if significant anemia develops, a temporary subischial urethrotomy has been successful in a number of affected geldings. With sedation and epidural or local anesthesia, one makes a vertical incision down to a catheter placed in the urethra. The surgical wound requires several weeks to heal, and moderate hemorrhage from the corpus spongiosum penis is apparent for the first few days after surgery. Additional

treatment consists of local wound care and prophylactic antibiotic treatment (typically a trimethoprim-sulfonamide combination) for7 to 10 days. Hematuria should resolve within a week following this procedure. Limiting the incision to entry of the corpus spongiosum penis but not into the urethral lumen also has been a successful treatment and is now the preferred surgical treatment.[29] This treatment option provides support for the blowout cause and lessens the risk of urethral stricture formation. Furthermore, the treatment decreases the chance of a permanent fistula forming at the site, a surgical complication that has occurred in two geldings.

Figure 17.8-2 Cross-sectional diagram of the equine penis at the level of the ischial arch shows a defect between the corpus spongiosum penis (csp) and the urethral lumen *(U)*. The corpus spongiosum penis is a cavernous tissue surrounding the urethra that is distinct from the corpus cavernosum penis (CCP); the corpus spongiosum penis is also adjacent to the bulbospongiosus muscle (BS) caudally. (From Schumacher J, Varner DD, Schmitz DG et al: Urethral defects in geldings with hematuria and stallions with hemospermia, *Vet Surg* 24:250, 1995.)

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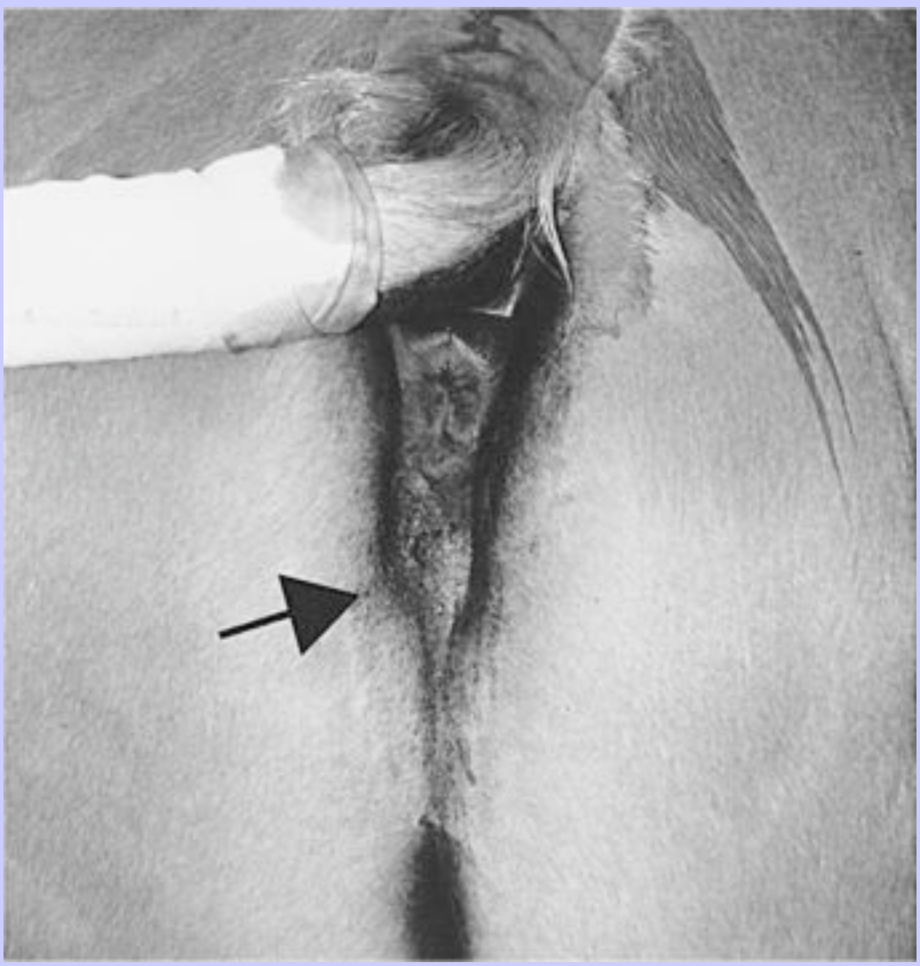
17.8.3 Idiopathic Renal Hematuria

Idiopathic renal hematuria is syndrome characterizedby sudden onset of gross, often life-threatening hematuria.[33,34] Hemorrhage arises from one or both kidneys and is manifested by passage of blood clots in urine. Endoscopic examination of the urethra and bladder usually reveals no abnormalities of these structures, but one may see blood clots exiting one or both ureteral orifices. Although one may establish a definitive cause of renal hemorrhage in some horses (e.g., renal adenocarcinoma and arteriovenous or arterioureteral fistula),[35,36] the disorder is termed *idiopathic* when one cannot find a primary disease process. Both sexes, a wide age range, and several breeds of horses (including a mammoth donkey and a mule) have been affected. However, more than 50% of animals with idiopathic renal hematuria have been Arabians.

Figure 17.8-3 Perineum of Paint gelding with hematuria associated with a proximal urethral defect; the asymmetry of the perineal musculature is visible at the level of the ischial arch. (Arrow shows where asymmetry is more prominent on left side.)



Use of the term *idiopathic renal hematuria* to describe this syndrome of horses was adopted from its use in human patients and dogs with severe renal hemorrhage.[37–44] *Benign essential hematuria* and *benign primary hematuria* are other terms that describe less severe hematuria that is not associated with trauma or other obvious causes of hematuria. In the latter species, hematuria is more commonly a unilateral than a bilateral problem, similar to what has been observed in the few affected horses. The pathophysiology remains poorly understood, but macroscopic hematuria has been associated with immune-mediated glomerular damage (e.g., acute postinfectious glomerulonephritis, membranoproliferative glomerulonephritis, and immunoglobulinA nephropathy or Berger's disease), thin basement membrane nephropathy, and the loin pain–hematuria syndrome in human patients.

Although hematuria and pigmenturia can accompany a number of systemic diseases in horses,[45–47] patients affected with idiopathic renal hematuria appear to have spontaneous, severe hematuria in the absence of other signs of disease. Although one report suggested that severe renal hemorrhage resulted from pyelonephritis,[15] supportive data were lacking. In cases managed by the author, neither urinary tract infection nor lithiasis has 1273
been detected and the magnitude of hematuria often resulted in need for repeated blood transfusions. As with 1274
hemorrhage associated with guttural pouch mycosis, the syndrome may produce episodic hemorrhage. Initially, one notes hemorrhage by finding a large amount of clotted blood in stall bedding or in the pasture. However, other client complaints (e.g., depression, anorexia, and weight loss) are typically absent. Examination may reveal dried blood at the end of the penis or in the sheath of males or on the vulvar lips and between the hindlimbs of mares. In both sexes neoplasia of the external genitaliaor urinary tract is an important differential diagnosis, whereas in mares one also must consider varicositiesin the area of the vestibulovaginal sphincter, especially in multiparous mares. When one does not detect bloodin the sheath or vulvar areas, further evaluation may be unrewarding because the renal bleeding may cease spontaneously. Bleeding has been attributed anecdotally to cystitis and pyelonephritis in the absence of positive urine culture results because hemorrhage stops during a course of antimicrobial therapy. More likely, spontaneous resolution has occurred. Furthermore, the magnitude of hematuria is considerably greater with idiopathic renal hematuria than with most urinary tract infections, pyuria is absent, and urine culture results are negative. In the author's experience, one or two initial episodesof hemorrhage are followed by a more severe hemorrhagic crisis within months to several years after observation of the initial bleeding episode. Interestingly, renal colic has been notably absent in the history of affected horses.

One diagnoses idiopathic renal hematuria by exclusion of systemic disease, other causes of hematuria, and alterations in hemostasis. Physical examination may reveal tachycardia, tachypnea, and pale membranes consistent with acute blood loss. Rectal palpation may reveal an enlarged, irregular bladder because of the presence of blood clots. Azotemia is inconsistent. Endoscopic examination is important to document that hematuria is originating from the upper urinary tract and to determine whether hemorrhage is unilateral or bilateral (Figure 17.8-4). Repeated examinations may be required to answer the latter question. Ultrasonographic imaging is necessary to rule out nephrolithiasis or ureterolithiasis and occasionally may reveal a distended vascular space or renal vascular anomaly as the cause of hematuria. Renal scintigraphy can be a useful technique in affected horses because it may provide semiquantitative information about renal function when a nephrectomy is being considered. Renal biopsy and immunofluorescent staining may assist in documenting immune-mediated glomerular injury, but the significance of such results is not well understood at this time.

Treatment for idiopathic renal hematuria consists of supportive care for acute blood loss, including blood transfusions. Medications intended to promote hemostasis (e.g., α-amino-caproic acid and formalin) also have been administered, but their efficacy has not been validated. In addition, treatment with corticosteroids may be

useful in cases with potential immune-mediated mechanisms as the cause of bleeding. Because the condition may be self-limiting in some patients, supportive care is warranted. With severe and recurrent hematuria of unilateral renal origin, a nephrectomy may be indicated, but one should warn owners of the risk of hematuria developing in the contralateral kidney. In the author's experience, risk of contralateral renal bleeding appears to be greater in the Arabian breed.

Figure 17.8-4 Endoscopic image of a large blood clot exiting the right ureteral opening of a gelding with idiopathic renal hematuria.

17.8.4

Exercise-Associated Hematuria

Exercise is accompanied by increased filtration of red blood cells and protein across the glomerular barrier in a large percentage of human and equine athletes.[4,48] Typically the hematuria is microscopic, but occasionally one may observe gross discoloration of urine. Gross hematuria more often may result from bladder erosions, which may be induced traumatically by the abdominal contents pounding the bladder against the pelvis during exercise.[49] Detection of focal bladder erosions or ulcers with a contrecoup distribution and a history of emptying the bladder immediately before the exercise bout would be characteristic for this problem. A 1274

diagnosis of exercise-associated hematuria should be one of exclusion after diagnostic evaluation has ruled out other causes of hematuria such as presence of a cystolith.

17.8.5 Pigmenturia Associated WithSystemic Disease

With any systemic disease that may lead to hemolysis, thrombocytopenia, coagulopathy, or alterations in vascular permeability, hematuria or hemoglobinuria may develop. Discolored urine has the potential to be accompanied by a degree of nephrotoxicity because of interaction of iron ions of the heme molecules with surface molecules on proximal tubular epithelial cells. With transient pigmenturia (as with exercise-associated hematuria), changes in renal function may not be apparent, but with more severe disease processes, acute renal failure may develop. In human beings, a syndrome of hemolytic anemia and thrombocytopenia leading to development of acute renal failure has been termed the *hemolytic-uremic syndrome*.[50] The syndrome is recognized more commonly in children than in adults and is the most common cause of acute renal failure in children. Similar syndromes have been described in a limited number of horses.[45–47] Furthermore, hemolysis and hemoglobinuria may be recognized with liver disease, medications, or intoxications (e.g., ingestion of red maple leaves). Finally, conditions accompanied by extensive rhabdomyolyis also may result in pigmenturia and acute renal failure.[51] Assessment of muscle enzyme activity in these cases usually is rewarding in establishing myoglobin as the most likely cause of pigmenturia. In addition, one can use the Blondheim test (ammonium sulfate precipitation) to differentiate myoglobinuria from hemoglobinuria.[52]

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17.9

17.9—Polyuria and Polydipsia

Harold C. Schott, II

For small animals, polyuria and polydipsia have been defined as urine output exceeding 50 ml/kg/day and fluid intake of more than 100 ml/kg/day.[1,2] These values would equate to 25 L of urine production and50 L of water

consumption for a 500-kg horse. Compared with normal values for daily urine production and water consumption of 5 to 15 L and 20 to 30 L, respectively,[3–10] these definitions of polyuria and polydipsia appear applicable to horses as well. One must remember that urine production and water consumption vary with age, diet, workload, environmental temperature, and gastrointestinal water absorption. For example, urine production increases by 50% to 100% when the diet is changed from a grass to a legume hay.[11] Although this increase in urine production has been associated with higher dietary protein intake and urinary nitrogen excretion,[12] increases in calcium intake and urinary calcium excretion maybe another contributing factor. Similarly, horses that are exercised heavily, are stabled in hot climates, or have chronic diarrhea may have a water intake exceeding100 L per day yet produce normal volumes of urine.[13]

A brief review of water turnover by the equine kidneys provides insight into how a small change in renal water reabsorption can lead to a dramatic increase in urine production (polyuria). In normal horses, glomerular filtration rate exceeds 1000 L per day, a volume that is 10 times greater than the total extracellular fluid volume; however, approximately 99% of this water is reabsorbed in the renal tubules and collecting ducts, resulting in production of between 5 and 15 L of urine daily. The result is urine that is 3 to 4 times more concentrated than plasma (urine osmolality of 900 to 1200 mOsm/kg and a specific gravity of 1.025 to 1.050). Futhermore, urea (in urine) has replaced sodium (in plasma) as the most important solute. If only 98% of water is reabsorbed, urine volume would double and the additional water would result in more dilute urine (urine osmolality of 450 to 600 mOsm/kg and a specific gravity of 1.015 to 1.025). If water reabsorption decreased to 96% of filtered water, the horse would produce approximately 40 L of urine with a urine osmolality of 225 to300 mOsm/kg and a specific gravity of 1.005 to 1.010 (Table 17.9-1). In the latter case, urine is more dilute than plasma (hyposthenuria) and the kidneys are excreting or losing water actively. Under certain conditions, active water excretion by the kidneys is important for maintenance of plasma osmolality in the normal range. The best example is a neonatal foal that may ingest a volume of milk exceeding 20% of its body weight daily.[14] This ingestion equates to a fluid intake approaching250 ml/kg/day, and failure to produce a large volumeof hyposthenuric urine could result in water retention, decreased plasma osmolality, and clinical hyponatremia (manifested by neurologic signs).

Determining that a horse is producing more urine than normal is often difficult, especially in horses kept at 1276
pasture. Owners may report that a horse is polyuric when in fact the frequency of urination has increased 1277
(pollakiuria) rather than the volume. Pollakiuria occurs with conditions such as cystitis and urolithiasis or during estrus in the mare. Horses housed in stalls bedded with straw are difficult to evaluate because excessive urine may not be obvious to the casual observer. For those bedded on shavings or sawdust, excessively wet bedding may be easier to recognize, but this is a subjective impression. Occasionally in a horse the polyuria may be so severe that urine may flow from the stall into the barn aisle. When doubt exists as to whether a horse has polyuria and polydipsia, documentation of water consumption over one or more 24-hour periods may be necessary.[6] Futhermore, one can quantify urine production by collecting urine over a 12- or 24-hour period. For geldings and stallions, one can construct a collection device by cutting off the bottom of a large plastic bottle, padding it, and fitted it over the prepuce. One covers the opening of the bottle with a rubber tube and clip to allow removal of urine every few hours. In mares, one can place an indwelling Foley catheter in the bladder or apply a urine collection harness.[15–19] During the collection period, horses usually are tied to minimize interference with the collection device.

TABLE 17.9-1 Relationship of the Percentage of Water Filtered That Is Reabsorbed to Daily Urine Output and Renal Water Absorption

GLOMERULAR FILTRATION RATE (L/DAY)	FILTERED WATER REABSORBED (%)	URINE PRODUCTION (L/DAY)	URINARY OSMOLE EXCRETION (mOsm)	URINE OSMOLALITY (mOsm/kg)	RENAL WATER REABSORPTION* (L/DAY)
1000	99	10	10,000	1000	23.3
1000	98	20	10,000	500	13.3
1000	96	40	10,000	250	−6.7

* Renal water reabsorption (the inverse of free water clearance) is a calculated volume of water that is retained or lost by the kidney. Renal water reabsorption is calculated from actual urine volume and the calculated volume of urine required to excrete all osmoles in urine that is isosmotic with plasma. In this table a urine osmolality value of 300 mOsm/kg is assumed to be isosmotic to plasma, 1 kg of water is assumed to equal 1 L of water, and a total of 10,000 Osm is assumed to be excreted daily. Thus when 98% of filtered water is reabsorbed, the 20 L of urine produced (if isosmotic) would contain 6000 mOsm. Because an additional 13.3 L of water would be needed to excrete the remaining 4000 mOsm (as isosmotic urine), the kidneys are considered to be actively reabsorbing 13.3 L of water.

The major causes of polyuria in horses include renal failure, pituitary adenoma (Cushing's disease), and primary or psychogenic polydipsia.[15,20] Less common causes include excessive salt consumption, central and nephrogenic diabetes insipidus, diabetes mellitus, sepsis and endotoxemia, and iatrogenic causes (sedation with α_2-agonists, corticosteroid therapy, or diuretic use).

17.9.1 Renal Failure

Horses with acute renal failure usually have a transient period of anuria or oliguria. If horses survive the acute phase of renal disease, tubule damage results in a subsequent period during which impaired concentrating ability results in polyuria.[15,21] Urine is frequently hyposthenuric during this period of tubular repair. Owners should provide horses recovering from acute renal failure with adequate water, salt, and a low-nitrogen (protein) and low-calcium diet. Such a diet consists of good-quality grass hay or nonlegume pasture. Repair of tubules and return of concentrating ability may take several weeks. Although these animals appear to have normal renal function after this recovery period, a permanent reduction in total renal function likely persists because most animals can maintain apparently normal health with only about 30% to 50% of functioning nephrons.

Chronic renal failure may develop following damage from nephrotoxins. In addition, immune-mediated mechanisms, chronic infection, and nephrolithiasis also may give rise to chronic renal failure.[21–23] Horses that do not recover from the ischemic renal damage occurring with hypovolemic or endotoxic shock also may progress to chronic renal failure. Signs vary and include polyuria and polydipsia in some cases. When present, polyuria and polydipsia are usually moderate compared with the dramatic increases in urine production observed with primary polydipsia or diabetes insipidus. Most horses with chronic renal failure also exhibit other signs, including poor performance, weight loss, and ventral edema. A variable degree of azotemia is present,

and urinalysis reveals isosthenuria (urine is isosmotic with plasma[260 to 300 mOsm/kg] with a specific gravity of1.008 to 1.014).

The mechanisms of polyuria following acute and chronic renal failure are not entirely clear.[24] Increased tubular flow rate in surviving nephrons is one possible mechanism that would result in less time for water removal from tubular fluid. Next, medullary hypertonicity may decrease because of diminished transport of sodium and chloride out of tubular fluid passing through the ascending limb of Henle's loop (diluting segmentof the 1277
nephron) along with increased blood flow through the remaining medullary tissue. A third possibility is 1278
impaired response of collecting ducts to vasopressin (acquired nephrogenic diabetes insipidus). Although all these mechanisms may contribute to the polyuria of renal failure, which one may be most important is not known. Futhermore, because the horse can produce hyposthenuric urine during the recovery phase of acute renal failure, the mechanisms of polyuria differ somewhat for acute and chronic renal failure.

17.9.2 Pituitary Adenoma

Pituitary adenomata are common in older horses and result in a syndrome of hyperadrenocorticism (Cushing's disease).[25–33] Although the most consistent clinical sign is hirsutism, hyperadrenocorticism often is accompanied by polydipsia and polyuria. In addition, affected horses may show weight loss, lethargy, laminitis, and recurrent infections. A horse occasionally may have neurologic signs, including blindness and seizures. Diagnosis is based on presence of excessive hair growth, one or more of the other clinical signs, and supportive laboratory data. In addition to hyperglycemia, neutrophilia, lymphopenia, and mild anemia often are present. Serum activity of hepatic enzymes also may be elevated. One may confirm the diagnosis by several endocrinologic tests, including measurement of elevated plasma adrenocorticotropin concentration,[34] a failure of plasma cortisol concentration to decrease (suppress) after dexamethasone administration,[25,26,35] or an exaggerated cortisol response to administration of thyrotropin-releasing hormone.[36] Treatment with serotonin antagonists (cyproheptadine) or dopamine agonists (bromocriptine or pergolide) may modify the clinical signs but does not achieve a cure because the pituitary lesion continues to grow slowly.[25,26,37]

In one review of 17 horses with Cushing's disease caused by pituitary adenoma, polyuria and polydipsia were found in 13 (76%)[33]; however, in another series of 21 cases of pituitary adenoma, polyuria and polydipsia were not a historical complaint in any of the affected horses.[32] Thus the polyuria and polydipsia associated with Cushing's disease are generally less severe than that observed with primary polydipsia or diabetes insipidus. Pituitary adenomata may lead to polyuria by several mechanisms. First, polyuria may result from actions of hormones derived from proopiomelanocortin, most specifically adrenocorticotropin. Hyperadrenocorticism resulting from excessive adrenocorticotropin activity on the adrenal cortex can lead to hyperglycemia, which may exceed the renal tubular threshold for reabsorption. The renal threshold for glucose in horses appears to be lower than in small animals (about 150 mg/dl).[38] When plasma glucose concentrations exceed this threshold value, the resultant glucosuria can lead to an osmotic diuresis. Although commonly implicated as the cause of polyuria in horses with pituitary adenomata, glucosuria was found in only one of five affected horses in a recent clinical report.[32] Futhermore, horses with hyperglycemia and glucosuria still may be able to concentrate their urine in response to water deprivation.[29] A second mechanism implicated in the development of polyuria is cortisol antagonism of the action of vasopressin on the collecting ducts. Although frequently cited as the mechanism of polyuria in canine hyperadrenocorticism, experimental evidence to support this mechanism is lackingin dogs and horses. Futhermore, considerable species heterogeneity exists in the effects of corticoids on vasopressin activity, and in some species a primary dipsogenic effect may be more important. Next, growth of the adenoma may lead to impingement on the posterior pituitary and hypothalamic nuclei (located immediately

dorsal to the pituitary gland), the sites of vasopressin storage and production, respectively. Decreased vasopressin production and release would result in a partial central diabetes insipidus as a third mechanism for polyuria.[26] Central diabetes insipidus, however, is not the cause of polyuria in all cases because some affected horses can concentrate their urine when deprived of water.[29] Consequently, polyuria and polydipsia seenin many horses with pituitary adenomas is likely the combined result of several mechanisms.

17.9.3

Primary Polydipsia

Although rare, primary or psychogenic polydipsia is probably the most common cause of polyuria and polydipsia in adult horses for which clients have a primary complaint of excessive water consumption and urination.[15,20] Primary polydipsia can be attributed to the fact that horses that exhibit this problem are generally in good body condition and are not azotemic. Futhermore, the magnitude of polyuria typically is much greater than that observed with renal failure or a pituitary adenoma. Owners may report that horses with primary polydipsia drink 2 to 3 times more water than their stablemates and their stalls often are flooded with urine. In some instances, primary polydipsia appears to be a stable vice that reflects boredom in affected horses, whereas in other cases it may develop following a change in environmental conditions, stabling, diet, or medication administration. Anecdotally, primary polydipsia is reported to be more common in southern states during periods of high temperature and humidity. In human beings, primary polydipsia can be a compulsive behavior associated with mental illness or may be caused by a primary abnormality in the osmoregulation of thirst, in which case it is referred to as *dipsogenic diabetes insipidus*.[39] The latter may be idiopathic or may follow 1278

1279 neurologic disease involving the hypothalamic osmoreceptors regulating thirst. Excessive water consumption causes expansion and dilution of body fluids, leading to a decrease in plasma osmolality and suppression of vasopressin release. With low plasma vasopressin concentrations, collecting ducts become impermeable to water and hyposthenuria is induced to allow rate of water excretion to balance intake. In human beings, the magnitude of polydipsia and resultant polyuria vary considerably between affected persons, and similar variation, although undocumented, likely occurs in affected horses as well.

One diagnoses primary polydipsia by exclusion of renal failure and hyperadrenocorticism. In addition, one must exclude other factors such as salt supplementation and medication administration. One excludes diabetes insipidus and confirms the diagnosis by demonstrating urine-concentrating ability after water deprivation.[7,9] Specific gravity should exceed 1.025 after water deprivation of sufficient duration (12 to 24 hours) to produce a 5% loss in body weight. In cases of long-standing polyuria, the osmotic gradient between the lumen of the collecting tubule and the medullary interstitium may be diminished (medullary washout). In these cases vasopressin activity may not lead to an increase in urine specific gravity to values greater than 1.020. Consequently, in horses with primary polydipsia of several weeks in duration that fail to concentrate their urine after24 hours of water deprivation, one may try a modified water deprivation test. One performs the test by restricting water intake to approximately 40 ml/kg/day for 3to 4 days. By the end of this time, urine specific gravity should exceed 1.025 in a horse that has had medullary washout. If the urine specific gravity remains in the isosthenuric range (1.008 to 1.014), one should evaluate the polyuric horse further for early chronic renal failure, in which urine-concentrating ability may be compromised before the onset of significant azotemia. In theory, chronic renal failure could occur when between two thirds and three fourths of functional nephrons have been lost. Subtle signs of decreased performance and mild weight loss also would support early renal failure. Finally, horses with primary polydipsia typically produce hyposthenuric urine. Although such dilute urine would be an unlikely finding in the early stages of chronic renal failure, it could be found in the polyuric recovery phase following acute renal failure. In the latter instance a thorough history should reveal whether any event that may have been complicated by acute renal failure may have affected the horse recently.

Management of horses with primary polydipsia is empiric. Because the diagnosis is one of exclusion, once one establishes that the horse is not suffering from a significant renal disease, one may safely consider restricting water intake to meet maintenance, work, and environmental requirements of the horse. In addition, one should take steps to improve the attitude of the horse by reducing boredom. Increasing the amount of exercise or turning the horse out to pasture are possible options, as is providing a companion or toys in the stall. Increasing the frequency of feedings or the amount of roughage in the diet also may increase the time spent eating and thus reduce the habitual drinking.

17.9.4 Excessive Salt Consumption

In an occasional case of apparent primary polydipsia, polyuria and polydipsia may be attributed to excessive salt consumption and are manifested by increased fractional sodium clearance. Such psychogenic salt eaters appear to be less common than psychogenic water drinkers, for the former would have to consume a substantial amount of salt to develop polyuria. In fact, the authors are aware of only one well-documented report of psychogenic salt eating in which a yearling Paint filly drank in excess of 500 ml/kg/day and had excessive urination when provided free access to salt.[40] The fractional clearances of sodium (3.4%) and chloride (2.6%) were increased and supported excessive intake. Although salt intake was not quantified for this filly, it may have exceeded 10% of the dry matter intake and appeared to be associated causally with muscle fasciculations and a stilted gait. Such a high intake is suggested because no increases in water consumption or urine volume were detected in one study in which ponies were fed diets containing 1%, 3%, and 5% of sodium chloride.[41] The 5% sodium chloride diet contained 5 to 10 times the daily requirement of sodium chloride and was similar to feeding about 350 g sodium chloride daily to a 500-kg horse. Similar to cases of primary polydipsia, the filly described in this report was able to concentrate urine in response to water deprivation, and the problem was managed successfully by limiting water intake to 50 ml/kg/day and preventing access to salt.

17.9.5 Diabetes Insipidus

Diabetes insipidus results in polyuria and polydipsia because of vasopressin deficiency or insensitivity of the renal collecting duct epithelial cells to vasopressin. In human beings, vasopressin deficiency, or neurogenic diabetes insipidus, is the more common form, with hereditary and acquired forms being described. The hereditary form appears to result from decreased numbers of neurosecretory neurons in the supraoptic nuclei of the hypothalamus and is inherited in an autosomal dominant fashion. However, polyuria and polydipsia do not develop until after the first few years of life in affected persons, suggesting progressive loss of neurosecretory 1279
tissue. The acquired form of neurogenic diabetes insipidus results from degeneration of neurons in the 1280
supraoptic nuclei following trauma, vascular abnormalities, infection, or a variety of tumors.[39,42,43] As with the hereditary form, polyuria and polydipsia usually are not manifested until 80% to 90% of the neurosecretory neurons are destroyed.

In equids, two well-documented cases of neurogenic diabetes insipidus have been described.[44,45] Neither animal could concentrate urine in response to water deprivation, but administration of exogenous vasopressin resulted in an increase in urine concentration and a decrease in urine volume. In a Welsh pony in which the condition was considered idiopathic, the absence of an increase in plasma vasopressin concentration after water deprivation (compared with control ponies) further supported a diagnosis of neurogenic diabetes insipidus.[44] Acquired neurogenic diabetes insipidus following encephalitis was confirmed histologically in the other horse.[45] Two other reports of diabetes insipidus in horses more likely described cases of primary polydipsia because

both animals demonstrated an ability to concentrate urine during water deprivation or had random urine specific gravities greater than 1.020.[46,47]

Nephrogenic diabetes insipidus results from resistance of the cortical and medullary collecting ducts to the antidiuretic action of vasopressin.[39,42,43] In the absence of systemic disease, nephrogenic diabetes insipidus is most commonly a familial disorder in human beings with an X-linked semirecessive mode of inheritance. As such, the disorder is carried by females and expressed in male offspring.[48] Schott, Bayly, Reed, et al. previously described nephrogenic diabetes insipidus in sibling Thoroughbred colts, suggesting that an inherited form of nephrogenic diabetes insipidus also may occur in horses.[49] These colts could not increase urine concentration in response to water deprivation, although they did show appropriate increases in plasma vasopressin concentration. Futhermore, minimal response to exogenous vasopressin administration confirmed resistance of the cortical and medullary collecting ducts to the antidiuretic action of vasopressin. Nephrogenic diabetes insipidus also maybe acquired following drug therapy or a variety of metabolic, infectious, or mechanical (postobstruction) disorders. Anomalous or neoplastic disorders resultingin structural deformation of the kidneys are another potential cause of nephrogenic diabetes insipidus.[39,42,43]

As far as pathophysiology the neurogenic and nephrogenic forms of diabetes insipidus are similar to each other. Polyuria because of a lack of vasopressin activity results in net water loss and an increase in plasma osmolality. As plasma osmolality increases, stimulation of thirst results in a compensatory increase in water consumption. In normal individuals and those with nephrogenic diabetes insipidus, the osmoreceptors in the hypothalamus sense the increase in plasma osmolality and subsequently signal for vasopressin release. As little as a 1% increase in plasma osmolality (about 3 mOsm/kg) results in a1 pg/ml increase in plasma vasopressin concentration. In normal individuals, this small change is substantial enough to increase urine osmolality and decrease urine flow. With greater increases in plasma osmolality, more vasopressin is secreted. In human beings, urine osmolality approaches a maximum after an increase in vasopressin concentration to about 5 pg/ml (from a resting value of about 1.0 pg/ml).[39,43]

Limited studies in ponies and horses suggest that a similar degree of vasopressin release occurs in response to minor increases in plasma osmolality[50–52]; however, vasopressin also appears to be a “stress hormone” in horses because substantially greater concentrations (tenfold higher than those induced by water deprivation) have been measured after application of a nose twitch, nasogastric intubation, or exercise.[53] Thus increases in plasma vasopressin concentration following water deprivation would be expected to vary in horses, and separating osmotic effects from stress effects in an individual horse may be difficult. A word of caution also is warranted about subjecting horses suspected of having diabetes insipidus to water deprivation. Because urine-concentrating ability may show minimal improvement with either form of diabetes insipidus, affected horses may continue to excrete excess water in the face of water deprivation. As a result, they may become substantially dehydrated (10% to 15%) within the first 12 hours of water deprivation. Thus one carefully should monitor horses suspected of having diabetes insipidus during the period of water deprivation to decrease the risk of inducing serious hypertonic dehydration. In addition to assessing the effects of water deprivation on urine-concentrating ability and plasma vasopressin concentrations, the final diagnostic manipulation is often administration of exogenous vasopressin. A suggested regimen for an adult horse is administration of 60 IU of exogenous vasopressin (Pitressin synthetic, Parke-Davis, Morris Plains, N.J.) in oil every6 hours combined with monitoring urine specific gravity.

The medullary interstitial osmotic gradient develops as a result of countercurrent exchange, and the magnitude of the gradient is related inversely to tubular flow. Medullary washout may occur with the high tubular flow rates that can accompany renal disease. Although partial medullary washout may contribute to the

concentrating defect in diabetes insipidus, the rapid response to exogenous vasopressin administration in cases of neurogenic diabetes insipidus (increase in urine osmolality to900 mOsm/kg or greater) indicates that the medullary concentration gradient is not severely compromised.[39,54] This response further explains why in some 1280
cases of diabetes insipidus urine osmolality can be greater than plasma osmolality after water deprivation. The 1281
mechanism for this response is thought to be a decrease in tubule flow rate that allows more time for passive water extraction from the hypoosmotic tubular fluid. With nephrogenic diabetes insipidus, one also may observe mild improvement in urine-concentrating ability (an increase in urine osmolality up to 500 mOsm/kg) in response to exogenous vasopressin administration. This response has been attributed to partial sensitivity of the collecting ducts to vasopressin and to vasopressin activity at other portions of the renal tubule.[39,42]

Treatment of diabetes insipidus should aim to manage polydipsia and polyuria. With neurogenic diabetes insipidus, recovery of vasopressin secretion is rare once the secretory neurons have degenerated to the degree that polyuria and polydipsia become apparent. Consequently, treatment consists of hormone replacement. In the past, intramuscular injection of Pitressin tannate in oil every 2 to 3 days was successful in limiting polyuria; however, development of resistance or allergic reactions was an occasional problem. With the development of potent vasopressin analogs (desmopressin), effective treatment by nasal insufflation is now available.[39] The use of desmopressin for diagnosis and treatment of neurogenic diabetes insipidus in small animals has been described.[54,55] Largely by chance, other oral medications, including chlorpropamide and clofibrate, have been found efficacious in treating neurogenic diabetes insipidus. The mechanism of action of these drugs is uncertain, but they are thought to potentiate the effect of vasopressin on the collecting ducts.[39,54]

With nephrogenic diabetes insipidus, replacement hormone therapy is ineffective, and the only practical form of treatment for many years has been to restrict sodium and water intake or to administer thiazide diuretics. The latter treatment may reduce polyuria by 50% in many cases.[39] Thiazide diuretics inhibit sodium reabsorption in the distal tubule (diluting segment of the nephron) and increase solute delivery to the collecting duct. The mechanism by which such therapy paradoxically benefits patients with nephrogenic diabetes insipidus is ill understood. Explanations include enhanced proximal tubular fluid reabsorption (via glomerulotubularbalance) and decreased glomerular filtration and tubular flow (via a greater osmotic stimulus to the macula densaand subsequent tubuloglomerular feedback).[39,43] Recently, treatment with prostaglandin inhibitors or amiloridehas decreased polyuria in patients with nephrogenic diabetes insipidus. The former agents probably work by decreasing renal blood flow and glomerular filtration, whereas the latter drug, a sodium channel blocker, is thought to act in a manner similar to the thiazide diuretics.[39]

17.9.6 Diabetes Mellitus

Diabetes mellitus is a state of chronic hyperglycemia usually accompanied by glucosuria.[56,57] The resultant osmotic diuresis is an occasional cause of polyuria and polydipsia in horses that has been described to result in a water intake exceeding 80 L per day.[58] Type I (insulin-dependent) diabetes mellitus results from a lack of insulin that in human beings is usually attributable to viral or autoimmune disease. Persons with type II (non–insulin-dependent) diabetes mellitus have normal to high insulin concentrations but their tissues are insulin insensitive. Thus the response to an oral carbohydrate load or an intravenous glucose challenge is impaired, resulting in prolonged hyperglycemia.[56] The mechanism of insulin resistance is not well documented for horses but may result from decreased numbers of insulin receptors or lack of insulin receptor activationin response to insulin binding. The most common cause of equine non–insulin-dependent diabetes mellitus is pituitary

adenoma.[27–31] With Cushing's disease, elevated plasma cortisol concentrations appear to antagonize the effect of insulin, leading to hyperglycemia and glucosuria.

Although uncommon, a few reports describe insulin-dependent and non–insulin-dependent diabetes mellitus that were not caused by a pituitary adenoma andthat resulted in polyuria and polydipsia as one of the presenting complaints.[58–62] Diagnostic evaluation reveals consistent hyperglycemia but negative dexamethasone suppression test results (a normal decrease of plasma cortisol to low concentrations). Treatment in most instances is supportive, although replacement insulin therapy may be helpful in cases in which measured serum insulin concentrations are low (insulin-dependent diabetes mellitus). Insulin therapy may be of some benefit in horses with elevated serum insulin concentrations because pharmacologic doses in part may overcome the insulin insensitivity. In such cases, synthetic insulin products may be preferable over protamine zinc insulin, for one horse with a pituitary adenoma and secondary diabetes mellitus developed antiinsulin antibodies and had a relapse in clinical signs after 7 weeks of insulin therapy.[31]

17.9.7 Sepsis and Endotoxemia

Polyuria and polydipsia also have been reported as clinical signs in horses with sepsis or endotoxemia, although other clinical signs such as fever, abdominal pain, and weight loss predominate.[63] The mechanism is unclear but may result from endotoxin-induced prostaglandin production. Prostaglandin E_2 is a potent renal vasodilating agent in laboratory animals, and it antagonizes the effects of antidiuretic hormone on the collecting 1281
ducts.[64] Some horses with chronic gram-negative bacterial infections (such as peritonitis or pleuritis) may have 1282
low-grade or intermittent endotoxemia as a mechanism for polyuria, similar to the polyuria observed with canine pyometra.[65]

17.9.8 Iatrogenic Polyuria

Finally, polyuria can be iatrogenic, following a number of management practices or medical treatments. The most obvious iatrogenic cause is fluid therapy, for which polyuria is a desired response. Polyuria also has been observed with exogenous corticoid administration, although as for pituitary adenomata the mechanism remains unclear. Human beings and dogs appear to experience a potent thirst response to exogenous corticoids; thus polydipsia may be an important cause of the polyuria observed. In horses receiving long-term dexamethasone treatment for immune-mediated disorders, one may observe profound glucosuria (2 to 3 g/dl) that leads to osmotic diuresis in these patients. Finally, transient diuresis or polyuria accompanies sedation with the α_2-agonists xylazine and detomidine.[66,67] Although these agents cause hyperglycemia, and occasionally glucosuria, a more likely mechanism for the transient polyuria is the existence of α_2-adrenoreceptors on collecting duct epithelial cells. Activation of these receptors is another mechanism of vasopressin antagonism.[68]

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17.10

17.10—Renal Tubular Acidosis

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The veterinary profession recognizes well that horses with acute or chronic renal failure are frequently acidotic. These animals are almost invariably azotemic, hypochloremic, and normo- or hyperkalemic and frequently have significant abnormalities on urinalysis. A less frequent renal disease associated with acidosis is renal tubular acidosis (RTA). Renal tubular acidosis is a clinical syndrome of impaired renal acidification characterized by a hypokalemic, hyperchloremic acidosis without azotemia. Affected horses frequently have normal urinalysis. The condition has been well-described in human beings,[1–3] and several case reports documenting its existence in horses have appeared since the mid-1980s.[4–7]

The causes and pathogenic mechanisms responsible for the development of RTA are ill understood. In human beings RTA may be primary (genetic or idiopathic) or secondary, following a variety of conditions, including hyperglobulinemia, various autoimmune disorders, kidney disease such as polynephritis and obstructive uropathy, cirrhosis, drug- or toxin-induced nephropathies (including amphotericin B toxicity), metabolic disorders involving nephrocalcinosis, and a number of genetically transmitted diseases. All reported equine cases apparently have been idiopathic because no signs of primary renal, hepatic, or autoimmune disease or disturbance of calcium metabolism have been evident, and the horses have had no history of access to toxins. In some cases, however, low-grade renal tubular disease has been suspected because of mild proteinuria.

General agreement is that three types of RTA exist.[8] Type 1, which is also known as *distal* or *classic RTA*, arises from the inability of the cells of the distal tubule to establish a steep hydrogen ion gradient between the blood and the urine. This inability results from failure of normal H^+ excretion from the distal tubules. In many cases, this gradient may be less than 10:1. Whether this low ratio is caused by an insufficient number of proton-secreting pumps in the distal nephron or by H^+ diffusing back across the lumenal membrane after being secreted is not clear. Accelerated K^+ secretion occurs because of the existing electrochemical driving forces in the distal nephron and the lack of protons to offset them. In addition to having high urinary K^+ clearances, patients may be hypercalciuric and hyperphosphaturic, although in horses this may be difficult to assess. Equine urinary concentrations of K^+ and Ca^{2+} in particular are high. In human beings, about 70% of adults with distal RTA 1283
develop some form of urolithiasis.[9] This condition has not been recognized in the few reported equine cases and 1284
may not be, given the significant differences between the two species.

Type 2, or proximal, RTA is caused by a failure of HCO_3^- resorption in the proximal tubule. This part of the nephron usually reabsorbs the bulk of the filtered HCO_3^- via Na^+ and H^+ exchange and the subsequent breakdown of carbonic acid to carbon dioxide and water under the influence of carbonic anhydrase. Disruption of normal Na^+ and H^+ exchange or carbonic anhydrase activity therefore results in excess flow of HCO_3^- to the distal tubule where the ability to resorb the anion is poor. This bicarbonaturia also results in accelerated K^+ secretion and hypokalemia. In human beings with this form of the disease, the suggestion is that a reduction occurs in the threshold concentration at which HCO_3^- is reabsorbed from the proximal nephron. As a result, reabsorption resumes once serum HCO_3^- concentration drops below this threshold, and a new steady state is said to develop. More likely, because of the urinary HCO_3^- loss, plasma HCO_3^- concentration decreases, as does glomerular filtration of HCO_3^-. Eventually the distal tubule reaches a point at which it can handle the amount of

HCO_3^- presented. Urine pH may start to decrease because of the lower amount of HCO_3^- being excreted. A new steady plasma HCO_3^- concentration is established, albeit considerably below the normal range, and acid-base homeostasis may return gradually. Consequently, the type 2 condition often appears to be self-limiting in human beings.[10] This form of the disease occurs rarely by itself in human beings and almost always is associated with other signs of proximal tubule dysfunction such as defective resorption of glucose, amino acids, and phosphate (Fanconi's syndrome). Type 2 RTA has been reported in two horses.[4]

Type 4 RTA is characterized by hyperkalemic, hyperchloremic acidosis and is common in human beings, but it has not been reported in horses. Type 4 RTA apparently is associated with hypoaldosteronism or resistance of the distal nephron cells to the effects of aldosterone. As a result the renal clearance of K^+ and H^+ is reduced. The associated natriuresis ultimately may lead to a reduced ability to concentrate urine because of “washout” of the medullary concentrating gradient, if the condition becomes chronic. Another form of RTA (type 3), which formerly had been described as having characteristics of type 1 and type 2 RTA, now is considered to be a variation of type 1.[8]

TABLE 17.10-1 Features Used to Differentiate Type 1 and Type 2 Renal Tubular Acidosis

VARIABLE	TYPE 1	TYPE 2
Acidosis	Severe	Less severe, self-limiting
Hypokalemia	Severe	Mild to moderate
Glycosuria, proteinuria	Absent	Often present
Urine pH during		
Mild/moderate acidosis	Inappropriately high	Inappropriately high
Severe acidosis	Inappropriately high	Normal
Effect of alkali administration	Decreases or worsens hypokalemia, depending on stage of disease	Worsens hypokalemia
Amount of HCO_3^- needed to correct acidosis	Low	High
Ammonium chloride challenge	Failure to excrete acid (urine does not acidify)	Urine pH decreases
Bicarbonate loading	Filtered bicarbonate is reabsorbed	Bicarbonate is lost in the urine

Differentiating between the distal (H^+ retention) and proximal (HCO_3^--wasting) forms of RTA is theoretically important if the approach to the human form of the disease is any guide because in human beings the two types differ respecting clinical severity, treatment, and prognosis.[11] The ability to differentiate between the forms of the disease in horses appears to be less critical, for treatment does not seem to differ much according to the suspected type of RTA. Table 17.10-1 summarizes some differential characteristics of the two types of the disease. In human beings with type 2 RTA, plasma HCO_3^- concentrations tend to be higher than those associated with the distal

form of the disease; but this does not appear to be the case in horses, for all the horses the author has seen, plus those that have been described in the literature, have been severely acidotic (HCO_3^- concentrations of 10 mmol/L or less). In horses, differentiation between the distal and proximal types of the condition has been based on measurement of the urine pH. Evaluation of urine ammonium excretion, urine net charge or anion gap, and urinary P_{CO_2} have not been reported in horses, although they are regarded as critical to the specific differentiation of RTA in human beings.[12–14] Because of the alkalinity of normal equine urine, measurement of urine P_{CO_2} is probably of little benefit. With type 1 RTA, urine pH tends to stay high (i.e., in the normal to alkaline range). In 1284
type 2, urine pH is generally neutral or slightly acidic. A suggested way of making this differentiation is to assess 1285
the urinary response to the administration of the urine-acidifying agent ammonium chloride (0.1 g/kg). Typically, one gives this solution orally and it should lower the urine pH to less than 7.0, which it supposedly does in normal horses and those with type 2 RTA. In cases of distal (type 1) RTA, the urine pH remains high in the face of the increased acid load. Because the administration of such acidifying agents potentially could worsen the degree of acidosis in cases of type 2 RTA because of reduced buffering capacity, performance of this test is not recommended until one has attempted at least partial replacement of the HCO_3^- deficit. In the author's experience, the ammonium chloride challenge test has proved unreliable, having failed to acidify the urine of normal healthy horses even when given intravenously in a 4-L solution. The dose and rate of administration may need to be increased before the test can be considered worthwhile. A more suitable alternative may be to infuse sodium sulfate as is done sometimes in human beings.[1] The author could find no reports of the use of this test in horses.

Another diagnostic option that may be worthy of investigation in horses is calculation of the urine net charge. As in any body fluid, the sum of urinary cations must equal the sum of its anions. Therefore in urine,

$$Na^+ + K^+ + Ca^{2+} + Mg^{2+} + NH_4^+ = Cl^- + HPO^- + SO^- + \text{other urine buffers} + \text{organic anions}$$

In human beings, Ca^{2+} and Mg^{2+} tend to be present in urine in small, constant quantities. Excretion of HPO^-, SO^-, titratable buffers, and organic anions is also constant and exceeds the Ca^{2+} and Mg^{2+} by an amount that is referred to as the urine anion gap (AG_u). Thus

$$Na^+ + K^+ - Cl^- = AG_u - NH_4^+$$

The expression ($Na^+ + K^+ - Cl^-$) is referred to as the *urine net charge* and reflects the excretion of NH_4^+.[13] Given the aforementioned plasma electrolyte and acid-base abnormalities, type 2 RTA has been shown likely to exist when the urine net charge is negative (i.e., $Cl^- > [Na^+ + K^+]$) because NH_4^+ is greater than AG_u. In other words, H^+ continues to be secreted normally from the distal tubule, and plenty of NH_4^+ is being produced. When ($Na^+ + K^+$) is greater than Cl^-, the net charge is positive, indicating that NH_4^+ excretion is low because of failure to excrete H^+ (i.e., type 1 or distal RTA exists).

Horses with RTA usually have a history of severe, progressive weakness, depression, and anorexia. Various other signs such as chronic weight loss, ataxia, dysphagia, and periodic collapse also have been noted. The latter three signs may be manifestations of severe weakness, which probably is caused by the severe hypokalemia. The reduced K^+ concentration also may be associated with bradycardia.

On clinical examination, affected horses are usually afebrile. Mild icterus is common, presumably because of the anorexia, although significant indirect hyperbilirubinemia and increases in serum concentrations of γ-

glutamyltransferase and alkaline phosphatase have been observed. Although cirrhosis of the liver has been associated with the development of RTA in human beings, the equine cases of which the author is aware have demonstrated no clinicopathologic changes compatible with severe liver disease. Sulfobromophthalein clearance half-time was normal in the one case in which it was measured.[5]

One should suspect RTA whenever a horse has a severe hyperchloremic metabolic acidosis in the absence of any obvious cause of extrarenal hypovolemia such as diarrhea or small intestinal ileus. In such cases the plasma anion gap usually is widened, whereas with RTA it remains normal. (However, hypoalbuminemia may mask widening of the anion gap because albumin is normally responsible for much of the normal anion gap.[15] Therefore the anion gap value should be smaller in hypoalbuminemic horses.) Azotemia has not been recognized in cases of equine RTA, although signs of tubular dysfunction such as mild proteinuria and increases in fractional K^+ clearance and urine γ-glutamyltransferase activity have been observed. The results from renal biopsy and ultrasonic examination of the kidneys are normal.

Definitive diagnosis of RTA is based on the demonstration of severe hypokalemia (2.3 mmol/L or less) and a urine pH that is generally neutral or alkaline in the face of a severe acidosis (venous blood pH less than 7.15), although theoretically one could observe a slight aciduria with type 2 RTA. Hypokalemia in the face of severe acidosis is uncommon. Classically, the reverse is expected because of intracellular buffering of H^+ and the reciprocal role of H^+ and K^+ in maintaining the electric neutrality of the extracellular fluid. Occasionally, serum chloride concentrations are in the high normal range rather than being increased. Blood gas measurement usually reveals some degree of compensatory respiratory alkalosis (P_{CO_2} equals 25 to 35 torr).

The differential diagnosis of RTA includes those of any animal that has sudden weakness, depression, and possible collapse: cardiovascular failure, neurologic disease (including rabies), hypoglycemia, and peracute toxemia or endotoxemia. One usually can rule out allof these based on a thorough physical examination that finds nothing to support their possible existence. These findings, plus demonstration of the aforementioned electrolyte
and acid-base abnormalities, suggest RTA. Conditions such as hypokalemic periodic paralysis, Addison's disease, 1285
adenoma of the pars intermedia of the pituitary, and chronic corticosteroid or diuretic abuse could cause some of 1286
these clinicopathologic disturbances, but not the combination of hyperchloremia, acidosis, hypokalemia, and a normal plasma anion gap.

Prompt recognition of the disease and quick institution of therapy are important because the untreated condition is potentially fatal. All reported equine cases of RTA have responded satisfactorily to treatment, as have the cases the author has seen. Although the ability to differentiate between type 1 and type 2 RTA may be important in human beings from a therapeutic perspective, the distinction has not seemed to be essential in horses. Regardless of the suspected type of the disease, treatment of the condition in horses is associated with HCO_3^- and K^+ replacement therapy. Although the administration of $KHCO_3$ would seem to be ideal, the lack of ready availability of this substance and the need for prompt therapy means that treatment usually involves a combination of orally administered KCl and intravenously administered $NaHCO_3$ and KCl. That one should not give $NaHCO_3$ without some form of potassium supplementation deserves emphasis, to ensure that the hypokalemia does not get worse. Generally, a steady improvement in the condition of the patient occurs after 12 to 24 hours of treatment. All fluids usually are given 4 to 6 times per day for the first 48 hours, whereas intravenous fluids frequently are given at a slow but steady rate. Inclusion of glucose or dextrose in the latter helps to promote uptake of K^+ by the cells. The serum chloride concentration usually decreases as HCO_3^- concentration increases. In the author's experience, a steady improvement occurs in HCO_3^- values, and these tend to improve more rapidly than the K^+ concentrations. Serum concentrations of the latter appear to be slower to return to normal, possibly because intracellular stores are

replaced first. Estimating the total potassium deficit before the onset of treatment is impossible because of the huge intracellular deficit these animals suffer. In the author's experience, total deficits of this cation frequently exceed 4000 mmol.

With the correction of the acid-base and electrolyte disturbances, muscle strength and appetite generally return within 48 hours of the onset of treatment. At this point, one usually can reduce potassium supplementation and subsequently discontinue it once the forage intake of the horse has returned to normal. Obviously, giving the horse a diet that includes ample amountsof good-quality hay is important because of the high potassium content of this feedstuff. Bicarbonate therapy generally continues longer, although one usually discontinues intravenous assistance in the horse with return of the appetite. Periodic rechecking of the animal is advised until it becomes clear that its condition is stable. Long-term follow-up of cases the author has seen and those that have been reported in the literature suggest that the prognosis is favorable.

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17.11

17.11—Neoplasia of the Urinary Tract

Harold C. Schott, II

Although neoplasia of the urinary tract is uncommon in horses,[1–6] reports exist of tumors involving the upper and lower urinary tract in this species. Renal neoplasms, which represent fewer than 1% of all equine tumors, include adenomata, renal cell carcinomata, and nephroblastomata.[7] Renal adenomata are small, well-circumscribed lesions in the renal cortex, that are usually incidental necropsy findings.[8] The best-described renal tumor in the horse is renal cell carcinoma or adenocarcinoma.[9–16] These lesions arise from epithelium of the proximal convoluted tubules in most cases. In human beings, renal cell carcinomata are known as the "internist's tumor" because of their diverse and often obscure presenting signs. Although a classic triad of symptoms including flank pain, gross hematuria, and palpable renal mass has been described, these symptoms occur in fewer than 10% of affected human beings at presentation.[17] Similarly, affected horses usually have nonspecific presenting complaints, including poor performance, depression, weight loss, and recurrent colic (Table 17.11-1). Signs that increase suspicion of a primary urinary tract problem include hematuria (7 of 12 cases in Table 17.11-1) and detection of a palpable mass on rectal examination (8 of 12 cases in Table 17.11-1). Renal cell carcinomata are typically unilateral, and the contralateral kidney maintains normal renal function. Although nephrectomy is the treatment of choice in human beings, tumors are typically large and adherent to surrounding organs by the time they are detected in horses. Thus surgical removal usually is not possible. Furthermore, frequent metastases (8 of 12 cases in Table 17.11-1) are another indication that renal adenocarcinoma is usually not a treatable disease. The poor prognosis can be attributed to the fact that clinical signs of intraabdominal neoplasia in horses often are not apparent until the disease is advanced.[18] In one report of renal carcinoma, clinical signs of the tumor were absent until the horse was anesthetized for laryngeal surgery. After an uncomplicated surgery, the horse was repositioned for recovery but died shortly thereafter. Compression of the caudal vena cava by a large renal carcinoma was suspected to have led to a decrease in venous return as the cause of sudden death.[19] Other neoplastic diseases that may affect the kidneys include nephroblastoma,[20–22] transitional cell carcinoma,[18,23] and squamous cell carcinoma.[24] Nephroblastoma (Wilms' tumor) is an embryonal tumor that arises in primitive nephrogenic tissue or in foci of dysplastic renal tissue, whereas the latter tumor types arise from the uroepithelium of the renal pelvis or ureter.[8] Neoplastic involvement of the upper urinary tract also may occur with dissemination of lymphosarcoma, hemangiosarcoma, melanoma, or adenocarcinoma arising from other tissues in the abdomen.[2,7,8,18,25] Finally, although they are not truly cancerous disease processes, mucinous hyperplasia of the renal pelvic and proximal ureteral uroepithelium or ureteropelvic polyp formation also can lead to development of a tissue mass in either kidney, ureteral obstruction, and hydronephrosis.[26,27]

In addition to rectal palpation for a mass in the area of either kidney and examination of urine for red blood cells (hematuria), ultrasonographic evidence of a tissue mass destroying the normal architecture of the kidney would support renal neoplasia. Unfortunately, attempts to establish a definitive antemortem diagnosis were successful in only two previous reports. In the first case, neoplastic cells were detected on analysis of a sample of peritoneal fluid,[12] whereas percutaneous biopsy of the tissue mass in a second horse demonstrated neoplastic tissue. Thus these procedures are warranted in all horses with a mass consistent with a renal neoplasm. In addition to analyzing urine for hematuria, cytologic examination of urine for neoplastic cells is also warranted.[28]

The most common presenting complaint for bladder neoplasia is hematuria.[28–30] Unlike dogs, in which transitional cell carcinoma is the most commonly described bladder neoplasm, squamous cell carcinoma has been

reported most frequently in horses.[28,29] Other types of bladder neoplasms affecting horses include transitional cell carcinoma, lymphosarcoma, leiomyosarcoma, and fibromatous polyps. In contrast to cattle, which develop bladder neoplasia in association with chronic ingestion of bracken fern and other plants (enzootic hematuria), dietary factors have not been incriminated in development of bladder cancer in horses.[8] One can establish diagnosis of bladder neoplasms by palpation or ultrasonographic imaging of a bladder mass, endoscopic examination and biopsy (Figure 17.11-1), and urine cytologic examination. Treatment has included partial bladder resection or intravesicular instillation of 5-fluorouracil, but successfuloutcomes have not been reported.[28] Again, a poor prognosis likely is related to the extensive bladder involvement by the time clinical signs are apparent.

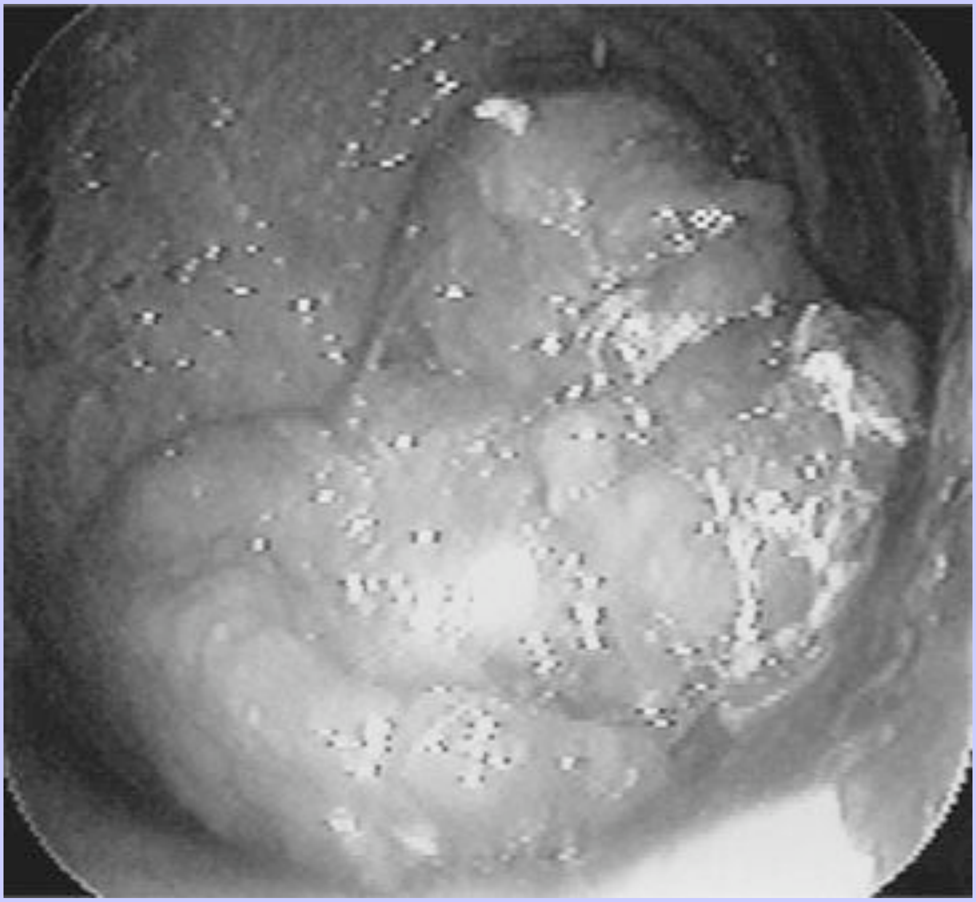
Tumors of the urethra and external genitalia are the most common urinary tract neoplasms of horses. Although reports describe a paraurethral lipoma[31] and fibrosarcomata[32] resulting in frequent urination or dribbling and urinary tract infection, respectively, tumors of the external genitalia are more common than urethral tumors.[33–35] 1287
Tumors affecting the external genitalia include squamous cell carcinoma, sarcoid, melanoma, mastocytoma, 1289
hemangioma, and papillomata or warts. Habronemiasis used to be a significant cause of genital lesions before the use of ivermectin, and the disease should remain on the differential list because it can be distinguished from squamous cell carcinoma or sarcoid only by examination of a biopsy sample. Breeds with nonpigmented genitalia (Appaloosas and Paints) appear to be predisposed to squamous cell carcinoma. Similarly, a predilection for geldings and stallions has been associated with a carcinogenic potential of smegma.[36] Affected horses may have a malodorous sheath or hematuria if the distal urethra is involved. Urinary tract obstruction is uncommon unless the tumors are large. Diagnosis is by visual examination and collection of a biopsy sample. Collection of the latter often is by complete excision of the lesion. In addition to surgical excision, a rather high rate of recurrence has led to use of a number of adjunct therapies, including immunotherapy, cryotherapy, hyperthermia, and radiation therapy. Because all treatment combinations have had variable success, some authors recommend aggressive surgical treatment early in the course of the disease.[37] Most recently, local injection of cisplatin, a cytotoxic antineoplastic agent, has been used with a high success rate to treat equine sarcoid and squamous cell carcinoma.[38,39]

TABLE 17.11-1 Clinical Features of 12 Horses With Renal Cell Carcinoma

BREED	AGE (YEARS)	SEX	UNILATERAL OR BILATERAL	PRESENTING COMPLAINT	WEIGHT LOSS	PAIN/COLIC	HEMATURIA	PALPABLE MASS ON RECTAL EXAMINATION?	AZOTEMIA	POSTMORTEM FINDINGS	REFERENCE
American Saddlebred	15	G	Bilateral	Recurrent colic	Yes	Yes	No	Yes	No	25-cm diameter renal calculus, metastases to liver and other kidney	9
Albino	10	F	Unilateral, left	Abortion and ascites	No	No	No	Yes	Mild	75-cm diameter, 31-kg mass, metastases to peritoneum	10
Standardbred	16	F	Unilateral, right	Weight loss, hematuria	Yes	No	Yes	Yes	No	30-cm diameter, 8-kg mass, adhesions to liver and bowel, no metastases	10
Thoroughbred	16	F	Unilateral, left	Weight loss	Yes	No	Yes	Yes	Mild	35-cm diameter, 20-kg mass, hemoperitoneum, no metastases	11
Thoroughbred	16	F	Unilateral, right	Weight loss, soft feces	Yes	No	No	No	Mild	Metastases to liver and lungs	12
Pony	15	G	Unilateral, left	Weight loss, hematuria	Yes	No	Yes	Yes	No	6.6-kg mass, metastases to liver and lungs	13
Pony	10	G	Unilateral, right	Weight loss, back pain, colic, polyuria/polydipsia	Yes	Yes	Yes	No	No	6-cm diameter mass hemoperitoneum, no metastases	13
Thoroughbred	4	G	Unilateral, left	Respiratory noise	No	No	NR	NR	No	30-cm diameter mass, local invasion of sublumbar muscles	13
Grade	7	G	Unilateral, right	Hematuria, follwed by weight loss and colic	Yes	Yes	Yes	No	Mild	40-cm diameter, 23-kg mass, hemothorax and hemoperitoneum, metastases to liver and lungs	13
Thoroughbred	9	F	Unilateral, right	Recurrent colic, weight loss	Yes	Yes	No	Yes	No	30-cm diameter mass, metastases to omentum and muscle	14
Shire	4	F	Unilateral, right	Hematuria, followed by weight loss	Yes	Yes	Yes	Yes	No	65-cm diameter, 47.7-kg mass, adhesions to liver and bowel, no metastases	15
Grade	14	F	Unilateral, left	Pyrexia, hematuria, diarrhea	Yes	No	Yes	Yes	No	5-kg mass; metastases to liver, pancreas, and lungs	16

G, Gelding; *F*, mare; *NR*, not reported.

Figure 17.11-1 Leiomyosarcoma of the urinary bladder resulted in hematuria. (Courtesy R. MacKay, University of Florida.)



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17.12

17.12—Urinary Incontinence and Bladder Dysfunction

Warwick M. Bayly

Loss of control of bladder function is an infrequent problem in horses. When the condition is recognized, it is usually because a degree of incontinence develops, which by definition means that intravesicular pressure exceeds resting urethral pressure. Although a number of recognized abnormalities of bladder emptying can afflict human beings, dysfunctions of equine bladder control and micturition tend to fall into one of three categories, with the extent to which they are recognized depending on clinical signs. Basically, the three types of problems are (1) the reflex or upper motor neuron (UMN) bladder (also known as *spastic* or *autonomic bladder*);(2) paralytic or lower motor neuron (LMN) bladder; and (3) the myogenic or nonneurogenic bladder. Either of the last two conditions can be associated with the atonic bladder syndrome, which is probably appropriate given the similarity of clinical signs and subsequent treatment once either of the two types of conditions is recognized. In fact, although signs of UMN bladder problems are initially different from those of the other two groups, this condition in horses also usually is not recognized until a degree of incontinence develops. A final form of bladder neuropathy that has been described in human beings and dogs, but not in horses, is reflex dyssynergia. In this condition, loss of coordination of detrusor contraction and urethral relaxation occurs, with the result that the animal may make efforts to urinate but fail to do so. Such cases frequently appear similar to those of urethral obstruction, and one must distinguished between them.

Although treatment options are limited and tend to be the same, regardless of what type of condition is responsible for the incontinence, determining the origin of the problem is prognostically important. To accomplish this, the internist needs to have a good working knowledge of how micturition normally is controlled.

17.12.1 Control of Micturition

From a neurologic perspective, one can regard the bladder as having a body and an outlet, which can be divided further into the neck (or trigone) and the proximal urethra. Functionally, the bladder alternates between filling and storage and emptying and elimination phases.[1] Dysfunction in any of these areas or phases may result in clinical problems. Somatic innervation is primarily to the striated muscle of the urethra via a branch of the pudendal nerve, which originates from the sacral cord (S1 to S2). Other branches of this nerve go to the anal sphincter and perineum. The sympathetic nerve supply is provided via the hypogastric nerve, with the preganglionic fibers arriving from L1 to L4 and synapsing in the caudal mesenteric ganglion. From there, postganglionic fibers supply the bladder (β_2-receptors) and proximal urethra (primarily α_1- and some α_2-adrenergic receptors).[2] Parasympathetic innervation originates in the sacral cord also, with neurons combining to form the pelvic nerve. Many complex interneuronal connections exist between sympathetic and parasympathetic nerves in the wall of the bladder, as well as small adrenergic cells that facilitate contact between sympathetic and parasympathetic pathways.[3] As a result, complete denervation of the bladder is virtually impossible. During the filling phase, the tone of the smooth and striated muscles that together comprise the external and internal urethral sphincters increases. These muscles are innervated by the pudendal 1290
nerve and the sympathetic nerves, respectively. Contraction of these muscles during filling maintains 1291
continence. Although the striated muscle forms a definite sphincter around the pelvic urethra, the anatomic existence of a bona fide internal sphincter is debatable.[4] However, the restriction to urine outflow that follows stimulation of the α-adrenergic receptors at the neck of the bladder has a sphincterlike effect. The smooth muscle of the bladder, referred to as the detrusor muscle, is innervated bythe parasympathetic pelvic nerve and β_2-adrenergic postganglionic fibers.

The storage or filling phase is dominated by sympathetic nerve activity and provides an excellent example of the effects of reciprocal innervation.[5] During filling, the detrusor muscle relaxes because of α-receptor–mediated inhibition of pelvic nerve afferents and stimulation of sympathetic β_2 receptors in the smooth muscle of the bladder body. The latter is a reflex response that involves sensory input from bladder stretch and pressure receptors via the afferent pelvic nerve fibers to the sacral cord, interneurons in the cord, and pre- and postganglionic sympathetic axons in the hypogastric nerve. Relaxation of this muscle allows accumulation of large volumes of urine with little or no increase in intravesicular pressure.

Intravesicular pressure starts to rise once detrusor muscle fibers are stretched fully. Receptors in the bladder wall detect these increases, and impulses are transmitted via the pelvic nerve and ascending spinoreticular cord tracks to the pons, cerebrum, and cerebellum, where they are interpreted as the sensation of bladder fullness. Signals responsible for voluntary micturition originate in the cerebrum and exert their influence via the brainstem, from which upper motor neurons descend in reticulospinal tracts to the sacral parasympathetic nuclei. This triggers the emptying phase. From these sacral segments, pelvic nerve impulses stimulate detrusor muscle contraction, action potentials traveling via parasympathetic ganglia in the pelvic plexus or bladder wall to postganglionic fibers that stimulate the smooth muscle. Depolarization waves spread throughout the bladder via tight junctions, resulting in a strong, coordinated, contractile process. Simultaneous inhibition of the pudendal nerve and hypogastric α- and β_2-sympathetic activity further facilitates detrusor muscle activity and relaxation of the external and internal urethral sphincters, respectively. Part of this inhibitory activity represents reflex neuronal activity linking pelvic and pudendal nerve axons in the sacral cord and inhibiting internuncial connections between sacral segments and the sympathetic neurons in the lumbar cord. Urethral sphincter relaxation also is coordinated centrally in a number of areas, including the cerebellum. Detrusor muscle

contraction pulls the bladder neck open, and micturition occurs. The emptying phase ends when the bladder stretch receptors sense that the bladder is empty and afferent parasympathetic (pelvic nerve) impulses cease. Pelvic nerve efferent activity also stops, and pudendal motor and sympathetic nerve activity resumes (because it is no longer inhibited), with the result that the detrusor muscle relaxes, restoring external and internal urethral sphincter tone.

17.12.2 Clinical Signs of Bladder Dysfunction

Control of bladder function obviously is complex, and many sites exist from which disruption of normal micturition could originate. In reality, problems are usually detectable in horses only when a degree of incontinence develops, which usually is manifested as constant or periodic dribbling of urine from the vulva or penis. In chronic cases, one frequently finds evidence of scalding and associated depilation of the perineum in mares and of the ventral abdomen in males, as well as the insides of the rear limbs in all animals. Any activity that results in increased intraabdominal pressure, such as coughing or exercise, may exacerbate signs or else be associated with their initial observation. Adult horses develop these problems much more frequently than do foals.

Academically, being able to differentiate between the different neurogenic forms of bladder dysfunction and those of myogenic origin may seem important. In reality, the clinical signs and treatments tend to be the same, regardless of cause. Upper motor neuron disease is characterized by increased urethral resistance, despite the presence of a full bladder, and may make catheterization or manual emptying of the bladder via rectal compression difficult. The condition usually occurs in association with broad, deep spinal cord lesions. In horses, these problems rarely are recognized in such cases because of the severe nature of associated clinical problems such as recumbency and myopathies. Frequently, such situations are deemed incompatible with life. (Possibly, a focal lesion caused by a disease such as equine protozoal myeloencephalopathy or an aberrant parasite migration could result in the development of spastic bladder without associated neurologic signs.)

In the event that horses suffering from this type of problem are able to stand, are kept in a sling, or have an isolated problem, in time they may develop the abilityto urinate reflexively. This reflex develops because ofthe stimulation of pressure receptors connected topelvic nerve afferents, which reflexively activate pelvic (parasympathetic) nerve efferents and the pudendal nerve. This activation results in detrusor muscle contraction and relaxation of the striated urethral muscle (external sphincter) leading to frequent urination, especially if the increases in abdominal pressure are regular, as may occur with any movement. These patients usually have 1291
some residual volume in the bladder after these voiding episodes. In such cases, urine dribbling usually is 1292
reported to occur intermittently, which is an important part of differentiating this type of dysfunction from paralytic (i.e., LMN) causes of incontinence.

As for the LMN bladder, lumbosacral trauma, equine herpes virus 1 myeloencephalitis, and cauda equina neuritis are probably the most common causes of this type of dysfunction, although in some parts of the world Sudan grass toxicity[6] and sorghum cystitis-ataxia (enzootic ataxia cystitis)[6,7] are major problems. Tumors of the lumbosacral spinal cord, such as lymphosarcoma and melanomas, are also capable of inducing paralytic bladder. Finally, iatrogenic paralysis following epidural administration of alcohol occasionally has been reported in show horses. Because this practice is taboo, how frequently the procedure leads to this problem is impossible to know.

With a paralytic (LMN) bladder, one usually sees other signs of LMN and lumbosacral dysfunction, including all or some of the following: loss of anal sphincter tone, tail paralysis, analgesia or hypalgesia over the perineum, atrophy of the muscles of the hip and hindleg, and hindlimb weakness. Damage to the pudendal

nerve and loss of external urethral sphincter integrity are therefore particularly important in the development of this problem. The bladder is atonic and distended with the urethral muscles relaxed, which results in urine dribbling because of overflow from the bladder. This incontinence may appear continuous, which helps to differentiate it from spastic (UMN) dysfunction. Sometimes the penis or vulva may appear paralyzed also. The prognosis is usually poor because of the development of secondary cystitis and general damage to the bladder wall and the detrusor muscle.

Myogenic problems occur mainly in geldings. Although myogenic problems can occur following obstructive effects of cystic calculi and cystitis, the problem generally lacks a specific identifiable cause. The condition generally develops slowly, in association with the accumulation of large amounts of sabulous or mucoid urinary sediment or sludge in the bladder, which mainly is comprised of calcium carbonate crystals. In time, the weight and volume of this material, when coupled with that of normal urine accumulation, progressively stretch the detrusor muscle. Incontinence is not normally notable until the cranial aspect of the bladder begins to protrude over the edge of the pubis, which results in craniad and ventrad displacement of the sediment, with the result that the bladder muscle is stretched beyond its normal modulus of elasticity and normal contraction and micturition is no longer possible. Severe distention and stretching also lead to a breakdown in tight junctions that prevents depolarizing waves from passing from muscle fiber to muscle fiber. Eventually, overdistention becomes so significant that the ability to maintain sphincter function is lost and incontinence develops. These cases usually occur without any other signs of neurologic disease; however, because of the lack of any identifiable cause or specific pathophysiologic mechanism, specific focal lesions of a peripheral nerve suchas the hypogastric nerve in fact could lead to a similar syndrome. Certainly, retention of urine in the bladder for any period results in the deposition of large quantities of sediment that in turn exacerbate the problem. One must recognize that part of the problem in cases of myogenic atony may stem from secondary cystitis, which develops following retention of urine for any period. The urea in retained urine breaks down to ammonia, which is irritating to the mucosal wall. Subsequent inflammation helps to damage the bladder musculature further.

Cystitis and chronic urethritis per se can be nonneurogenic, nonmyogenic causes of apparent incontinence. Irritation of stretch receptors in the bladder wall apparently causes regular stimulation of stretch receptors in parasympathetic afferents and stimulates detrusor contractions that cannot be inhibited voluntarily. This effect results in an apparent increase in the frequency of urination (pollakiuria) and an inability to control when it occurs. The condition frequently is referred to as urge incontinence and also may occur in association with a unilateral ectopic ureter in which the bladder is much smaller than usual (because of disuse) and incapable of storing a normal volume of urine. The frequency of micturition again is increased.

Hypoestrogenism also has been reported as a cause of nonneurogenic incontinence in an 18-year-old female Shetland pony.[8] A similar condition has been noted in older spayed bitches. The pathophysiologic mechanism of the condition is not known but probably is linked to a modulating effect of estrogen on the effects norepinephrine exerts on α-adrenergic receptor activity in the internal urethral sphincter.[9] In the documented case, the patient responded well to small doses (2 mg) of estradiol cypionate given intramuscularly every other day.

A transient postoperative condition that results in urine retention following abdominal or perineal operations has been noted in human beings.[10] The cause has not been explained, although the hypothesis is that a reflex depression of parasympathetic nerves may be responsible for detrusor muscle stimulation. This condition is distinct from that which may be associated with pain and reluctance to contract abdominal muscles following surgery. Whether such a condition exists in horses is unknown.

17.12.3 Diagnosis

Careful general physical and neurologic examinations are the basis for characterization of the type of bladder dysfunction that exists in the equine patient and the basis for any efforts aimed at identifying a cause. Although never good, the prognosis appears to be most positive in cases in which the horse still can generate significant increases in intravesicular pressure. Therefore performance of tests aimed at evaluating bladder and urethral pressure-generating capacities may be diagnostically useful. Cystometry, which involves the inflation of the bladder with volumes of sterile water, isotonic saline, or carbon dioxide, has been described in horses and pony mares.[11,12] Briefly, one introduces a large-gauge (No. 30 French) catheter into the bladder and connects it via a three-way stopcock to an infusion pump and pressure transducer. The pressure transducer is connected to a chart recorder. One fills the bladder until micturition occurs. Intravesicular pressure is recorded continuously. Usually a gradual increase in pressure is related to the infusion of fluid until the pressure suddenly rises, reflecting the onset of detrusor muscle contraction. The pressure at the point of this sharp increase is regarded as the contraction threshold. In normal horses, this threshold is about 90 ± 20 cm H_2O.[11] One determines the urethral pressure profile in the same test by using a catheter with multiple side openings and positioning the tip at the urethral sphincter. One then fills the catheter with fluid and withdraws it at a constant rate while recording the intraurethral pressure. Normal values are usually greater than 50 cm H_2O. Pressures were significantly lower in recordings of three incontinent mares.[12] Although these tests have not been used widely, they have considerable prognostic potential. Put simply, the higher the pressures are, the better the prognosis is. One also can determine the distance over which high pressure is present in the urethra from these tests. Such determination also maybe important, for it gives some further information on the integrity of the urethral sphincters.

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17.12.4 Treatment

Some horses with UMN disease recover gradually, especially if one can determine and treat a specific cause. A major complication with paralytic and myogenic forms of bladder dysfunction is that cases often are not recognized until the atonia is irreversible. By that time the prognosis tends to be poor and identification of an initiating cause is difficult—and likely irrelevant. In such cases, the treatment is often futile; however, one often can determine this definitively only by assessing the response to attempted treatment. Regardless of whether dealing with UMN, LMN, or myogenic disorders, treatment tends to be the same concerning the bladder.

If one identifies a definitive cause such as equine herpes virus 1 myeloencephalitis or equine protozoal myeloencephalopathy, then one should institute the specific treatment for that disease. Concerning bladder dysfunction, the basic aim is to provide support while hoping for spontaneous recovery, which may take a long time (e.g., months in cases of pelvic nerve damage). A basic aim of therapy is to avoid retention of urine because of secondary problems that this entails. Therefore promotion of bladder emptying is an important goal that one may attempt to reach by regular catheterization or the use of an indwelling catheter. (In males, one normally inserts such catheters via a perineal urethrostomy.) Although facilitating drainage, intermittent or indwelling catheterization appears to predispose the horse to secondary bacterial cystitis and therefore should not be used without some forethought. Some horses that are chronically incontinent seem to survive comfortably without any catheterization.[7] Antimicrobial therapy is important for treating any incontinence cases but is especially critical when one uses indwelling or regular catheterization.

One may give the α-adrenergic blocker phenoxybenzamine (0.7 mg/kg per os every 6 hours) to eliminate any urethral resistance, thus facilitating emptying in the reflex UMN problem or in situations of great atonic overdistention. Bethanechol chloride is a parasympathomimetic agent resistant to the action of acetylcholinesterase and apparently has a selective effect on the smooth muscle of the gastrointestinal tract and bladder. The drug is used principally to stimulate detrusor muscle activity, and it acts by stimulating postganglionic parasympathetic effector cells rather than motor end plates. The recommended dose ranges from 0.25 to0.75 mg/kg subcutaneously 3 times a day. Starting the horse on the smallest dose is recommended. This drug reportedly has varying results.[13] Bethanechol has no effect when the bladder is completely atonic or areflexic. If the muscle is capable of generating weak contractions, then bethanechol may be useful. One could determine whether contractions are possible by cystometry. Although bethanechol can increase intravesicular pressure, whether it helps evacuate the bladder also depends on the status of the urethral sphincter and striated muscle. One must remember that drugs such as phenoxybenzamine and bethanechol can have undesired side effects in other body systems. The use of general muscle relaxants and an α-adrenergic blocker may be useful for achieving urethral muscle relaxation. Diazepam (0.2 to 0.5 mg/kg intravenously) and dantrolene are the most commonly used relaxants. Diazepamis effective in large doses, which also usually result in sedation. Dantrolene (10 mg/kg per os loading dose; 2.5 mg/kg per os maintenance every 6 hours) slows release of calcium from the sarcoplasmic reticulum and has been tried in dogs with varying effects.

Surgical removal of the sabulous sludge found in a number of these horses has been tried via perineal 1293

urethrostomy or cystotomy, with poor results.[14] Removal via cystotomy is not recommended because of 1294
difficulties in evacuating the material without contaminating the peritoneal cavity. Perineal urethrotomy, combined with irrigation with large volumes of fluid while the horseis anesthetized, seems to be the most effective way of removing this material. However, the prognosis is still poor because of chronic irreversible changes in the bladder wall, which seem to prohibit any possible return of normal detrusor muscle function.

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18 CHAPTER 18 DISORDERS OF THE ENDOCRINE SYSTEM

Ramiro E. Toribio

18.1 18.1—Calcium Disorders

Ramiro E. Toribio

18.1.1 Calcium

Calcium (molecular weight 40.08) is one of the most abundant elements in nature. In vertebrates, calcium is important in physiologic functions such as muscle contraction, hormone secretion, enzyme activation, cell division, cell membrane stability, neuromuscular excitability, and blood coagulation.[1] Calcium also regulates processes that result in cell injury and cell death, such as free radical production, cytokine release, protease activation, vasoconstriction, and apoptosis. Because of the importance of calcium in normal intracellular and extracellular processes, maintenance of a steady concentration of calcium is important.

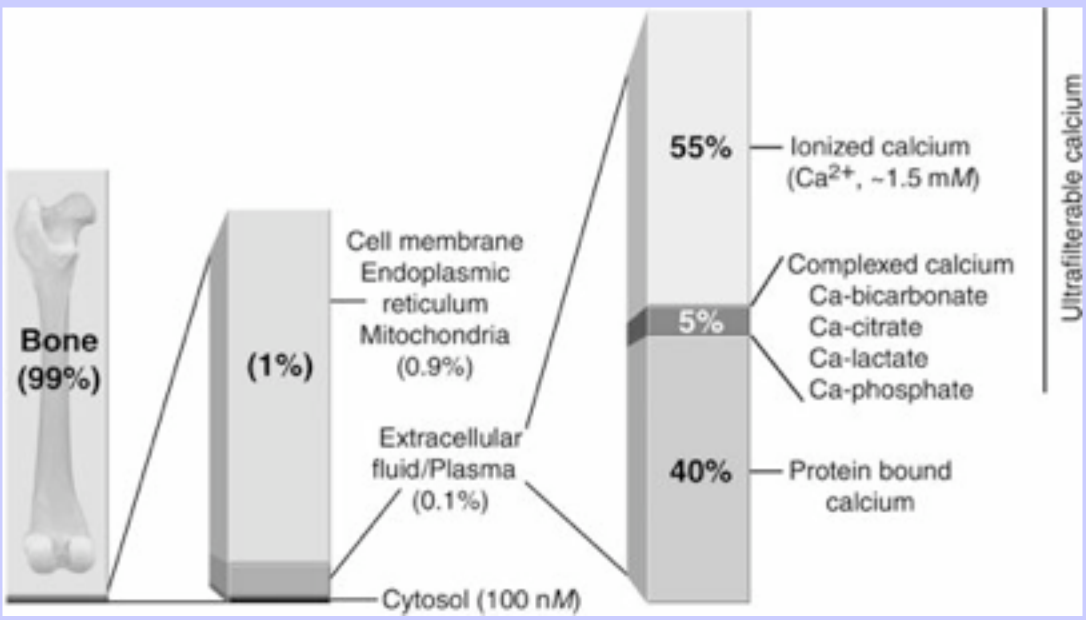
18.1.1.1 DISTRIBUTION AND PHYSICAL PROPERTIES OF CALCIUM

In the body, calcium has structural and nonstructural functions and is found in three main compartments: the skeleton, soft tissues, and the extracellular fluid. The skeleton contains approximately 99% of the total body calcium as hydroxyapatite crystals ($Ca_{10}[PO_4]_6[OH]_2$). Calcium is a major component of the skeleton, providing support against gravity, protection of vital internal organs (brain, spinal cord, thoracic organs), and a niche for blood-forming elements. The skeleton also acts as a reservoir for calcium. The nonstructural functions are related to calcium as a regulatory ion. The remaining calcium is present in the cell membrane, mitochondria, and endoplasmic reticulum (0.9%) and in the extracellular fluid (0.1%).[1] In blood, virtually all calcium is in plasma, in which calcium exists as a free or ionized form (50% to 55%), bound to proteins (40% to 45%), and complexed to anions such as citrate, bicarbonate, phosphate, and lactate (5% to 10%) (Figure 18.1-1).[1,2] In horses, serum ionized calcium represents 55% to 58% of the total serum calcium concentration.[3,4] Free or ionized calcium (Ca^{2+}) is the biologically active form of calcium. Of the protein-bound calcium, approximately 80% is associated with albumin and 20% with globulins.

Calcium binds to negatively charged or anionic proteins, and the affinity of calcium for anionic sites is pH-dependent. During acidosis, Ca^{2+} binding to anions decreases because of increased H^+ concentrations, resulting in increased plasma Ca^{2+} concentrations. Alkalosis results in lower Ca^{2+} concentrations. In human beings, for each 0.1 unit change in the blood pH, an inverse change of 0.2 mg/dl (0.05 mmol/L) in Ca^{2+} concentrations has been calculated to occur. In healthy horses, Kohn and Brooks[3] found that the plasma Ca^{2+} percentage (Ca^{2+}/total calcium) was weakly correlated with plasma pH. However, in vitro manipulation of serum pH did not reveal a statistically significant relationship between serum pH and Ca^{2+} percentage. The authors speculated that the lack of relationship between serum pH and Ca^{2+} percentage probably resulted from heterogeneity of Ca^{2+} percentage among horses and from the presence of other unmeasured variables that may affect Ca^{2+} binding. Hypoalbuminemia results in total hypocalcemia (pseudohypocalcemia), with Ca^{2+} concentrations remaining within the normal range, unless the primary cause of hypoalbuminemia also is

altering calcium homeostasis (sepsis, systemic inflammatory response syndrome, severe gastrointestinal disease).

Figure 18.1-1 Calcium distribution in the body. The skeleton contains approximately 99% of the total body calcium. The remaining calcium is present in the cell membrane, mitochondria, endoplasmic reticulum, and in the extracellular fluid. In blood, calcium exists as a free or ionized form, bound to proteins, and complexed to anions such as citrate, bicarbonate, phosphate, and lactate. In horses, serum ionized calcium represent 55% to 58% of the total serum calcium concentration.



Cytosolic calcium concentrations ($Ca^{2+}{}_i$) are low (1×10^{-6} to 10^{-3} *M*) compared with extracellular Ca^{2+} concentrations (1×10^{-3} *M*). This results in a 10,000-fold calcium gradient between the extracellular fluid and the cytosol that makes calcium an important cation in signal transduction. In the cytosol, $Ca^{2+}{}_i$ is buffered by phospholipids, dicarboxylic acids, and several calcium-binding proteins. Approximately 95% of $Ca^{2+}{}_i$ is in the endoplasmic reticulum and mitochondria (see Figure 18.1-1). Low $Ca^{2+}{}_i$ concentrations are maintained by calcium-binding proteins (caldindin, calmodulin, calsequestrin, caldecrin), calcium pumps (Ca^{2+}-ATPase), and calcium exchangers (Na^+/Ca^{2+} exchangers).

18.1.1.2 CALCIUM SIGNALING

In most cells the signaling properties of calcium depend on increasing Ca^{2+}_i. Calcium concentrations within the cells depend on mechanisms that regulate calcium influx, efflux, and calcium release.[5] Mechanisms that control changes in Ca^{2+}_i concentrations include Ca^{2+} channels in the plasma membrane, the endoplasmic reticulum, and the sarcoplasmic reticulum. Some of the mechanisms that remove Ca^{2+}_i from the cytosol include Ca^{2+}-ATPases in the plasma membrane, endoplasmic reticulum, and sarcoplasmic reticulum, and Na^+/Ca^{2+} exchangers on the plasma membrane. The mitochondria are also important in regulating Ca^{2+}_i by providing energy for active calcium transport and by acting as a calcium storage.

18.1.1.3 CALCIUM ENTRY FROM THE EXTRACELLULAR COMPARTMENT

Calcium enters the cell through cation channels, such as the fast sodium channels; however, most Ca^{2+} influx occurs through Ca^{2+}-specific channels. A vast number of Ca^{2+}-specific channels are present on the cell membrane. Voltage-operated (voltage-gated) Ca^{2+} channels (VOCCs) are present in excitable cells and are activated by depolarization. Ligand- or receptor-operated Ca^{2+} channels include a large number of channels that are present primarily in secretory cells and nerve terminals. Examples of receptor-operated Ca^{2+} channels include the nicotic acetylcholine and the *N*-methyl-d-aspartate receptors. These channels are activated by binding of an agonist (serotonin, glutamate, acetylcholine) to an extracellular domain. Mechanically activated Ca^{2+} channels are present in cells responsive to changes in cell shape such as smooth muscle cells and cardiac myocytes.

18.1.1.4 CALCIUM RELEASE FROM INTRACELLULAR STORES

The release of calcium from intracellular stores depends on messenger-activated channels present in the
endoplasmic reticulum, sarcoplasmic reticulum, and mitochondria. For example, by interacting with specific 1296
cell membrane receptors, hormones may activate phospholipase C to hydrolyze phosphatidylinositol 4,5- 1297
biphosphate to inositol 1,4,5-triphosphate (IP_3) and diacylglycerol. Inositol 1,4,5-triphosphate is highly diffusible and interacts with IP_3 receptors on the endoplasmic reticulum and sarcoplasmic reticulum, opening calcium channels and increasing the Ca^{2+}_i. The ryanodine receptors (activated by the plant alkaloid ryanodine) are similar to the IP_3 receptors; however, they are present in excitable cells (muscle and neurons), and their activation also increases the Ca^{2+}_i.

Calcium is introduced into the cytoplasm in short time (millisecond) pulses (plumes) around the mouth of the different calcium channels. These signals have to be short (spikes) because high Ca^{2+}_i is toxic to the cell. The frequency of the spikes is proportional to cell stimulation, and the sensors for the spikes are the calcium-binding proteins. The calcium-calmodulin complex then activates enzymes, resulting in protein phosphorylation. Striated muscle also has a pulsatile Ca^{2+} signaling system activated by depolarization. With the depolarization of the sarcolemma, Ca^{2+} enters the cell via VOCCs, and the VOCCs also interact with the ryanodine receptors, inducing Ca^{2+}_i release from the sarcoplasmic reticulum (calcium-induced calcium release) to promote muscle contraction.

18.1.1.5

MEMBRANE POTENTIALS

Electric potentials are present in every cell in the body, and they result from the diffusion of ions across the cell membrane. The membrane potentials result primarily from the diffusion of K^+ and Na^+ because these ions are subject to higher electrochemical gradients. Some cells (neurons and muscle cells) are excitable and capable of self-generating electric impulses because their cell membrane is “leaky” to these ions. Concentrations of K^+ are higher inside the cell, and as these ions diffuse outward, they line up along the outside of the membrane, carrying positive charges; the result is an electropositive membrane on the outside and electronegative membrane in the inside because anions remain inside. Concentrations of Na^+ are higher in the outside and their diffusion to the inside can create electropositivity inside the cell. A potential in the cell prevents the net diffusion of ions across the membrane in either direction; this is the Nernst potential that one can calculate by the *Nernst equation*. The membrane potential in nerve fibers is around –90 mV, meaning that the potential inside the fiber is 90 mV more negative than the outside. Cells maintain their membrane potentials by transporting Na^+ and K^+ across the membrane by different mechanisms. The Na^+, K^+-ATPase is an electrogenic pump that pumps three Na^+ outside the cell for each two K^+ to the inside, creating large concentrations gradients across the nerve membrane (Na^+, 140 mEq/L outside, 14 mEq/L inside; K^+, 4 mEq/L outside, 140 mEq/L inside). The K^+/Na^+ leak channels are in the nerve membrane and are more permeable to K^+ than to Na^+; this leakage is important in determining the membrane potential. The resting membrane potential in large skeletal muscle fibers is similar to that of nerve fibers (–90 mV). In small striated fibers, small nerves, neurons in the central nervous system, and smooth muscle, the membrane potential ranges between –40 to –60 mV. The role of calcium in the resting membrane potential is minimal.

18.1.1.6

ACTION POTENTIAL

Excitable cells are particular for their ability to propagate a signal in the form of action potentials, which are rapid changes in the cell membrane potential. The action potential has a resting stage in which the membrane is polarized. In the depolarization stage, the membrane becomes rapidly permeable to Na^+, positive charges move inside the nerve fiber, and the polarized state (–90 mV) is lost. Immediately after depolarization, the Na^+ channels become less permeable to Na^+, and K^+ channels open to return the cell membrane to the resting stage (repolarization). Under resting conditions, the cell membrane is relatively impermeable to Ca^{2+}, and many cells have a Ca^{2+} pump that extrudes Ca^{2+} from the inside to the outside (or into the endoplasmic reticulum or sarcoplasmic reticulum), creating a large Ca^{2+} gradient across the cell membrane that is important for Ca^{2+} to act as a signaling ion. Calcium channels (VOCCs) are slightly permeable to Na^+ and are also known as Ca^{2+}/Na^+ channels or slow channels (Na^+ channels are fast channels). Calcium ion channels are abundant in cardiac and smooth muscle. Few fast Na^+ channels are present in smooth muscle cells, and the action potential in these cells primarily results from activation of the Ca^{2+} channels.

During hypocalcemia, the fast Na^+ channels open with small changes in the membrane potential and the nerve fibers become excitable or discharge spontaneously, causing tetany. Calcium ions bind to the exterior of the Na^+ channels, altering the electric state of the channel itself and increasing the voltage (threshold) required for opening. By increasing the threshold potential, Ca^{2+} stabilizes the cell membrane, and this is the mechanism by which Ca^{2+} antagonizes the effects of hyperkalemia. Hyperkalemia increases the resting

membrane potential closer to threshold, thus increasing membrane excitability, whereas calcium administration raises the threshold potential, stabilizing membrane excitability.[2]

18.1.1.7 NEURONS

In addition to its effects on cell membrane excitability, Ca^{2+} is required for the release of neurotransmitters. When the neuronal action potential reaches the nerve terminal, VOCCs in the presynaptic membrane open to 1297
increase the Ca^{2+}_i. As a result, vesicles with neurotransmitters fuse with the terminal membrane, causing 1298
exocytosis into the synaptic space.[6]

18.1.1.8 SKELETAL MUSCLE

In the muscle fiber, Ca^{2+} is important in determining the threshold potential. Minimal increases in Ca^{2+}_i results in a series of events associated with excitation-contraction coupling. In addition, Ca^{2+}_i acts as a cofactor for enzymes important in energy release. Abnormalities in muscle fiber function are often an early sign of hypocalcemia. One may observe increased membrane excitability and muscle weakness.

In the skeletal muscle fiber the Ca^{2+} cycle starts with the depolarization of the sarcolemma and the transverse tubular system (T system), which leads to Ca^{2+} entry via VOCCs and Ca^{2+}_i release from the sarcoplasmic reticulum via the ryanodine and the dihydropyridine receptors. Approximately 95% of the Ca^{2+}_i required for muscle contraction is released by the sarcoplasmic reticulum. In the sarcoplasm, Ca^{2+}_i interacts with troponin C, causing conformational changes that release myosin-binding sites on the actin molecule, resulting in contraction. Therefore the higher the concentrations of Ca^{2+}_i, the greater the number of troponin free binding sites and the greater the force of contraction. Muscle contraction is terminated by the active pumping of Ca^{2+}_i into the sarcoplasmic reticulum. Calcium also activates glycogenolysis, providing energy for adenosine triphosphate generation, and therefore calcium works actively in skeletal muscle contraction and in the generation of energy necessary to sustain contraction.

18.1.1.9 CARDIAC MUSCLE CELLS

In the cardiac myocyte, Ca^{2+} also mediates excitation-contraction coupling. The action potential extends down T tubules where VOCCs are opened. The Ca^{2+} influx diffuses to the sarcoplasmic reticulum, triggering the release of Ca^{2+}_i (calcium-induced calcium release). As in skeletal muscle, intracellular stores of calcium are responsible for most of the calcium required for excitation and contraction. Calcium also contributes to the spontaneous depolarization of cardiac muscle through slow cation channels. Spontaneous depolarization results from a gradual increase in conductance of Na^+ and Ca^{2+}, the affinity for Na^+ being greater. Once threshold is achieved, fast Na^+ channels open, resulting in the fast upstroke (phase 0) of the cardiac action potential. As the membrane potential passes –30 to –40 mV mark, VOCCs open, allowing Ca^{2+} influx into the cell. These calcium channels remain open for a long time, resulting in the plateau (phase 2) of the cardiac action potential.

Hypocalcemia decreases the rate of the slow inward calcium current, prolonging the plateau phase of the cardiac action potential and the QT and ST segments of the electrocardiogram. With profound hypocalcemia, this prolonged phase results in slowing the heart rate, heart block, and arrhythmias and in decreasing the strength of contraction, or contractility, because of the lack of calcium to bind troponin C. Hypercalcemia, such as that induced by rapid administration of calcium for treatment of hyperkalemia, shortens the QT and ST segments, flattens the T wave, and may cause first-degree heart block. Severe hypercalcemia may cause asystole or ventricular arrhythmias, increasing heart rate and contractility. Therefore monitoring the heart rate of the horse whenever one administers calcium is always advisable.

18.1.1.10 SMOOTH MUSCLE CELLS

The smooth muscle fiber is small compared with the skeletal fiber, and Ca^{2+} ions can diffuse to most parts of the cell to induce contraction. The time for calcium to diffuse is the latent period (200 to 300 ms), which is the period before contraction begins. The latent period in smooth muscle cells is 50 times greater than in skeletal muscle.[6] Smooth muscle cells are stimulated by voltage, stretching, and hormones. Smooth muscle cells have more VOCCs and less voltage-gated Na^+ channels than skeletal muscle fibers, and therefore Na^+ is of less importance in generating the action potential. Hormones such as epinephrine, norepinephrine, acetylcholine, serotonin, vasopressin, and histamine interact with specific receptors to stimulate or inhibit Ca^{2+} influx into the cell.

In skeletal muscle almost all Ca^{2+} required for excitation and contraction comes from the sarcoplasmic reticulum. In contrast, in the smooth muscle cell the sarcoplasmic reticulum is rudimentary and most of the Ca^{2+} required for contraction comes from the extracellular fluid. This has clinical implications, for hypocalcemia may result in ileus.

18.1.1.11 COAGULATION

Calcium ions (factor IV) are necessary for the coagulation process to occur, and Ca^{2+} is a cofactor required by factors II, VII, IX, X, XI, XII, and XIII, and without $^{2+}$ blood clotting does not occur. However, the Ca^{2+} concentration required for coagulation is minimal, and even during severe hypocalcemia, low Ca^{2+} concentrations do not seem to interfere with the clotting process. Other clinical conditions associated with hypocalcemia are more likely to result in animal death than hypocoagulation.

18.1.1.12 LACTATION

Calcium demands in the growing neonate are high, and they must be provided by the mother. As a result of the high calcium needs, the daily requirements for calcium in the lactating mother at the onset of lactation are twice maintenance. Mare's milk calcium concentration ranges from 1.3 g/kg of fluid milk during the first 2 1298
weeks of lactation to 0.8 g/kg of milk on weeks 15 to 17.[7,8] In a 500-kg mare producing 15 kg of milk per 1299
day with a calcium concentration of 1.2 g/kg of milk this represents a demand of 36 g of calcium (15 kg of milk × 1.2 g of calcium/50% absorption) a day, in addition to maintenance. Most of the calcium present in milk is complexed to organic bases and casein.

18.1.2 ## Calcium in the Horse

18.1.2.1 ### REQUIREMENTS

Calcium and phosphorus requirements in horses and ponies depend on the age, physiologic status, and amount of work or exercise performed (Table 18.1-1). Horses do not have a nutritional drive to meet their calcium requirements and depend highly on the amount of calcium present in their diet. Because extracellular Ca^{2+} is under the homeostatic control of several factors, serum Ca^{2+} concentration is not a reliable indicator of dietary calcium intake. An acceptable diet for horses must contain 0.15% to 1.5 % of calcium in feed dry matter and 0.15% to 0.6% of phosphorus in feed dry matter (Table 18.1-2). A calcium/phosphorus ratio less than 1:1 may have negative effects on calcium absorption and skeletal development; however, a calcium/phosphorus ratio as high as 6:1 for growing horses may not be detrimental if phosphorus intake is adequate.[9] Adult horses should receive approximately 40 mg/kg/day of calcium. These requirements depend on the physiologic status of the animal; pregnant mares require around 50 to 60 g of calcium per day, whereas lactating mares and growing horses may require 50 to 75 g of calcium per day. The average horse has been estimated to need to absorb 20 to 25 mg of calcium and 10 to 12 mg of phosphorus per kilogram per day to balance losses.[10–12] According to the National Research Council,[13] the maximum tolerable amount of phosphorus in horses fed adequate amounts of calcium is 1%.Tables 18-3 and 18-4 present the calcium and phosphate content in some mineral supplements and equine feeds.

TABLE 18.1-1 Calcium and Phosphorus Requirements in Horses

AGE/CONDITION OF HORSE	PERCENT IN THE DIET		DAILY INTAKE (g)	
	Ca	P	Ca	P
Foals (<6 months)	0.80	0.55	33	20
Weanlings	0.60	0.45	34	25
Yearlings	0.50	0.35	31	22
Two-year-old	0.40	0.30	25	17
Mare, late pregnancy	0.45	0.30	34	23
Mare, lactation	0.45	0.30	50	34
Mature horses	0.30	0.20	23	14

Adapted from Schryver HF, Hintz HF: Minerals. In Robinson NE, editor: *Current therapy in equine medicine,* ed 2, Philadelphia, 1987, WB Saunders.

TABLE 18.1-2 Acceptable Ranges of Minerals and Vitamins in Feed of Horses[23,24,208–210]

MINERAL/VITAMIN	IN DRY MATTER
Calcium	0.25%-1.5%
Potassium	0.15%-0.6%
Magnesium	0.08%-0.16% (14 mg/kg body mass)
Vitamin D	300–800 IU/kg

18.1.2.2 ABSORPTION

Equids absorb a larger proportion of dietary calcium compared with ruminants.[14] Horses absorb calcium and phosphorus with high efficiency and with little effect of age.[12,14] Studies in horses of different ages revealed that horses fed diets with adequate amounts of calcium absorb 50% to 75% of the calcium and less than half the phosphorus present.[12,15] Calcium in most forages for horses is more than 50% to 60% digestible (Table 18.1-5). The efficiency of calcium absorption is related inversely to the calcium content of the diet. The proximal half of the small intestine is the main site for calcium absorption in the horse, followed by the distal small intestine and the dorsal colon.[12,16] The amount of calcium absorbed in the dorsal colon is minimal, and the cecum and ventral colon are mainly secretory sites for calcium (Figure 18.1-2). A high content of phosphate (or phytate) inhibits calcium absorption,[17] whereas a high content of calcium in the diet has minimal effect on phosphorus absorption.[18] Oxalate reduces calcium absorption; a 1% oxalate content in an 1299
equine diet reduced calcium absorption by 66% and increased fecal calcium excretion.[19] Oxalate content in 1300
the diet higher than 0.5% or a calcium/oxalate ratio less than 0.5 can result in a negative calcium balance.[20] Diets containing 0.5% and 0.87% of oxalate had no effect in calcium absorption when the calcium/oxalate ratios were higher than 1.7.[21] Table 18.1-6 lists some plants containing harmful amounts of oxalate.

TABLE 18.1-3 Calcium and Phosphorus Content (Percent) in Some Mineral Supplements

MINERAL SUPPLEMENT	CALCIUM CONTENT	PHOSPHORUS CONTENT
Calcium carbonate	34	0
Defluorinated phosphate	32	15
Bone meal	30	14
Dicalcium phosphate	27	21
Monocalcium phosphate	17	21
Monosodium phosphate	0	22
Calcium gluconate 23%	2.14*	0

Adapted from Schryver HF, Hintz HF: Minerals. In Robinson NE, editor: *Current therapy in equine medicine,* ed 2, Philadelphia, 1987, WB Saunders.

* Elemental calcium calculated based on the molecular weight of calcium gluconate hemicalcium salt. Each milliliter of the 23% solution contains 21.4 mg of elemental calcium.

TABLE 18.1-4 Mineral Composition of Some Equine Feeds on a Dry Matter Basis

SOURCE	Ca (%)	P (%)	Mg (%)
Alfalfa	1.71	0.30	0.36
Alfalfa hay	1.41	0.21	0.34
Timothy	0.40	0.26	0.16
Timothy hay	0.51	0.29	0.13
Bluegrass	0.50	0.4	0.18
Oat hay	0.32	0.25	0.29
Orchard grass	0.25	0.39	0.31
Barley	0.05	0.37	0.15
Corn	0.05	0.60	0.03
Oats	0.09	0.38	0.16
Wheat	0.05	0.42	0.14
Cottonseed meal	0.18	1.22	0.59
Linseed	0.43	0.90	0.67
Skim milk	1.36	1.09	0.13
Soybean meal	0.40	0.71	0.31
Molasses, cane	1.10	0.15	0.47
Wheat bran	0.14	1.27	0.63

Adapted from the National Academy of Sciences: *Nutrient requirements of horses,* ed 5, Washington, DC, 1989, National Research Council.

The dietary cation-anion balance (DCAB) affects Ca^{2+} absorption; a low DCAB increases intestinal Ca^{2+} absorption, whereas a high DCAB has the opposite effect. A decrease in DCAB in horses resulted in increased Ca^{2+} concentrations.[22]

TABLE 18.1-5 Availability (Percent) of Calcium and Phosphorus in Some Equine Feeds and Supplements

SOURCE	Ca	P
Corn	—	38
Timothy hay	70	42
Alfalfa hay	77	38
Milk products	77	57
Wheat bran	—	34
Limestone	67	—
Dicalcium phosphate	73	44
Bone meal	71	46
Monosodium phosphate	—	47

Adapted from Schryver HF, Hintz HF: Minerals. In Robinson NE, editor: *Current therapy in equine medicine*, ed 2, Philadelphia, 1987, WB Saunders.

TABLE 18.1-6 Plants Containing Harmful Amounts of Oxalate

COMMON NAME	SCIENTIFIC NAME
Bermuda grass	*Cynodon dactylon*
Buffel grass	*Cenchrus ciliaris*
Dallas grass	*Paspalum* spp.
Foxtail grass	*Setaria* spp.
Greasewood	*Sarcobatus vermiculatus*
Halogeton	*Halogeton glomeratus*
Kikuyu grass	*Pennisetum clandestinum*
Lambsquarters	*Chenopodium* spp.
Napier grass, mission grass	*Pennisetum* spp.
Pangola grass	*Digitaria decumbens*
Panic grass	*Panicum* spp.
Para grass	*Brachiaria* spp.
Purple pigeon grass	*Setaria incrassata*
Purslane	*Portulaca oleracea*
Red-rooted pigweed	*Amaranthus* spp.
Rhubarb	*Rheum rhaponticum*
Russian thistle, tumbleweed	*Salsola* spp.
Setaria	*Setaria sphacelata*
Sorrel	*Rumex* spp.
Soursob, Shamrock	*Oxalis* spp.
Sugar beet	*Beta vulgaris*

These plants have an oxalate content higher than 0.5% dry matter or have a calcium/oxalate ratio of less than 0.5. Ingestion of these plants can result in calcium deficiency and clinical signs consistent with nutritional secondary hyperparathyroidism. Some of these plants also may cause gastrointestinal irritation and diarrhea.

Information on the effect of magnesium on calcium and phosphorus absorption in the horse is limited,[23,24] and no controlled studies have evaluated the effects of excessive dietary magnesium. No changes in serum calcium and phosphorus concentrations were detected in foals fed a magnesium-deficient diet; however, mineralization of the aorta was present.[23]

Glucocorticoids affect the calcium metabolism in the horse. Dexamethasone administration to pony foals resulted in decreased intestinal absorption of calcium, decreased bone resorption, and an increased urinary excretion of calcium.[25,26]

Phosphorus absorption in the horse ranges from 30% to 55% and occurs in the small and large intestines. The dorsal colon and small colon, followed by the distal small intestine, are the most effective sites for phosphorus absorption in the horse[12,27,28] (Figure 18.1-3). The proximal small intestine, cecum, and ventral colon secrete phosphorus into the lumen, and most of this phosphorus is reabsorbed in the next segment of intestine. High aluminum and phytate contents in the diet reduce phosphate absorption. Some phytase activity is present in the large colon of the horse and can release some phytate phosphorus to be absorbed.[29] The mechanisms responsible for calcium and phosphorus absorption are discussed elsewhere in this section. 1300

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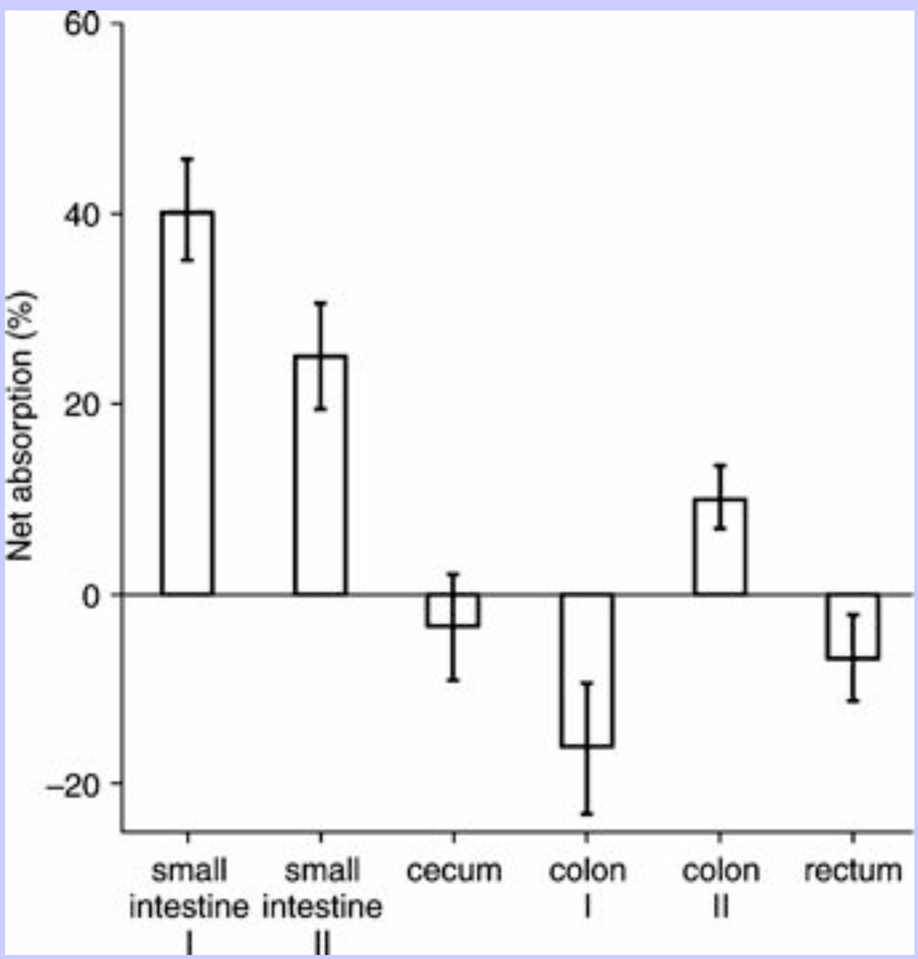
Figure 18.1-2 Net absorption of calcium from the intestine of the horse. The intestine was divided in six regions: proximal *(small intestine I)* and distal *(small intestine II)* halves of the small intestine, cecum, proximal *(colon I)* and distal *(colon II)* large colon, and transverse colon and rectum *(rectum)*. (Adapted from Schryver HF, Craig PH, Hintz HF et al: The site of calcium absorption in the horse, *J Nutr* 100:1127–1131, 1970.)

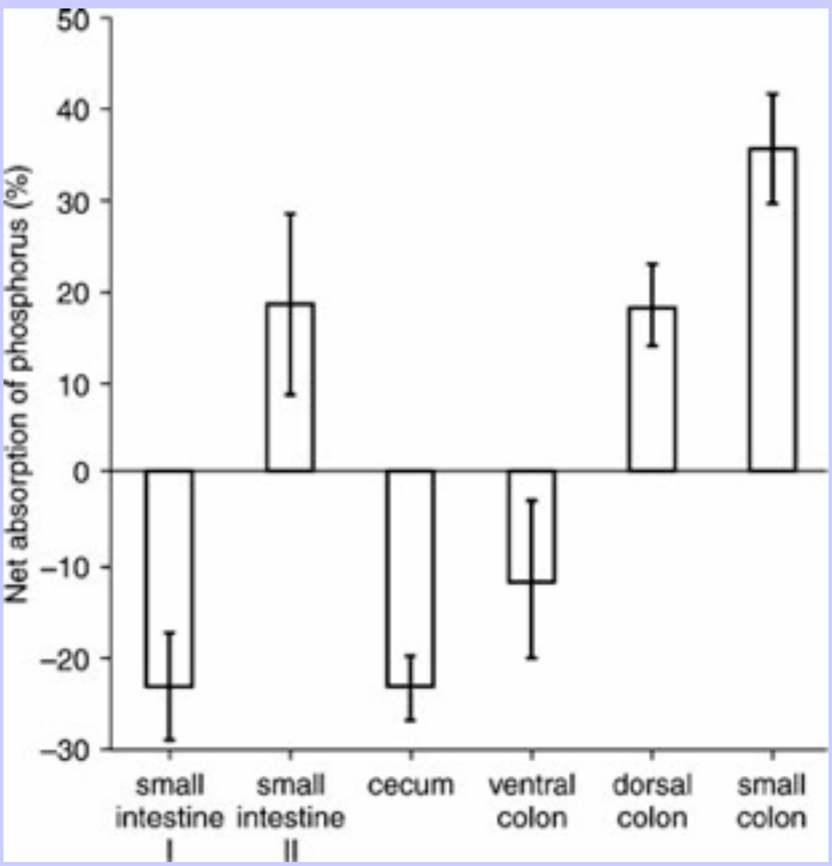


18.1.2.3 ELIMINATION

Calcium is eliminated from the animal through the kidneys, milk, sweat, feces, and the fetus. Because horses absorb a greater proportion of their dietary calcium, their feces contain a lower concentration of calcium and a lower Ca:P ratio than feces of ruminants when these animals are fed equivalent diets.[12,14]

Endogenous losses of calcium in horses have been estimated at 20 to 25 mg/kg of body mass per day.[10] Assuming a 50% calcium digestibility, a 500-kg horse would require 20 g of calcium to replace losses, or 40 mg/kg/day; growing and lactating horses can double these requirements. The amount of calcium eliminated depends on the physiologic status of the animal and on the amount of calcium ingested, the presence of substances that may interfere with calcium absorption (phosphates, oxalates, phytates), and disease. If horses receive adequate amounts of calcium and phosphorus, the urinary excretion of calcium exceeds that of phosphorus.[30,31] The urinary fractional clearances of calcium and phosphorus and the ratio of urinary to serum calcium and phosphorus have been proposed as methods to estimate calcium and phosphorus intake.[31] Interpretation of the fractional clearance of calcium could be difficult because horses eliminate large amounts of calcium in urine.[32]

Figure 18.1-3 Net absorption of phosphorus from the intestine of ponies fed an alfalfa diet.



One can calculate the fractional clearance of calcium or phosphorus using the following formula:

$$\frac{\text{Urine Ca}^{2+}\text{(or P)}}{\text{Serum Ca}^{2+}\text{(or P)}} \times \frac{\text{Serum creatinine}}{\text{Urine creatinine}} \times 100$$

18.1.2.4 DEFICIENCY

Calcium deficiency can be acute or chronic. Chronic calcium deficiency generally is manifested as abnormal cartilage and bone development (developmental orthopedic disease) and lameness. Horses with acute calcium deficiency have clinical signs associated with neuromuscular excitability. When one suspects a calcium deficiency, feed analysis is recommended to determine whether dietary calcium content is adequate.

18.1.2.5 PHOSPHORUS

The clinical signs associated with phosphorus excess generally result from calcium deficiency and include lameness, abnormal cartilage and bone development, fractures, and osteodystrophia fibrosa (nutritional secondary hyperparathyroidism). Phosphorus deficiency can be manifested as pica and developmental orthopedic disease. Serum inorganic phosphorus (P_i) concentration is more indicative of dietary phosphorus intake and status than serum calcium because the homeostatic control for P_i is not as precise as that of calcium. 1301

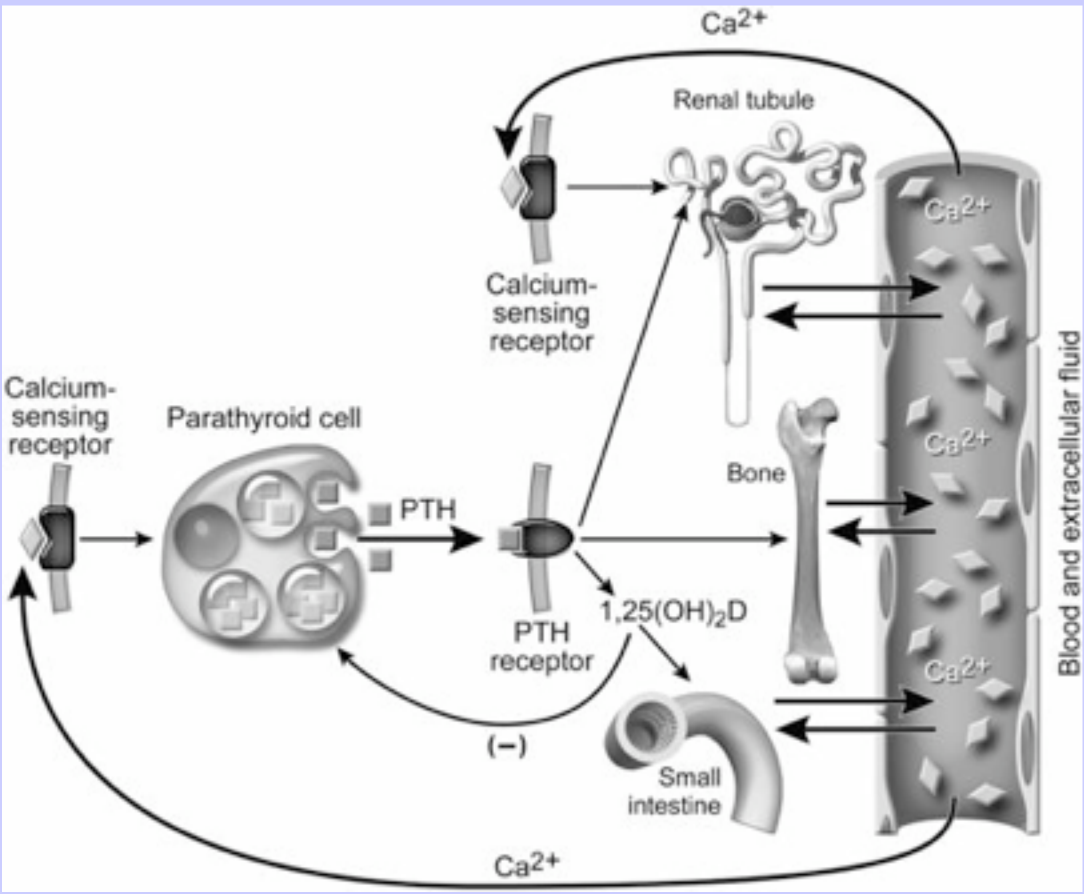
18.1.3 Calcium Homeostasis 1302

Regulation of extracellular ionized calcium (Ca^{2+}) concentration is controlled by a complex homeostatic system that includes three major hormones: parathyroid hormone (PTH), calcitonin, and 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] or calcitriol).[2,4,33] Parathyroid hormone–related protein (PTHrP) is a recently discovered protein that shares considerable homology with PTH, binds and activates the PTH-1 receptor, and is important for cell growth and differentiation and in calcium transport across membranes.[34,35] Parathyroid hormone increases during hypocalcemia, whereas calcitonin increases during hypercalcemia.

18.1.3.1 PARATHYROID HORMONE

Parathyroid hormone is responsible for the minute- to-minute regulation of extracellular Ca^{2+} concentrations. The biologic functions of PTH include stimulation of osteoclastic bone resorption, thereby increasing Ca^{2+} release into circulation, stimulation of Ca^{2+} reabsorption and inhibition of phosphate reabsorption in the renal tubules, and stimulation of calcitriol synthesis in the kidney (Figure 18.1-4). Calcitriol then increases Ca^{2+} and phosphate absorption in the intestine and inhibits PTH secretion in the parathyroid gland.[1]

Figure 18.1-4 Parathyroid hormone and calcium homeostasis. The biologic functions of parathyroid hormone include stimulation of osteoclastic bone resorption, stimulation of renal Ca^{2+} reabsorption, inhibition of phosphate reabsorption in the renal tubules, and stimulation of calcitriol synthesis in the kidney. (Adapted from Marx SJ: Hyperparathyroid and hypoparathyroid disorders, *N Engl J Med* 343:1863-1875, 2000.)



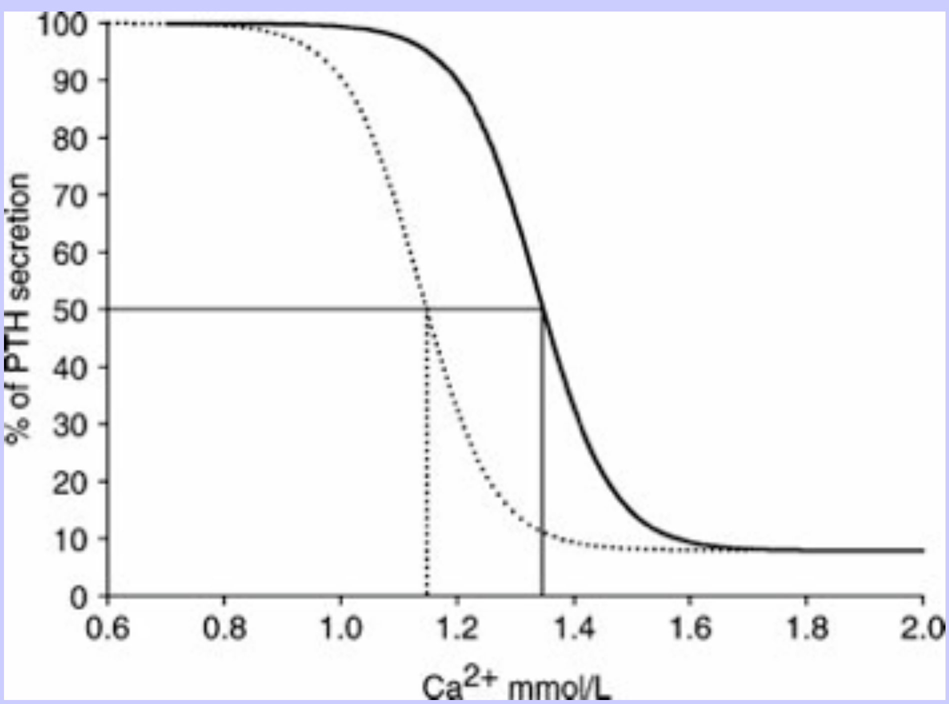
Parathyroid hormone is secreted by the chief cells of the parathyroid gland in response to small changes in extracellular Ca^{2+} concentrations. The relationship between serum Ca^{2+} concentrations and PTH secretion is inverse and sigmoidal in different species, including the horse (Figure 18.1-5).[4,36–40] This relationship rapidly enables the parathyroid gland to respond to minimal changes in Ca^{2+} concentrations. Changes in extracellular Ca^{2+} concentrations are detected by a Ca^{2+}-sensing G protein–linked cation receptor (CaR) in the parathyroid cells.[41]

18.1.3.1.1

Parathyroid Gland Anatomy and Function

In mammals the parathyroid glands develop from the endoderm of the third and fourth pharyngeal pouches and migrate during embryonic development.[2,42] Some evidence indicates that the parathyroid glands contain endodermal and ectodermal element markers (neuron-specific enolase, dihydroxyphenylalanine (DOPA) decarboxylase, chromogranin).[42–44] Human beings with multiple endocrine neoplasia develop 1302
tumors of neuroendocrine origin (medullary thyroid C carcinoma, pheochromocytoma) and often develop 1303
parathyroid gland neoplasia.[42] Multiple endocrine neoplasia recently was reported in the horse.[45]

Figure 18.1-5 Schematic representation of the relationship of Ca^{2+} and parathyroid hormone *(PTH)* and the Ca^{2+} set-point in healthy horses, human beings, and dogs. The Ca^{2+} set-point is the serum Ca^{2+} concentration at which serum parathyroid hormone concentrations are 50% of maximal during induction of hypocalcemia.[40] Horses have a sigmoidal relationship between serum Ca^{2+} and parathyroid hormone concentrations and a Ca^{2+} set-point (1.37 mmol/L) that is higher *(solid line)* than the set-point reported for human beings and dogs (1.0 to 1.2 mmol/L) *(dotted line)*.[39,40,128]



During development, the parathyroid glands III remain connected to the thymus and migrate caudally, whereas the parathyroid glands IV develop in relation to the thyroid gland and are more constant in their location.[46] In the horse the parathyroid glands IV (cranial or upper) usually are located in the fat craniodorsolaterally to the cranial pole of the thyroid gland and along the thyroid artery; however, they can be found in any location around the thyroid gland. In the author's experience, a few horses also may have parathyroid gland tissue embedded within the thyroid gland. One can locate the parathyroid glands III (lower, caudal) at the bifurcation of the bicarotid trunk, at the cranial pole of the thymus, or embedded in the thymus (young horses), and generally they are twice the size of the upper parathyroid glands.

The parathyroid gland consists of chief cells, oxyphil cells, and clear cells, which probably represent different morphologic and metabolic stages of the same parenchymal cells. These cells have a secretory cycle from inactivity under steady state conditions to an active secretory phase when extracellular Ca^{2+} concentrations are low.[47] Parathyroid chief cells have the ability to detect changes in extracellular Ca^{2+} concentrations by a calcium-sensing system, which includes CaR and megalin/gp330 (a glycoprotein of the low-density-lipoprotein–receptor superfamily).[41,48,49]

18.1.3.1.2

Parathyroid Hormone Synthesis and Secretion

Parathyroid cells in human beings and animals store small amounts of preformed PTH and rapidly respond to changes in Ca^{2+} concentrations. Mammalian PTH is synthesized from a 115–amino acid preprohormone, and after enzymatic cleavage in the endoplasmic reticulum and in the Golgi apparatus, the mature hormone is a straight chain of 84 amino acids (intact PTH; molecular weight 9500 d in human beings and 9393 d in the horse). Other forms of PTH (amino- and carboxy-terminal peptides) can be found in circulation after cleavage in different organs.[47] Fifty percent to 90% of PTH immunoreactivity in blood is from carboxy-terminal fragments.[50–52]

The plasma half-life of PTH in different species is approximately 2 minutes[52–55]; however, equine PTH half-life has not been determined. The rapid metabolism of PTH, together with the rapid response of the parathyroid chief cells to changes in Ca^{2+} concentrations, ensures that PTH concentrations can adjust rapidly to changes in Ca^{2+}. Sixty percent to 70% of PTH is removed in the liver, around 25% by the kidneys (glomerular filtration) and the rest by other organs.[52,53,55] In the liver, Kuppfer's cells are responsible for PTH clearance and proteolysis (a major source of circulating carboxy-terminal fragments), whereas in the kidney, PTH is cleared by glomerular filtration and reabsorbed by the renal tubules for extensive degradation, so low amounts or no PTH appear in the urine.[53,55] The plasma half-life of amino-terminal fragments is shorter than the inactive carboxy-terminal fragments, and carboxy-terminal fragments particularly are degraded in the kidney, and renal diseases may result in accumulation of carboxy-terminal PTH fragments.[51]

The PTH gene is expressed almost exclusively by the parathyroid glands and is under the influence of extracellular Ca^{2+} concentrations and $1,25(OH)_2D_3$. Information on the role of vitamin D metabolites on equine parathyroid gland function and calcium regulation is limited, and additional studies are necessary. However, $1,25(OH)_2D_3$ is well accepted as decreasing PTH gene transcription and PTH messenger RNA expression.[56] This concept is important in understanding the role of the kidney in vitamin D synthesis and metabolism and in parathyroid gland physiology. In the chief cells, vitamin D binds to the vitamin D

receptor, which interacts with specific DNA sequences (vitamin D responsive elements) to decrease PTH gene transcription. Unlike vitamin D, the effects of Ca^{2+} concentrations regulating PTH secretion are not mediated by affecting gene transcription but rather by altering PTH mRNA stability.[57] In the horse the effect extracellular Ca^{2+} concentrations on PTH mRNA expression has not been evaluated in vivo; however, from in vitro studies the author has found that low extracellular Ca^{2+} concentrations increase PTH mRNA expression in equine parathyroid chief cells.[58] 1303

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18.1.3.1.3 Physiologic Actions of Parathyroid Hormone

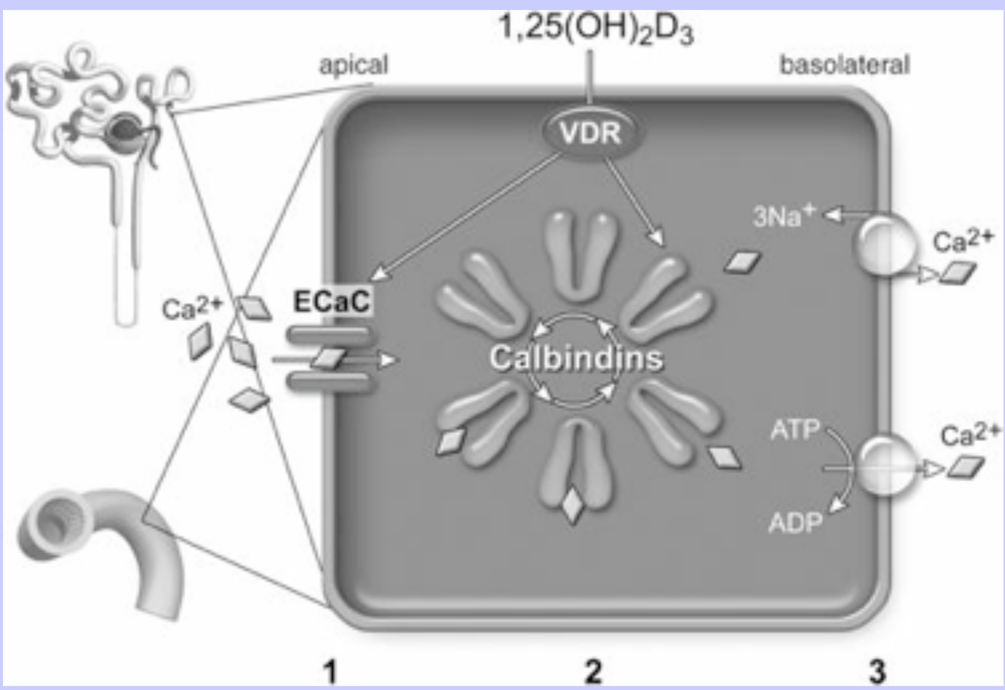
18.1.3.1.3.1 Kidney

The kidney is considered the major target organ for PTH. In the kidney, PTH regulates Ca^{2+} and phosphate reabsorption and $1,25(OH)_2D_3$ synthesis. These effects are mediated by the PTH-1 receptor, which is distributed widely in different segments of the nephron.[59] The PTH-1 receptor is coupled to G proteins, activating adenylate cyclase and increasing intracellular cyclic adenosine monophosphate (cAMP). Parathyroid hormone also activates phospholipases (A_2, C, D), protein kinase A, and protein kinase C.[59,60] The activation of any of these pathways depends on which segment of the nephron PTH is acting.

18.1.3.1.3.1.1 Effect on Ca^{2+} and Mg^{2+} Reabsorption.

Sixty percent of the filtered calcium and 20% of the filtered magnesium have been estimated to be reabsorbed in the proximal tubules. The reabsortion process in the proximal convoluted tubules is passive and is driven by the luminal positive voltage and the increased luminal concentrations of Ca^{2+} and Mg^{2+}.[61,62] In the proximal convoluted tubules, Ca^{2+} and Mg^{2+} reabsorption is paracellular, and the difference between Ca^{2+} and Mg^{2+} reabsorption in this segment of the nephron results from differential permeability. In the cortical thick ascending loop of Henle, Ca^{2+} and Mg^{2+} transport is also passive, but permeability is greater for Mg^{2+} (60% for Mg^{2+} versus 20% for Ca^{2+}). The lumen in the cortical thick ascending loop of Henle is positively charged, and the driving force for Ca^{2+} and Mg^{2+} reabsorption is the transepithelial voltage gradient generated by the $Na^+/K^+/2Cl^-$ cotransporter.[63] The effects of PTH on Ca^{2+} reabsorption in the cortical thick ascending loop of Henle are not completely clear, but apparently PTH increases the transepithelial voltage gradient, enhancing paracellular calcium transport.[62] Paracellin-1 is one of the newly discovered proteins that mediate the passive paracellular permeability of Ca^{2+} and Mg^{2+} in the cortical thick ascending loop of Henle.[64] The major site for PTH-regulated Ca^{2+} transport is in the distal convoluted tubules of the nephron, in which the effects of PTH on transepithelial (transcellular) calcium transport are mediated by epithelial calcium channels (Figure 18.1-6).[65,66]

Figure 18.1-6 Schematic representation of the epithelial calcium channel and the transcellular transport of calcium in the small intestine and kidney. The transcellular transport of Ca^{2+} is considered a three-step process in which calcium *(1)* enters the cell passively, *(2)* diffuses through the cytosol facilitated by calcium-binding proteins (calbindins), and *(3)* is extruded at the basolateral membrane by a Ca^{2+}-ATPase and a Na^{+}/Ca^{2+} exchanger. $1,25(OH)_2D$ increases all three steps of transepithelial calcium transport. (Adapted from Hoenderop JG, Willems PH, Bindels RJ: Toward a comprehensive molecular model of active calcium reabsorption, *Am J Physiol Renal Physiol* 278:F352-F360, 2000.)



Reabsorption of Ca^{2+} and Mg^{2+} in the kidney also is regulated by PTH-independent mechanisms that depend on the activation of the CaR in the basolateral membrane of the loop of Henle.

18.1.3.1.3.1.2 Effects on Phosphorus.

The effect of PTH on renal phosphate handling has been known for some time. By increasing bone resorption and by increasing $1,25(OH)_2D_3$ synthesis, PTH also increases plasma phosphate concentrations. To avoid high phosphate concentrations that may interact with Ca^{2+} to form insoluble precipitates and decrease Ca^{2+} concentrations, PTH decreases phosphate reabsorption in the kidney 1304
and therefore increases phosphate elimination. The movement of phosphate (P_i) in the proximal 1305
tubule occurs against an electrochemical gradient (negative inside the cell) and is mediated by Na^+/P_i cotransporters driven by the transcellular Na^+ gradient. Parathyroid hormone lowers Na^+ and P_i cotransport across the brush border in the proximal convoluted tubule, apparently by inducing degradation of the Na^+/P_i cotransporters.[67,68] The end result is decreased P_i reabsorption and increased fractional urinary clearance of P_i.

18.1.3.1.3.1.3 Effects on Vitamin D Metabolism.

PTH increases the synthesis of $1,25(OH)_2D_3$ by increasing gene expression and synthesis of 1α-hydroxylase and by decreasing synthesis of 24-hydroxylase. These effects are mediated by cAMP.[59]

18.1.3.1.3.1.4 Other Functions.

Parathyroid hormone also has functions associated with Na^+ and H^+ excretion in that it induces natriuresis and diuresis and inhibits $HCO^-{}_3$ reabsorption. These actions result from inhibition of Na^+/P_i cotransporters, Na^+/H^+ apical exchangers, and basolateral Na^+,K^+-ATPase.[59] Most of the effects of PTH in the proximal tubules are mediated by an increase in intracellular cAMP levels.

18.1.3.1.3.2 Bone

The bone is an active organ in a continuous process of bone resorption and bone formation. In bone, Ca^{2+} is exchanged between the extracellular fluid and the bone fluid, which is a Ca^{2+}-rich medium in equilibrium with the bone surface.[33] The bone membrane represents the lining cells that cover the bone surface and separates the bone fluid from the extracellular fluid.[69,70] Movement of Ca^{2+} across these compartments is regulated by several factors, including PTH.

The osteoclasts are multinucleated cells of hematopoietic origin (monocyte/macrophage lineage) responsible for bone resorption, whereas the osteoblasts are derived from bone marrow stromal cells and are responsible for bone formation. In adult vertebrates the process of bone resorption is in equilibrium with bone formation. In growing animals, bone formation exceeds bone resorption, whereas in old animals and human beings the opposite occurs. The effect of PTH increasing bone resorption has been known for some time; however, bone biologists were intrigued that osteoclasts, despite lacking PTH receptors, were the effectors of bone resorption. One of the earliest findings that elucidated this question was the discovery of PTH receptors in osteoblasts (Figure 18.1-7). For osteoclasts to be active they must be recruited, and this process requires the presence of osteoblasts and direct osteoblast-to-osteoclast

contact. The osteoblast-dependency for bone resorption led to the discovery of factors secreted by osteoblasts that are important in inducing osteoclast differentiation and activity.[71] Osteoblasts secrete macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κβ (RANK) ligand (RANKL),[72] with RANKL also being known as osteoprotegerin ligand and TNF-related activation-induced cytokine (TRANCE).[71] These two factors (M-CSF and RANKL) are essential for osteoclast function. Osteoclasts express RANK, which is a transmembrane receptor for RANKL, and the interaction of RANKL with RANK, and M-CSF with its specific receptor results in osteoclast recruitment and activation. To keep a balance between bone formation and bone resorption, the osteoblasts also release osteoprotegerin, which is a soluble decoy receptor for RANKL, interfering with RANKL action on the osteoclasts.[73]

Factors that can stimulate osteoclast differentiation include PTH, $1,25(OH)_2D_3$, and interleukin-11 (IL-11). Inflammatory cytokines such as IL-1 and tumor necrosis factor α may be important factors in osteoclast activation during inflammatory bone disease.[74] Prostaglandin E_2 stimulates bone resorption through a direct effect on hemopoietic precursors, inducing osteoclastic differentiation. The interaction of PTH (and PTHrP) with PTH receptors in the osteoblasts results in increased synthesis and release of RANKL and M-CSF into the bone microenvironment. Subsequently, the cell-to-cell contact together with RANKL and M-CSF release activates osteoclasts, resulting in PTH-induced bone resorption.

Serum markers of bone formation measured in horses include osteocalcin, the N-terminal extension peptide of type I procollagen, and bone-specific alkaline phosphatase.[75–77] Serum markers of bone resorption include the cross-linked carboxy-terminal telopeptide of type I collagen and total deoxypyridinoline.[75,77]

18.1.3.1.3.3

Intestine

No PTH receptors are in the intestinal tract; however, PTH indirectly increases intestinal Ca^{2+} and phosphate absorption by increasing synthesis of $1,25(OH)_2D_3$ in the kidney.

18.1.3.2

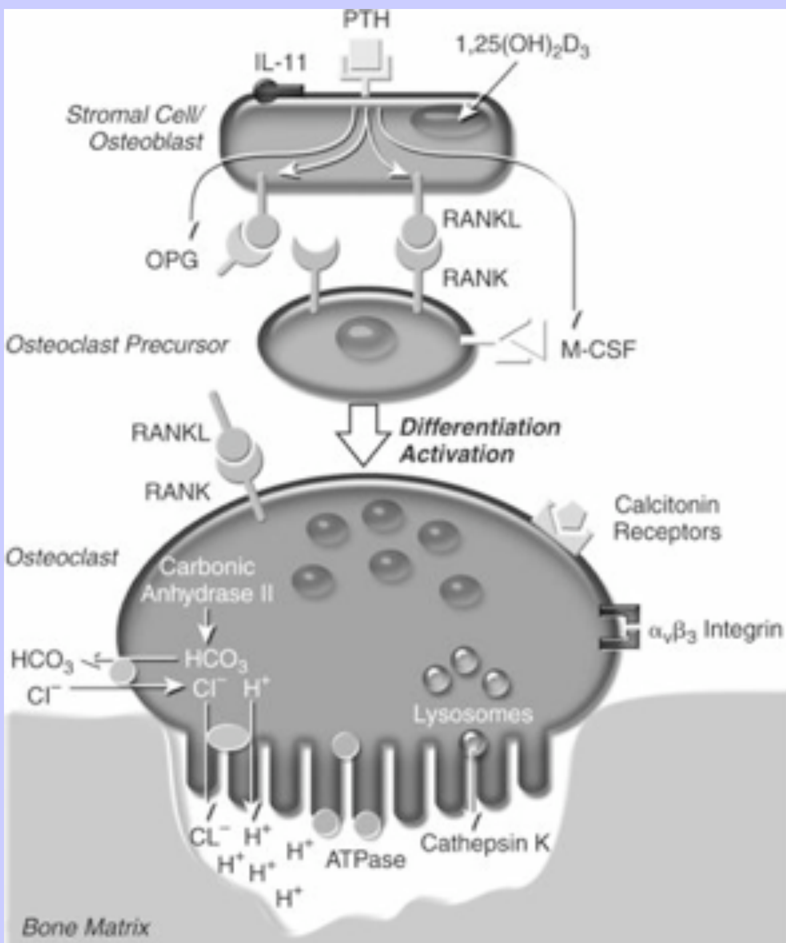
VITAMIN D

Vitamin D plays an important role in Ca^{2+} and phosphate homeostasis and to lesser extent probably in magnesium metabolism. Based on current knowledge of the physiology of vitamin D and its metabolites, vitamin D should be considered a hormone rather than a vitamin; nutritional supplementation is not required with sufficient exposure to sunlight.

The recognition by Mellanby[78] that a fat-soluble nutritional factor was associated with rickets was a major breakthrough in understanding calcium and phosphorus metabolism and bone biology. Additional studies on antirachitic substances led to the discovery of vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). These substances are secosterols derived from photolytic cleavage of the B rings of ergosterol (plants and 1305
yeasts) and 7-dehydrocholesterol (animals), respectively. The major circulating form of vitamin D in 1306
mammalians is 25-hydroxyvitamin D_3 ($25[OH]D_3$, calcidiol), which is produced primarily in the liver.[79] Soon after the discovery of $25(OH)D_3$ a new vitamin D_3 metabolite identified as 1,25-dihydroxyvitamin D_3 ($1,25[OH]D_3$, calcitriol) and mainly synthesized in the kidney was found to be the most active metabolite.[80]

The other major breakthrough in vitamin D physiology was the discovery of the vitamin D receptor.[81] Considerable analysis of this receptor revealed that unlike other hormone receptors, the vitamin D receptor is also a transcription factor. The vitamin D receptor was found initially in vitamin D target organs (intestines, bones, kidneys, parathyroid glands); however, vitamin D receptor expression also was found nontraditional target organs. Subsequently, other biologic functions for vitamin D have been described (immunomodulation, control of cell growth and differentiation), and this understanding has created new therapeutic approaches for using vitamin D and its derivatives for a variety of disorders (leukemia, hyperparathyroidism, autoimmune diseases). In veterinary medicine, the use of vitamin D metabolites is being considered for antiproliferative therapy in small animals.

Figure 18.1-7 Schematic representation of the cellular interactions responsible for osteoclastic activation and bone resorption. Factors that stimulate osteoclast differentiation include parathyroid hormone *(PTH)*, 1,25$(OH)_2$D, and interleukin-11 *(IL-11)*. Calcitonin inhibits osteoclastic activity. Osteoclast recruitment requires direct osteoblast-to-osteoclast contact. Osteoblasts secrete macrophage colony-stimulating factor *(M-CSF)* and receptor activator of NF-κβ *(RANK)* ligand *(RANKL)*. Osteoclasts express RANK, which is a transmembrane receptor for RANKL. Osteoblasts also release osteoprotegerin *(OPG)*, which is a soluble decoy receptor for RANKL. (Adapted from Rodan GA, Martin TJ: Therapeutic approaches to bone diseases, *Science* 289:1508-1514, 2000.)



18.1.3.2.1 Metabolism of Vitamin D

Vitamin D derives from dietary sources (vitamin D_2 comes from yeasts and plants and vitamin D_3 from animal-derived diets) and from activation of 7-dehydrocholesterol. In mammals, vitamin D_3 in produced in the skin by ultraviolet light (290 to 315 nm) photolytic cleavage of 7-dehydrocholecalciferol, producing 1306
previtamin D_3, which after thermal isomerization forms vitamin D_3.[82] From the skin, vitamin D_3 is 1307
translocated to the bloodstream by a vitamin D–binding protein. The next step in the formation of active vitamin D_3 is the hydroxylation of carbon 25, which occurs primarily in the liver, although other organs also may activate vitamin D_3 into 25-hydroxyvitamin D_3 [25(OH)D_3]. This reaction is catalyzed by microsomal and mitochondrial mixed function P-450 oxidases, also known as D-25-hydroxylases. The conversion of vitamin D_3 into 25(OH)D_3 is a poorly regulated reaction, and therefore plasma concentrations of 25(OH)D_3 are considered an indicator of the vitamin D status.

25-Hydroxyvitamin D_3 is transported in blood bound to vitamin D–binding protein, and at normal plasma concentrations this metabolite is considered to be inactive. The active metabolite of vitamin D_3, 1,25-dihydroxyvitamin D_3 (1,25[OH]$_2D_3$) is produced in the kidney by another cytochromone P-450 mixed-function oxidase, 25(OH)D-1α-hydroxylase (1α-hydroxylase). Under physiologic conditions the kidney is considered the main site for 1,25(OH)$_2D_3$ synthesis, although other organs (placenta, skin, monocytes) have 1α-hydroxylase activity. Unlike 25-hydroxylase, 1α-hydroxylase is a tightly regulated enzyme. Mutations of the 1α-hydroxylase gene in human beings and laboratory animals results in vitamin D-dependent type I rickets.[83] 25-Hydroxyvitamin D_2 is metabolized in the same pathways just described to produce 1,25(OH)$_2D_2$. The author will refer to both vitamin D active metabolites, 1,25(OH)$_2D_2$ and 1,25(OH)$_2D_3$, as 1,25-dihydroxyvitamin D (1,25[OH]$_2$D). In blood, 1,25(OH)$_2$D concentrations are one thousandth the concentrations of 25(OH)D. The half-life of 25(OH)D is measured in weeks, whereas the half-life of 1,25(OH)$_2$D is measured in few hours.

An alternative pathway in the metabolism of 25(OH)D and 1,25(OH)$_2$D is the 24-hydroxylation by a 25(OH)D-24-hydroxylase, which is the major catabolic enzyme in the kidney and other tissues (intestine, cartilage, bone, liver) to form the inactive metabolites 24,25-dihydroxyvitamin D (24,25[OH]$_2$D) and 1,24,25-trihydroxyvitamin D. Although a receptor for 24,25(OH)$_2$D has not been identified and this metabolite may play a physiologic role,[84] its function remains unclear.

Parathyroid hormone and hypophosphatemia induce 1α-hydroxylase activity in the kidney, whereas increased Ca^{2+} concentrations and 1,25(OH)$_2$D inhibit 1α-hydroxylase and stimulate 24-hydroxylase, making this an effective self-regulatory mechanism. The synthesis of 1,25(OH)$_2$D in other tissues is not regulated in the same manner or related to calcium homeostasis.

18.1.3.2.2 Transport of Vitamin D

Vitamin D and its metabolites are lipophilic compounds that are bound to specific plasma binding proteins, vitamin D–binding protein being the most important. Vitamin D–binding protein is synthesized in the liver and is related structurally to albumin and α-fetoprotein. The affinity of vitamin D–binding protein for vitamin D metabolites is as follows: 25(OH)D = 24,25(OH) = 25,26(OH)$_2$D > 1,25(OH)$_2$D > vitamin D.[85]

The affinity of vitamin D–binding protein for different vitamin D_2 and D_3 seems to be similar in mammals, whereas in birds, vitamin D–binding protein binds vitamin D_3 with 100 more times affinity than vitamin D_2. Because on the molar basis vitamin D–binding protein concentrations are 20 times greater than those of vitamin D metabolites, approximately 5% of the vitamin D–binding protein is occupied by vitamin D metabolites, and nearly all vitamin D is protein bound.[86] Furthermore, vitamin D–binding protein seems to be important to extend the plasma half-life of vitamin D.[87] The free, unbound vitamin D is the one that exhibits biologic activity, and in addition to buffer and transport vitamin D metabolites, vitamin D–binding protein has been proposed also to have a protective effect against vitamin D intoxication.[88] Vitamin D–binding proteins bound to vitamin D metabolites are filtered in the renal glomerulus and reabsorbed in the proximal tubular cells by an endocytic receptor (megalin). This receptor 1307
also is expressed in other cells (parathyroid gland and placenta), where it may have calcium-sensing 1308
properties.[48] The vitamin D–binding protein/megalin pathway seems to be important in vitamin D recycling and 25(OH)D activation by 1α-hydroxylase. Vitamin D metabolites also bind to albumin and lipoproteins with lesser affinity than vitamin D–binding protein; however, lipoproteins seem to be more important and efficient in transporting vitamin D_3 from the skin to the liver for 25-hydroxylation.

18.1.3.2.3 Regulation of Vitamin D Metabolism

Extracellular Ca^{2+} and phosphorus concentrations. control the metabolism of $1,25(OH)_2D$. These actions are mediated through organs involved in mineral metabolism (kidneys, intestines, bones, parathyroid glands). Blood concentrations of $1,25(OH)_2D$ are regulated by PTH, Ca^{2+}, phosphorus, and $1,25(OH)_2D$. The role of calcitonin on the vitamin D metabolism remains unclear.

18.1.3.2.3.1 Parathyroid Hormone

Low extracellular Ca^{2+} concentrations stimulate PTH release from the parathyroid gland. Subsequently, PTH interacts with PTH-specific receptors on the proximal tubular cells, increasing 1α-hydroxylase and suppressing 24-hydroxylase activities. The end result is increased $1,25(OH)_2D$ release from increased synthesis and decreased catabolism. These are indirect effects of extracellular Ca^{2+} on $1,25(OH)_2D$ synthesis.

18.1.3.2.3.2 Calcium

In addition to its indirect effects on $1,25(OH)_2D$ synthesis (via PTH), Ca^{2+} also has direct effects on $1,25(OH)_2D$ synthesis and metabolism that are mediated by a direct interaction of Ca^{2+} with the renal calcium-sensing receptors. Hypocalcemia stimulates and hypercalcemia suppresses 1α-hydroxylase synthesis.

18.1.3.2.3.3 Phosphate

Hypophosphatemia results in increased $1,25(OH)_2D$ concentrations in the blood by increasing 1α-hydroxylase activity in the kidney. The mechanism by which phosphate controls $1,25(OH)_2D$ synthesis is independent of PTH secretion. The effects of phosphate on $1,25(OH)_2D$ synthesis recently have been

found to be mediated by a type IIa sodium/phosphate cotransporter (NPT2); low phosphate directly stimulates 1α-hydroxylase synthesis and decreases 24-hydroxylase synthesis.[89]

18.1.3.2.3.4

1,25$(OH)_2$D

The synthesis of 1,25$(OH)_2$D is an autoregulatory mechanism. 1,25$(OH)_2$D exhibits effects at the level of gene expression; high 1,25$(OH)_2$D concentration decreases 1α-hydroxylase and increases 24-hydroxylase gene expression and protein synthesis. This mechanism seems to be the most important regulatory mechanisms in 1,25$(OH)_2$D synthesis when the daily intake of calcium and phosphate is adequate.

18.1.3.2.3.5

Calcitonin

The importance of calcitonin on vitamin D is unclear. Calcitonin is known to stimulate 1α-hydroxylase, inhibit phosphate reabsorption, and inhibit Ca^{2+} reabsorption. These functions are not known in the horse, and too many differences exist among species to draw any conclusions.

18.1.3.2.4

Actions of Vitamin D

Most of the biologic actions of vitamin D result from its active metabolite, 1,25$(OH)_2$D, which is important in Ca^{2+} and phosphate homeostasis. The most important function of 1,25$(OH)_2$D in Ca^{2+} metabolism is considered to be stimulation of intestinal Ca^{2+} and phosphate absorption, Ca^{2+} in the duodenum and phosphate in the jejunum and ileum. In the horse this is unclear. The actions of vitamin D are mediated by a specific receptor (vitamin D receptor) that acts as a transcription factor, controlling gene expression. Once 1,25$(OH)_2$D is bound to the vitamin D receptor, the vitamin D receptor forms a heterodimer with the retinoid acid receptor. This heterodimer binds to specific DNA sequences or vitamin D response elements, recruiting a series of transcription factors to regulate gene expression. The vitamin D receptor has been cloned in several species but not in the horse, and its control, expression, and regulation in the equine species is being evaluated currently in the author's laboratory. The vitamin D receptor is found in vitamin D target organs (intestines, bones, kidneys, parathyroid glands) and in other organs not involved in Ca^{2+} homeostasis, such as the skin, pancreas, immune system, and reproductive organs.[86]

In the intestine, as in the kidney and placenta, transepithelial Ca^{2+} transport is considered a three-step process in which calcium (1) enters the cell passively, (2) diffuses through the cytosol facilitated by calcium-binding proteins (calbindins), and (3) is extruded at the basolateral membrane calcium by a Ca^{2+}-ATPase and a Na^+/Ca^{2+} exchanger (see Figure 18.1-6.).[90,91] In the small intestine (duodenum), 1,25$(OH)_2$D increases all three steps of transepithelial calcium transport. Vitamin D increases expression of calbindins (calbindin D_{9k}) and the activity of other proteins (i.e., alkaline phosphatase, calmodulin, Ca^{2+}-ATPase) that may be important in calcium absorption. The recent discovery of an apical epithelial calcium channel (epithelial calcium channels) in 1,25$(OH)_2$D-responsive cells in the small intestine, kidney, and placenta[65,66] has provided significant understanding on the mechanism of calcium entry into epithelial cells, for until recently how calcium entered epithelial cells was unclear. The epithelial calcium channel is not a voltage-gated calcium channel; however, it is distributed in the same epithelial cells that

express calbindin and may be regulated by 1,25(OH)$_2$D. Furthermore, epithelial calcium channels have been proposed to be the gate-keeper for 1,25(OH)$_2$D-dependent transepithelial calcium transport.[65,92] In addition to its effects on intestinal calcium transport, 1,25(OH)$_2$D increases phosphate transport. These effects are mediated by 1,25(OH)$_2$D direct stimulation of the Na^+/P_i cotransporter.

Recent evidence indicates a rapid and nongenomic action of vitamin D on intestinal calcium transport. This effect is mediated by a vitamin D membrane receptor, which is an exception to the traditional understanding of steroid hormone action.[93] This nongenomic action of 1,25(OH)$_2$D on intestinal calcium absorption results in vesicular and paracellular calcium transport.

In the kidney, perhaps the most important effect of 1,25(OH)$_2$D is the suppression of 1α-hydroxylase and the stimulation of 24-hydroxylase gene expression and synthesis. In addition, 1,25(OH)$_2$D stimulates renal $^{2+}$ reabsorption by increasing the expression of calbindins (calbindin D$_{9k}$, calbindin D$_{28k}$) in the distal convoluted tubules of the nephron, which is the site with the highest vitamin D receptor concentration and where active transport of calcium occurs.[86] Horses have high concentrations of vitamin D receptor in the distal convoluted tubules of the nephron and collecting tubules (unpublished information). In the distal convoluted tubules of the nephron, epithelial calcium channels are distributed in the same cells with calbindin activity. The effect of 1,25(OH)$_2$D on renal phosphate tubular transport is considered to be minimum and mostly results from the suppressive effect of 1,25(OH)$_2$D on PTH synthesis and secretion. In addition, 1,25(OH)$_2$D increases Mg^{2+} reabsorption in the distal convoluted tubules of the nephron through gene activation and protein synthesis.[94]

In bone, 1,25(OH)$_2$D is an important hormone for skeletal development and bone mineralization. An 1308
example of vitamin D deficiency is rickets in young animals and osteomalacia in adult animals and human 1309
beings. The existence of rickets in the horse is controversial and remains to be documented. 1,25(OH)$_2$D induces bone matrix synthesis and mineralization by increasing blood concentrations of Ca^{2+} and phosphorus. In addition, 1,25(OH)$_2$D induces osteoclastogenic activity and bone resorption, which may be important to maintain normocalcemia and normophosphatemia. However, PTH is more important in bone resorption than vitamin D.

In the parathyroid gland, PTH synthesis is regulated negatively by 1,25(OH)$_2$D. Parathyroid hormone increases 1α-hydroxylase activity in the kidney, increasing plasma concentrations of 1,25(OH)$_2$D, which then inhibits PTH gene expression and synthesis and controls parathyroid gland chief cell growth and differentiation.[95] This mechanism is part of the rationale for the development of parathyroid hyperplasia in human beings and animals with chronic renal failure and vitamin D deficiency. As previously mentioned, the effects of vitamin D on parathyroid cell function are mediated through the vitamin D receptor. In the horse, what the direct effect of vitamin D metabolites is on parathyroid gland function is not known, although one may reasonably accept that the effect is similar to that in other species. The author's findings of abundant expression of the vitamin D receptor in the equine parathyroid gland support this postulation.

Vitamin D has other nonclassical functions. Anemia, predisposition to infections from impaired immune system, skeletal muscle weakness, cardiomegaly, pancreatic glucose-mediated insulin secretion, and infertility are among conditions reported with vitamin D deficiency.[86] Vitamin D is important in skin and hair development and in cell proliferation and differentiation, and significant efforts have been made in the

last few years to develop and use vitamin D analogs that have minimal or no effect on calcium metabolism but that retain their antiproliferative and prodifferentiation properties. These drugs are being used to treat different pathologic conditions in human beings (seborrhea, psoriasis, secondary hyperparathyroidism) to reduce cell proliferation in cancer (breast cancer, prostate cancer, leukemia).[86]

18.1.3.2.5

Vitamin D Metabolism in the Horse

The amount of information on vitamin D in the horse is limited compared with other species. However, an important point is that horses have unique features regarding calcium metabolism, including high serum total and ionized calcium concentrations, high urinary fractional clearance of calcium, a high Ca^{2+} set-point, and low serum concentrations of vitamin D metabolites (25[OH]$_2$D and 1,25[OH]$_2$D).[4,58,96–103] Intestinal absorption of Ca^{2+} in the horse also apparently is a poorly controlled mechanism.[12,96] As mentioned previously, 25(OH)D concentrations are considered an indicator of vitamin D status. Plasma concentrations of 25(OH)D in the horse are approximately one tenth the plasma concentrations of 25(OH)D in other species.[100–102] In human beings and in most domestic animals, plasma concentrations of 25(OH)D range from 50 to 100 nmol/L,[97] with minimal differences between young animals and adults,[98] and a plasma 25(OH)D concentration less than 40 nmol/L indicates vitamin D deficiency. In contrast, in the horse plasma concentrations of 25(OH)D_3 of 5 to 10 nmol/L or less are normal.[100–102] Despite the low vitamin D concentrations, rickets or osteomalacia are not reported in horses.[104]

When the effect of vitamin D on growth and bone development of young horses was evaluated for a period of 5 months, horses with limited access to sunlight and fed a vitamin D-deficient diet were found to have decreased growth, discomfort while standing, and lower bone density. Of interest from that study is that none of these horses developed clinical evidence of rickets, supporting the concept that rickets may be a rare finding in the horse. The authors concluded that vitamin D supplementation of horses with limited sunlight exposure is necessary.[104] In northern latitudes for example, the synthesis of 1,25(OH)$_2$$D_3$ in human beings is low during winter because of limited exposure to sunlight. When serum concentrations of 25(OH)D_2 and 25(OH)D_3 were measured in horses located in Finland during the summer and winter, 25(OH)D_3 concentrations were found to be significantly lower during winter,[99,100] suggesting that the low vitamin D_3 concentrations in horses during winter may result from limited sunlight exposure (Table 18.1-7). The authors also found that 25(OH)D_2 concentrations were low during both seasons and showed no seasonal variation, which may have resulted from small amounts of vitamin D_2 in forages. A more recent study on vitamin D metabolism also found that plasma 25(OH)D and 1,25(OH)$_2$$D_3$ concentrations were low or undetectable in healthy horses and ponies compared with vitamin D metabolite concentrations from other species.[96] No difference in plasma 25(OH)D concentrations between horses and ponies was found. In the same study, renal 1α-hydroxylase activity could not be detected, but renal 24-hydroxylase activity was present. Likewise, in a preliminary study in the author's laboratory, 1α-hydroxylase mRNA expression in equine kidneys was not detected, suggesting that perhaps 1α-hydroxylase is downregulated by the high extracellular Ca^{2+} concentrations of the horse or by other unknown mechanisms or that 1α-hydroxylase is expressed minimally in the equine kidney. Possibly some of the 1,25(OH)$_2$$D_3$ present in equine plasma results from extrarenal 1α-hydroxylase activity, as occurs in human beings and animals after nephrectomy.

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TABLE 18.1-7 Normal Serum Concentrations of Calcium, Phosphorus, Magnesium, Vitamin D, and Parathyroid Hormone in Healthy Horses

SOLUTE	CONCENTRATION
Total calcium	11.1–13.0 mg/dl
Ionized calcium	6.0–7.0 mg/dl
Phosphorus	1.2–4.8 mg/dl
Total magnesium	0.53–0.91 mmol/L
Ionized magnesium	0.46–0.66 mmol/L
Parathyroid hormone	<4.0 pmol/L (<40 pg/ml)
Calcitonin	<40 pg/ml
25-Vitamin D_3	<2.0 ng/ml[102]
	4.7 ±1.0 ng/ml[102*]
	1.90 ± 0.23 ng/ml, winter[99]
	2.43 ± 0.09 ng/ml, summer[99]
	4.2 ± 0.34 μg/L, winter[100†]
	6.2 ± 0.36 μg/L, summer[100†]
	11.42 ± 3.26 ng/ml[103]
1,25 Vitamin D_3	18.6 ± 7.3 ng/L, winter[100*]
	18.7 ± 8.0 ng/L, summer[100*]
	55.0 ± 24.0 pmol/L[96]

Values from the chemistry laboratory at the College of Veterinary Medicine, The Ohio State University.

* D2 metabolite.

† Includes D_2 and D_3 metabolites.

18.1.3.3

CALCITONIN

Coalcitonin (thyrocalcitonin) is a 32–amino acid peptide that functions primarily to inhibit osteoclast function during hypercalcemia. Calcitonin is secreted by the parafollicular cells (C cells) of the thyroid gland. The C cells are of neuroendocrine origin and are derived from the ultimobranchial body (ventral portion of the fourth pharyngeal pouch, also considered by some as the fifth pharyngeal pouch) that during embryologic development is incorporated into the thyroid gland.[43] Calcitonin structure is known in several species, and the author and colleagues recently cloned equine calcitonin.[105] Equine calcitonin has 90% homology with human calcitonin. Several factors control secretion of calcitonin, Ca^{2+} perhaps being the most

important. The C cells of the thyroid gland sense changes in extracellular Ca^{2+} concentrations by the same calcium-sensing receptor expressed in parathyroid chief cells and renal tubular cells.[106] Calcitonin interacts with calcitonin-specific receptors in osteoclasts and renal tubular cells, increasing cytosolic cAMP and $Ca^{2+}{}_i$ concentrations, and activating the inositol phosphate signaling pathway.

Calcitonin decreases plasma concentrations of Ca^{2+} and phosphorus by inhibiting osteoclastic bone resorption and to lesser extend by increasing urinary excretion of Ca^{2+} and phosphorus. Calcitonin also stimulates renal 1α-hydroxylase activity. Despite these effects on Ca^{2+} and phosphorus metabolism, the importance of calcitonin on mammalian Ca^{2+} homeostasis has not been established. Calcitonin is considered by some as a hormone "in search of a function." This has been demonstrated in human beings in which a minimal or no increase in plasma Ca^{2+} concentrations occurs after thyroidectomy.[2] Furthermore, some authors have concluded that calcitonin may be important in extreme hypercalcemia, but in the day-to-day Ca^{2+} variations, calcitonin may not play a relevant role.[2,107] Increases in serum calcitonin concentrations after eating suggests that calcitonin may be important in controlling postprandial increases in plasma Ca^{2+} concentrations. Gastrin, glucagons, and increases in Ca^{2+} concentrations appear to mediate the calcitonin release.

Unlike in terrestrial vertebrates, no doubt exists that calcitonin plays an important role in regulating Ca^{2+} concentrations in saltwater fish, where the ambient calcium concentrations are high (10 m*M;* 40 mg/dl). Few studies have evaluated calcitonin in the horse.[108–111] In an immunohistochemistry study, antibodies to human calcitonin cross-reacted with equine calcitonin.[108] Equine osteoclasts respond to calcitonin stimulation.[109] Serum concentrations of calcitonin in horses have been measured using human calcitonin radioimmunoassays.[112] However, defining the normal range of calcitonin is a downside of these studies. Serum calcitonin concentrations in horses have been evaluated using different human calcitonin immunoassays, resulting in a wide range of values among healthy horses (from undetectable to 40 pg/ml). Administration of calcium gluconate to healthy horses resulted in increased serum calcitonin concentrations but not of the same magnitude as reported in human beings.

18.1.3.4 PARATHYROID HORMONE–RELATED PROTEIN

The discovery of PTHrP in human beings with humoral hypercalcemia of malignancy (HHM)[34] was an important step in understanding Ca^{2+} homeostasis in health and disease. Parathyroid hormone and PTHrP share homology in their first 13 amino acids and act through the same receptor (PTH-1 receptor). In contrast to PTH, PTHrP is produced by almost every tissue in the body and has a broad range of functions, most of which have little to do with Ca^{2+} homeostasis.[33,35] Under physiologic conditions, PTHrP functions are considered to be paracrine, autocrine, and intracrine (inside the cell). For most part the endocrine functions of PTHrP are considered pathologic (HHM); PTHrP may be an important endocrine Ca^{2+}-regulating factor in the fetus. Three different isoforms of PTHrP have been identified in human beings,[35] but only one has been identified in the horse.[113] Through its different regions, PTHrP has different functions; the amino-terminal peptide interacts with the PTH-1 receptor, initiating PTH-like actions (bone resorption, renal Ca^{2+} 1310
reabsorption); the midregion PTHrP is important for transepithelial and placental Ca^{2+} transport; and the 1311
carboxy-terminal (osteostatin) inhibits osteoclastic bone resorption.[35] The functions of PTHrP can be grouped as (1) stimulation of transepithelial Ca^{2+} transport (kidney, placenta, mammary gland); (2) smooth

muscle relaxation (gastrointestinal tract, uterus, bladder, blood vessels); and (3) regulation of cell proliferation, differentiation, and death. High concentrations of PTHrP (10,000-fold higher than plasma) are found in milk of lactating human beings and animals, including the mare.[114] Although the functions of PTHrP in milk are unclear, perhaps PTHrP is important for Ca^{2+} transport into the milk, and for intestinal Ca^{2+} absorption in the newborn.

Humoral hypercalcemia of malignancy is a paraneoplastic syndrome that results from excessive production and secretion of PTHrP by some tumors. By interacting with PTH-1 receptors, PTHrP promotes bone resorption and inhibits renal Ca^{2+} excretion, causing hypercalcemia in human beings and in different domestic animals, including the horse.[115–120]

18.1.3.5 CALCIUM-SENSING SYSTEM

Mammalians maintain their extracellular Ca^{2+} concentrations within a narrow limit. Within the cells, cytosolic free or ionized calcium ($Ca^{2+}{}_i$) acts as a second messenger and as an enzymatic cofactor, controlling cellular functions such as hormone secretion, muscular contraction, cell differentiation, cell motility, and cell death. Cytosolic calcium concentration is around 100 n*M,* which is more than 10,000-fold lower than extracellular Ca^{2+} concentrations (>1 m*M;* 1.5 m*M* in the horse). Because $Ca^{2+}{}_i$ has many intracellular functions, sustained high $Ca^{2+}{}_i$ may be deleterious for the cell: rapid (greater than tenfold) and short time increases can occur. In contrast, extracellular Ca^{2+} concentrations change minimally (a few percent). Extracellular Ca^{2+} also has a large number of functions (blood clotting, skeletal integrity, membrane excitability, cell adhesion); however, it does do not change rapidly as $Ca^{2+}{}_i$ does. To support such physiologic processes, Ca^{2+} requires a tight control. Movement of $^{2+}$ across the kidney and intestine and from the skeleton is controlled, and such movement is sensed by the parathyroid gland. The molecular basis by which parathyroid cells detect changes in Ca^{2+} concentrations was not clear until a CaR was cloned.[41] In addition to being a second intracellular messenger, Ca^{2+} acts as a hormone on specific Ca^{2+} receptors, and therefore extracellular Ca^{2+} also should be considered a first messenger.[121]

Organs involved in Ca^{2+} homeostasis (parathyroid gland, thyroid gland, and kidney) express CaR. The author and colleagues have cloned a CaR from equine parathyroid gland, thyroid gland, and the kidney. Equine CaR has 90% homology with the human and bovine CaR.[58] Activation of the CaR by high extracellular Ca^{2+} concentrations inhibits PTH secretion by the parathyroid cells and stimulates calcitonin secretion from the C cells of the thyroid gland.[58,122] In the horse, hypercalcemia induces calcitonin secretion, although work from the author's laboratory indicates that the magnitude of calcitonin release in response to hypercalcemia in the horse is minimal (unpublished).

Cation receptor signaling includes the activation of phospholipases C, A_2, and D. In the parathyroid cells, phospholipase C hydrolyzes phosphatidylinositol 4,5-biphosphate to diacylglycerol and IP_3.[123] Inositol 1,4,5-triphosphate interacts with specific receptors to release $Ca^{2+}{}_i$ from intracellular stores,[124] whereas diacylglycerol activates protein kinases.[123] In addition, increased Ca^{2+} concentrations inhibit intracellular cAMP accumulation by inhibiting adenylate cyclase.[125]

In the kidney, CaR is expressed in almost all segments of the nephron, with the highest receptor concentration in the cortical thick ascending loop of Henle,[126] where Ca^{2+} is reabsorbed in a regulated manner.[90] Cation receptor is also present in the distal convoluted tubules of the nephron, where Ca^{2+} reabsorption is also stimulated by PTH. One should note that in the kidney, CaR regulates Ca^{2+} and Mg^{2+} reabsorption independently of PTH actions. By interacting with CaR, Ca^{2+} (and to lesser extent Mg^{2+}) inhibits the $Na^+/K^+/2Cl^-$ transporter, reducing transepithelial voltage gradient and decreasing Ca^{2+} and Mg^{2+} reabsorption. In fact, the effect of Ca^{2+} is similar to the effect that loop diuretics (furosemide) have in the cortical thick ascending loop of Henle; that is, the inhibition of the $Na^+/K^+/2Cl^-$ cotransporter decreases reabsorption of Na^+, $^-$, K^+, Ca^{2+}, and Mg^{2+} and causes diuresis. High concentrations of Ca^{2+} exert a diuretic effect that may result in volume depletion, as reported in human beings with hypercalcemia.[63] Calcium gluconate or calcium chloride infusions to healthy horses result in diuresis and in a rapid decrease in urine specific gravity (unpublished). The opposite is also true regarding Ca^{2+} reabsorption; hypocalcemia results in less activation of the CaR and more paracellular Ca^{2+} transport. In addition, hypocalcemia also increases PTH secretion, which stimulates the activity of the $Na^+/K^+/2Cl^-$ cotransporter in the cortical thick ascending loop of Henle, increasing Ca^{2+} reabsorption. CaR also regulates Mg^{2+} reabsorption by modulating Na^+, K^+, and $^-$ reabsorption.[127] The author has found evidence for these mechanisms in the horse, in which infusion of calcium salts (gluconate or chloride) resulted in increased fractional clearance of Ca^{2+}, Mg^{2+}, Na^+, K^+, and $^-$; diuresis; and a significant decrease in urine specific gravity. Furthermore, increasing Ca^{2+} concentrations (>7 mg/dl) decreased serum Mg^{2+} concentrations in horses receiving infusions of calcium salts. 1311

The inhibitory effects of high Ca^{2+} (and Mg^{2+}) concentrations on Ca^{2+} and Mg^{2+} reabsorption are mediated 1312
by CaR receptors located in the basolateral membranes of the cortical thick ascending loop of Henle.[61]

18.1.4 Calcium Disorders in the Horse

Abnormal Ca^{2+} homeostasis in the horse is associated with hypocalcemic or hypercalcemic disorders. Horses have unique features regarding calcium metabolism including high serum total and ionized calcium concentrations,[4] a poorly regulated intestinal Ca^{2+} absorption,[12] high urinary fractional clearance of calcium,[4] low serum concentrations of vitamin D metabolites (1,25[OH]$_2$D, 25[OH]D),[96,99–101] and an increased Ca^{2+} set-point.[58,128] Some pathologic conditions of the horse characterized by abnormal calcium homeostasis include idiopathic hypocalcemia of foals,[129] hypoparathyroidism,[130,131] primary hyperparathyroidism,[132] nutritional secondary hyperparathyroidism,[133] hypercalcemia of malignancy,[116,117,120] vitamin D toxicity,[134] renal failure,[135] exercise-induced hypocalcemia,[36,136] and sepsis.[4,137,138] Table 18.1-7 presents normal calcium concentrations for horses.

18.1.4.1 HYPOCALCEMIA

Hypocalcemia in the horse develops from different conditions (Box 18.1-1), and the clinical signs associated with hypocalcemia result from increased neuromuscular excitability and decreased smooth muscle cell contractility (Box 18.1-2). The Ca^{2+} concentrations in the extracellular fluid affect the voltage at which the Na^+ channels in nerve fibers are activated. Calcium ions bind to Na^+ channels, decreasing Na^+ permeability

and increasing the voltage required for channel opening. Calcium then is considered a Na^+ antagonist. When Ca^{2+} decrease, the Na^+ channels are activated by smaller changes in the resting potential and the nerve fibers become highly excitable, even discharging spontaneously. Spontaneous and continuous discharges may result in muscle fasciculation, tremors, and tetany. Tachycardia and cardiac arrhythmias may be present during hypocalcemia, although bradycardia may develop during severe hypocalcemia, probably from decreased cardiac muscle contractility.

18.1.4.1.1

Synchronous Diaphragmatic Flutter

Synchronous diaphragmatic flutter (SDF) or "thumbs" has been reported in horses with gastrointestinal disease,[4,139] lactation tetany (eclampsia),[140] after transport, with thoracic hematoma,[141] blister beetle toxicosis,[142] urethral obstruction,[143] endurance exercise,[144] primary hypoparathyroidism,[131,145] idiopathic hypocalcemia,[129] and sepsis.[4] Any horse with hypocalcemia potentially can develop SDF. The condition is frequent in horses after prolonged exercise in which significant amounts of electrolytes (Ca^{2+}, Na^+, K^+, Mg^{2+}, $^-$) are lost in the sweat.[146,147] In the case of SDF, depolarization of the right atrium stimulates action potentials in the phrenic nerve as it crosses over the heart. Clinically, a rhythmic movement on the flank results from diaphragmatic contractions that are synchronous with the heartbeat.

18.1.4.1.1.1

BOX 18.1-1 CLINICAL CONDITIONS IN THE HORSE IN WHICH HYPOCALCEMIA HAS BEEN REPORTED

Acute renal failure

After endurance exercise

Cantharidin toxicosis

Chronic renal failure

Colic

During lactation (lactation tetany)

During transport (transit tetany)

Dystocia

Endotoxemia

Enterocolitis

Excessive administration of $NaHCO_3$

Furosemide administration

Heat stroke

Hypomagnesemia

Late pregnancy

Liver disease

Magnesium toxicosis

Malignant hyperthermia

Oxalate ingestion

Pancreatitis

Pleuropneumonia

Postoperative myopathy

Primary hypoparathyroidism

Retained placenta

Rhabdomyolysis

Sepsis

During alkalosis, Ca^{2+} binding to plasma anions increases, in particular to albumin, resulting in ionized hypocalcemia. Exercising horses may develop alkalosis from hyperventilation (respiratory alkalosis) and from chloride losses in sweat (metabolic hypochloremic alkalosis),[141] and the alkalosis rather than the nature of the alkalosis is what contributes to ionized hypocalcemia and SDF. Serum ionized magnesium (Mg^{2+}) concentrations may be decreased in some horses with SDF, and as in hypocalcemia, hypomagnesemia also may increase neuromuscular excitability. Some of the mechanisms responsible for hypomagnesemia are similar to those causing hypocalcemia. One always should include hypomagnesemia in the differential diagnosis of hypocalcemia. 1312

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18.1.4.1.1.2

BOX 18.1-2 CLINICAL SIGNS REPORTED IN THE LITERATURE OF HORSES WITH HYPOCALCEMIA

Anxiety

Asphyxia

Ataxia

Bruxism

Cardiac arrhythmias

Colic

Convulsions

Death

Depression

Dysphagia

Dyspnea

Excitation

Hyperhidrosis

Hypersalivation

Hyperthermia

Ileus

Laryngeal spasm

Muscle fasciculations

Seizures

Stiff gait

Synchronous diaphragmatic flutter

Tachycardia

Tachypnea

Tetany

Tremors

Trismus

18.1.4.1.2

Hypocalcemic Tetany

Low Ca^{2+} concentrations increase cell membrane excitability. Some horses develop excessive and sustained skeletal muscular contractions or tetany. Lactation tetany occurs in mares from 2 weeks before foaling up to few days after weaning. The predisposing cause of lactation tetany is the calcium losses in milk. Mares producing large amounts of milk, eating a low-calcium ration or grazing lush pastures, and performing physical work (Draft mares) are more at risk. Some horses transported for long distances may develop hypocalcemia and transit tetany.[140] Hypocalcemic tetany can occur in any horse with hypocalcemia. Clinical signs may include anxiety, depression, ataxia, stiff gait, muscle fasciculations and tremors, tachypnea with flared nostrils, dyspnea, dysphagia, hypersalivation, and hyperhidrosis (see Box 18.1-2.).

18.1.4.1.3 Hypocalcemic Seizures

As with the peripheral nerves, decreased extracellular ${}^{2+}$ concentrations in the central nervous system may increase neuroexcitability. Hypocalcemic seizures have been reported in foals with idiopathic hypocalcemia and sepsis of unknown origin.[129] Clinical signs usually improve with calcium treatment, although some foals may require repeated treatments with calcium salts. Horses with hypocalcemic seizures have a poor prognosis for recovery.

18.1.4.1.4 Ileus

Smooth muscle cells have more voltage-gated Ca^{2+} channels and fewer voltage-gated Na^{+} channels than skeletal muscle fibers, and therefore Na^{+} is less important in the action potential. For this reason contractions are slower and prolonged (Ca^{2+} channels are slow channels). In skeletal muscle, almost all calcium ions required for contraction come from the sarcoplasmic reticulum. In contrast, in smooth muscle cells the sarcoplasmic reticulum is a rudimentary organelle and the cells depend on extracellular Ca^{2+} for contraction. For this particular reason, any pathologic condition that results in ionized hypocalcemia may affect smooth muscle contractility. This effect is evident in horses that develop ileus following hypocalcemia (after exercise, transport, sepsis). Horses with primary gastrointestinal disease (colic) may develop ileus from increased concentrations of inflammatory and pain mediators (from sepsis and endotoxemia) that affect gastrointestinal motility; however, in some of these horses the ileus may result from hypocalcemia. Treatment with calcium gluconate may restore gastrointestinal motility.

18.1.4.1.5 Retained Placenta

Incidence of retained placenta in mares has been reported to occur in up to 10% of foalings.[148] Low serum total and ionized calcium concentrations often occur in mares with retained placenta and in mares with acute endometritis. Decreased uterine tone and contractility possibly results from a mechanism similar to ileus. In a recent study, mares with retained placenta were found to have statistically lower serum total calcium concentrations than mares without retained placenta within 12 hours after foaling.[149] Furthermore, 64% of mares treated with a combination of oxytocin in a calcium/magnesium borogluconate solution responded to treatment compared with 44% of the mares treated with oxytocin in saline solution. No differences in serum magnesium concentrations were found in this study.

18.1.4.1.6 Treatment of Hypocalcemia

When treating hypocalcemia, one should consider calcium deficit, maintenance, losses, and sequestration. If parathyroid gland function is normal, the amount of calcium required is probably minimal. Calcium therapy is more critical in horses that develop rapid hypocalcemia and in horses that may have impaired parathyroid gland function (exercise-induced hypocalcemia, sepsis) to restore normocalcemia. One should 1313
base the decision to treat horses with hypocalcemia on the presence of hypocalcemia and not in the 1314
presence of clinical signs associated with hypocalcemia. In most cases, horses with ionized hypocalcemia do not show signs of hypocalcemia, or the signs are too subtle to be detected, and the lack of therapy may result in additional complications (in particular ileus). Horses with mild hypocalcemia in general restore their normocalcemia without calcium administration; however, as a good practice and to avoid

complications, one should consider calcium administration. Horses with functional kidneys can eliminate large amounts of calcium rapidly, and hypercalcemia from excessive calcium administration is rare, in particular if the horse is receiving fluid therapy. At the Ohio State University, the author and colleagues determine serum ionized calcium concentrations in every critically ill horse and foal admitted to the Veterinary Teaching Hospital and, with few exceptions, treat most horses with gastrointestinal disease with calcium gluconate.

Some important considerations when calculating Ca^{2+} deficits in horses with hypocalcemia are as follows. The use of standard formulae to calculate electrolyte deficits based on extracellular fluid and body weight may not apply to calcium. One problem when using this formula is that Ca^{2+} can be eliminated rapidly or sequestered in different compartments, and larger doses of calcium often are required. One can calculate the calcium deficit based on ionized calcium concentrations (milligrams per deciliter equals millimoles per liter × 4).

The following formula allows one to calculate the Ca^{2+} deficit:

$$\frac{(6.5 - Ca^{2+})(10)(0.3)(\text{body mass})}{Ca^{2+}\ \text{ratio}} = Ca^{2+}\ \text{deficit}$$

in which the difference between the measured Ca^{2+} and normal Ca^{2+} (6.5 mg/dl) is multiplied by the extracellular fluid volume, the body mass (kg), and a factor of 10 and then is divided by the Ca^{2+} ratio (Ca^{2+}/total calcium). For a 450-kg horse with a serum Ca^{2+} concentration of 4.5 mg/dl and a total calcium of 10 mg/dl, the estimated Ca^{2+} deficit will be 6000 mg.

$$\frac{(6.5 - 4.5)(10)(0.3)(450\ \text{kg})}{0.45} = 6000\ \text{mg}$$

This product is a deficit of elemental calcium, and calcium gluconate or calcium borogluconate are the salts of choice for parenteral treatment of hypocalcemia. Calcium gluconate contains 9.3% of elemental calcium; in other words, every 100 ml of calcium gluconate 23% solution contains 2.14 g of elemental calcium or 21.4 mg/ml. The horse in the example would require approximately 300 ml of calcium gluconate 23% solution over 24 hours to replace the Ca^{2+} deficit.

One can use total calcium concentration to estimate calcium deficit; however, total calcium concentration has more variability than Ca^{2+} concentration because it depends highly on albumin concentrations and it is not subject to tight control by the Ca^{2+}-sensing system. A horse may have total hypocalcemia, but serum Ca^{2+} concentrations may be within the normal range, and calcium administration may not be necessary. However, one must be realistic that measurement of Ca^{2+} concentrations is not readily available for many practitioners, and keeping this in mind, measurement of total calcium concentrations is acceptable. Replacing measured total calcium and normal total calcium (11.5 mg/dl) in the same formula and dividing by a 0.5 Ca^{2+} ratio gives a close approximation of calcium deficit.

Frequent monitoring of Ca^{2+} concentration is important to adjust dosage. Some horses with severe gastrointestinal disease and sepsis remain hypocalcemic despite aggressive calcium supplementation. These horses may have considerable calcium losses into the intestinal compartment and in general carry a poor prognosis. Rapid administration of calcium may result in cardiovascular complications, in particular

in septic horses, which may be more vulnerable to the toxic effects of calcium. The author holds that horses can handle calcium dosages (and serum calcium concentrations) higher than traditionally believed; however, close monitoring is advised. Toribio, Kohn, Sams, et al.[128] have induced severe ionized hypercalcemia (10 to 12 mg/dl) in a large number of healthy horses using rapid (5 minutes) and slow (120 minutes) calcium chloride or calcium gluconate infusions with no obvious complications; however, every time calcium is administered intravenously a potential risk exists for serious complications, in particular in diseased horses. Based on the authors' experiences treating critically ill horses, calcium doses of 2 mg/kg/hr are safe in the horse. This dose rate represents approximately 50 ml of calcium gluconate 23% solution for a 500-kg horse in 1 hour while receiving fluid therapy. Furthermore, the authors have treated horses with severe hypocalcemia with calcium doses of up to 4-6 mg/kg/hr.

When administering calcium, the author starts with a calcium dose of 1 mg/kg/hr (25 ml of calcium gluconate per hour for a 500-kg horse). A 500-kg critically ill horse receiving 5 L of crystalloids supplemented with 50 ml of calcium gluconate is receiving 1000 mg of calcium per 5 L bag. If that amount is given in 1 hour, then the calcium dose is 2.0 mg/kg/hr. Sometimes the author uses 100 ml of calcium gluconate per 5 L of crystalloids in horses that are volume depleted and have severe hypocalcemia. Calcium chloride may be a good option to treat hypocalcemia in horses; however, calcium gluconate is readily available and inexpensive compared with calcium chloride. In addition, calcium 1314
chloride is not available in large volumes, and at least in human beings it may cause irritation at the 1315
administration site. One must keep in mind not to add calcium salts to fluid solutions containing bicarbonate because calcium carbonate complexes may form and precipitate.

A number of studies have found that calcium administration to some septic patients may be detrimental because it may result in increased intracellular calcium concentrations, activation of proteases, cell death, and increased mortality.

Oral treatment with calcium salts is feasible in some horses with non–life-threatening hypocalcemia. One can use dicalcium phosphate and calcium carbonate (limestone) safely (see Table 18.1-3).

18.1.4.2 HYPOCALCEMIC DISORDERS

18.1.4.2.1 Hypoparathyroidism

Hypoparathyroidism is a condition characterized by hypocalcemia, hyperphosphatemia, hypomagnesemia, and decreased serum PTH concentrations. Primary hypoparathyroidism results from decreased synthesis and secretion of PTH, whereas secondary hypoparathyroidism most commonly results from hypomagnesemia. Magnesium has a permissive effect on PTH secretion, and low magnesium concentrations may impair PTH release from the parathyroid gland. Pseudohypoparathyroidism is a condition in which PTH cannot interact with its receptor, or an abnormal signal transduction exists after PTH interacts with the PTH-1 receptor. Pseudohypoparathyroidism also may result from magnesium deficiency.

18.1.4.2.2 Primary Hypoparathyroidism

Primary hypoparathyroidism recently was reported in horses. The animals have clinical signs consistent with hypocalcemia, which may include ataxia, seizures, hyperexcitability, SDF, tachycardia, tachypnea, muscle twitching or fasciculations, stiff gait, recumbency, ileus, and colic. The diagnosis in based on the

determination of serum concentrations of Ca^{2+}, Mg^{2+}, intact PTH, and phosphorus. Hypocalcemia, hyperphosphatemia, low serum PTH concentrations, and hypomagnesemia have been reported in horses with primary hypoparathyroidism.[131,145] Hypocalcemia results from decreased PTH concentrations, and hyperphosphatemia most likely results from the decreased fractional urinary clearance of phosphorus caused by low PTH concentrations. Low PTH concentrations may contribute to hypomagnesemia because PTH stimulates Mg^{2+} reabsorption in the distal convoluted tubules. The administration of magnesium sulfate may be useful in differentiating primary from secondary hypoparathyroidism; in the case of secondary hypoparathyroidism from hypomagnesemia a rapid release (within minutes) of PTH is expected after magnesium administration.

18.1.4.2.3 Secondary Hypoparathyroidism

Secondary (functional or acquired) hypoparathyroidism as a pathologic entity has not been reported in the horse; however, some septic horses with hypocalcemia and impaired parathyroid gland function most likely are suffering from a secondary hypopathyrodism resulting from hypomagnesemia and increased concentrations of inflammatory mediators. Toribio, Kohn, Chew, et al. have found that a number of septic horses with ionized hypocalcemia and inappropriately low serum intact PTH concentrations had hypomagnesemia,[4] suggesting an impairment of the parathyroid gland to secrete PTH. Increased blood concentrations of inflammatory mediators such as IL-1, IL-6, and tumor necrosis factor α may potentially decrease PTH secretion,[4,58,150,151] causing a hypoparathyroid state. The author, like others,[129] has observed a number of critically ill foals with clinical evidence of hypocalcemia in which serum intact PTH concentrations are not increased as expected, suggesting hypoparathyroidism. However, whether these foals have a primary parathyroid gland dysfunction or whether they have parathyroid gland dysfunction from hypomagnesemia, from increased inflammatory mediators, or both is unclear.

Secondary hypoparathyroidism with hypercalcemia was reported in an 11-year-old Andalusian gelding with chronic renal failure and polycystic kidney disease.[130]

18.1.4.2.4 Pseudohypoparathyroidism

Parathyroid hormone resistance is referred as pseudohypoparathyroidism. In human beings the condition is inherited in which signal transduction after PTH interacts with its receptor is abnormal. Hypomagnesemia also may impair PTH action in its target organs, resulting in pseudohypoparathyroidism.

18.1.4.2.5 Idiopathic Hypocalcemia

Beyer, Freestone, Reimer, et al. reported on a series of foals with clinical signs of hypocalcemia of unknown cause.[129] These foals had serum PTH concentrations that were undetectable or within the reference range, suggesting abnormal parathyroid gland function for the degree of hypocalcemia. Possibly these foals may have suffered some form of hypoparathyroidism; serum phosphorus concentrations were increased in some foals, as occurs in hypoparathyroidism. Serum Mg^{2+} concentrations were not measured and a distinction between primary and secondary hypoparathyroidism cannot be made. Neonatal hypocalcemia occurs in human infants born of hyperparathyroid mothers, and the hypocalcemia that these infants develop is believed to result from maternal hypercalcemia, which may suppress fetal parathyroid gland function.[152] Another human condition with similar clinical features is familial hypercalciuric 1315

hypocalcemia, in which activating mutations of the CaR result in mild to severe hypocalcemia, hyperphosphatemia, and low to normal PTH concentrations.[63,153,154]

The author believes that abnormal activation of the CaR may be involved in the pathogenesis of hypocalcemia in septic foals. The author has found that IL-1 and IL-6 decrease PTH secretion from equine parathyroid cells in vitro and that IL-1 increases the expression of CaR mRNA, which in turn decreases PTH secretion. No studies in the horse have been done to evaluate whether mutations of the CaR may be involved in the pathogenesis of neonatal hypocalcemia as occurs in human infants.

18.1.4.2.6 Sepsis

Sepsis is perhaps the most common cause of hypocalcemia in equine patients admitted to veterinary hospitals. For reasons not yet understood, hypocalcemia is a frequent finding in human beings and animals with sepsis.[4,137,138,155–157] Clinical observations have revealed that total and ionized hypocalcemia are common in horses with severe gastrointestinal disease and sepsis.[4,137,138] Hypocalcemia in septic patients often results in increased serum PTH concentrations to return serum Ca^{2+} concentrations to the physiologic range.[4,157]

Of horses with enterocolitis admitted to the Ohio State University Veterinary Teaching Hospital, 75% had total hypocalcemia, 80% had ionized hypocalcemia, and 70% had ionized hypomagnesemia.[4] Of interest from that study was that some horses with clinical evidence of sepsis and hypocalcemia had low serum PTH concentrations for the degree of hypocalcemia, indicating an inappropriate response of the parathyroid gland to low serum Ca^{2+} concentrations (parathyroid gland dysfunction). Hypocalcemia is also frequent in septic foals, and the author has some critically ill foals with ionized hypocalcemia that have low serum intact PTH concentrations for the degree of hypocalcemia. In horses with surgically managed gastrointestinal disease, serum Ca^{2+} concentrations were lower in those with more severe lesions.[137,138] Increased endotoxin concentrations may be the trigger that activates a series of mechanisms that result in hypocalcemia. Horses with gastrointestinal disease may have detectable concentrations of endotoxin in plasma.[158–160] The author,[58] like others, has found that parenteral administration of endotoxin to healthy animals results in hypocalcemia.[155,161]

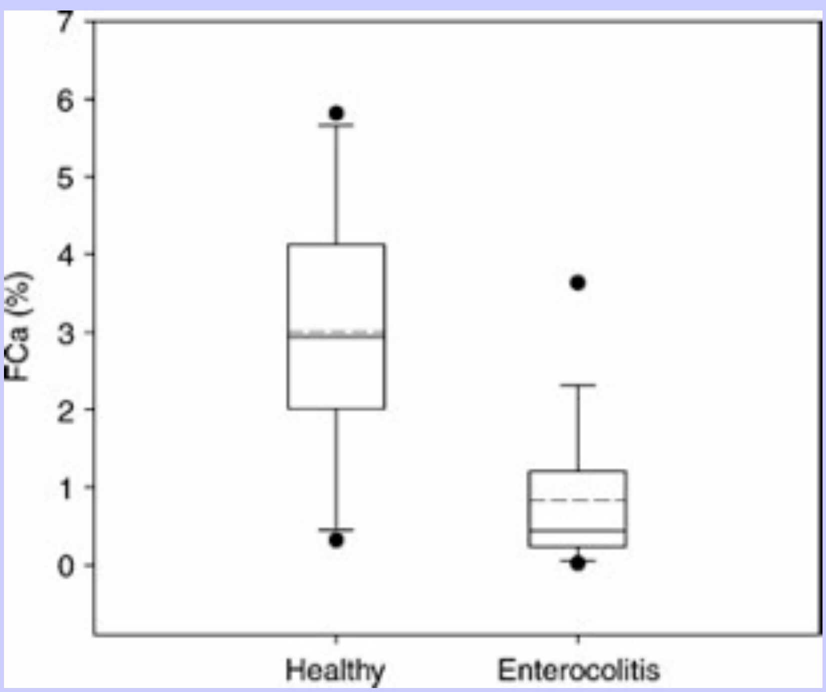
The mechanisms responsible for development of hypocalcemia during sepsis are not understood completely. Possible causes of hypocalcemia include renal loss of calcium,[162] calcium sequestration in the gastrointestinal lumen from poor absorption associated with inflammation,[161] intracellular calcium accumulation,[163] impairment in calcium mobilization,[164–166] tissue sequestration of calcium,[155,162] impairment of calcium release by the target tissue in response to PTH,[162] failure to synthesize $1,25(OH)_2D$,[156] and parathyroid gland dysfunction.[4] Renal loss of calcium as a cause of hypocalcemia in septic horses seems unlikely because horses with enterocolitis and hypocalcemia have been found to have low fractional urinary clearance calcium (Figure 18.1-8).[4]

Impaired calcium mobilization may result from impaired parathyroid gland secretion of PTH,[167] poor osteoblast-osteoclast response to PTH,[162] or high serum concentrations of calcitonin and procalcitonin.[164–166] Serum PTH concentrations are low in some human beings and horses with sepsis and hypocalcemia.[4,168] During sepsis, impaired PTH secretion could result from upregulation of the CaR in parathyroid

gland chief cells from intracellular accumulation of calcium and from hypomagnesemia.[58,151,169] Hypomagnesemia is a frequent finding in critically ill human beings.[4,138,156] Inflammatory mediators that may be increased in horses with sepsis such as IL-1 and IL-6 may increase intracellular calcium concentrations, upregulate the CaR in parathyroid chief cells, and suppress PTH secretion.[58,151,169] Upregulation of the CaR in equine parathyroid chief cells by IL-1 results in lower PTH secretion.[169]

Tissue sequestration as a cause of hypocalcemia has not been evaluated in the horse, although it may be
important in the pathogenesis of hypocalcemia. Administration of endotoxin to healthy pigs resulted in 1316
increased calcium accumulation in the liver and peritoneal fluid.[155] One proposal is that because 1317
interstitial fluid volume is much greater than the blood volume, interstitial accumulation of calcium could result in hypocalcemia.[155]

Figure 18.1-8 Box plot of fractional urinary clearance of calcium *(FCa)* in healthy horses (*n* = 20) and horses with enterocolitis (*n* = 20). Mean fractional urinary clearance of calcium was significantly lower (*P* < 0.05) in horses with enterocolitis. •, 5th and 95th percentiles. (Adapted from Toribio RE, Kohn CW, Chew DJ et al: Comparison of serum parathyroid hormone and ionized calcium and magnesium concentrations and fractional urinary clearance of calcium and phosphorus in healthy horses and horses with enterocolitis, *Am J Vet Res* 62:938-947, 2001.)



In human beings and animals with sepsis, intracellular calcium concentrations are increased in different cells, including hepatocytes, aortic smooth muscle cells, red blood cells, and skeletal muscle cells.[163,170,171] Intracellular accumulation of Ca^{2+} may play an important role in the progression from sepsis to multiorgan dysfunction syndrome, the most common cause of mortality in the intensive care unit.[163] Calcium accumulation in the cell membrane also may play some role in the pathogenesis of hypocalcemia and sepsis.

As previously mentioned, some studies indicate that calcium administration to some critically ill patients may be detrimental because it may increase the risk of mortality by increasing intracellular calcium concentrations and inducing cell injury.[156,172]

18.1.4.2.7 Exercise-Induced Hypocalcemia

Horses under intense exercise develop electrolyte and acid-base abnormalities. Unlike human beings, which may develop ionized hypo- or hypercalcemia, ionized hypocalcemia seems to be a more consistent finding in exercise in the horse. Common clinical signs in horses with exercise-induced hypocalcemia include SDF, muscle weakness, muscle fasciculation, ileus, colic, excitation, and cardiac arrhythmias.

Hypocalcemia in exercising horses may result from calcium losses into the sweat[146,173]; calcium movement to the intracellular compartment; increased calcium binding to plasma components such as albumin, lactate, phosphate, and bicarbonate,[174] which are increased during exercise; and from increased calcium binding to albumin during alkalosis. Exercising horses may develop respiratory alkalosis from hyperventilation, metabolic alkalosis from chloride losses in the sweat (hypochloremic), or a combined alkalosis. The effect of hyperalbuminemia on serum Ca^{2+} concentrations varies, and in some cases no relationship between serum albumin concentrations and Ca^{2+} has been found. Although some horses may develop alkalosis that may contribute to ionized hypocalcemia, most horses under intense exercise develop metabolic acidosis.[174]

More insight information on factors associated with calcium regulation (PTH, calcitonin, and $1,25[OH]_2D$) in the horse under exercise is lacking. In a recent study, serum Ca^{2+} and PTH concentrations were measured in jumping competition horses; serum Ca^{2+} decreased while serum PTH concentrations increased as expected, and the authors concluded that inadequate parathyroid gland function as the cause of hypocalcemia in these horses was unlikely.[36] However, the PTH increase (result of hypocalcemia or from parathyroid gland adrenergic stimulation) was not sufficient to restore normocalcemia. When serum PTH and Ca^{2+} concentrations were measured in horses competing in endurance rides,[136] serum PTH concentrations were found not to increase in all horses that developed hypocalcemia, which may indicate parathyroid gland dysfunction. In human beings, increased serum PTH concentrations are a consistent finding during exercise. Although acid-base and electrolyte disturbances are important in the development of ionized hypocalcemia, some other factors may be impairing the ability of these horses to correct their hypocalcemia. Increased serum concentrations of calcitonin may inhibit osteoclastic bone resorption. During intense exercise in human beings, serum concentrations of gastrin and glucagon increase, probably from increased energy demands and the result of transient gastrointestinal ischemia. Gastrin and glucagon cause a release of calcitonin from the thyroid gland C cells. Increased serum concentrations of gastrin[175] and glucagon[176] have been documented in exercising horses. Whether a relationship between exercise,

gastrin and glucagon release, calcitonin increase, and hypocalcemia exists in the horse still remains to be established. Renal losses of calcium as a cause of hypocalcemia during exercise are unlikely because urinary losses of calcium decrease in exercising horses.[147]

18.1.4.2.8
Vitamin D Deficiency

No evidence indicates that horses develop hypocalcemia from vitamin D deficiency. Horses have low concentrations of vitamin D metabolites, and their role in the critically ill horse is questionable. Horses with chronic renal failure and with bilateral nephrectomy[177,178] often develop hypercalcemia and hypophosphatemia, which may result from decreased glomerular filtration rate rather than from decreased $1,25(OH)_2D$ concentrations, emphasizing the important role of the kidney in calcium excretion.

18.1.4.2.9
Oxalate Toxicity

Oxalate excess reduces calcium absorption; 1% of oxalate or higher can reduce calcium absorption by 66% or more.[19] The equine diet must contain less than 0.5% of oxalate and a calcium/oxalate ratio greater than 1.0. Diets containing 0.87% of oxalate had no effect in calcium absorption when the calcium/oxalate ratios were higher than 1.7.[21] Clinical signs associated with oxalate excess are phosphate excess, calcium deficiency, and nutritional hyperparathyroidism. Oxalates are present in several tropical grasses, toxic plants, and in alfalfa hay (see Table 18.1-6).

18.1.4.2.10
Tetracycline Administration

Tetracycline chelates calcium, and its rapid intravenous administration can result in cardiac arrhythmias, recumbency, sudden collapse, or even death in horses. In most cases, these effects are transient and no treatment is necessary; however, in some cases calcium administration may be required. 1317 1318

18.1.4.2.11
Furosemide Administration

The administration of loop diuretics is a common practice in performance horses. Furosemide inhibits the $Na^+/K^+/2Cl^-$ cotransporter in the cortical thick ascending loop of Henle, decreasing the transepithelial voltage gradient required for Ca^{2+} and Mg^{2+} transport. The end result is increased Ca^{2+} and Mg^{2+} wasting into the urine. This effect can last up to 8 hours in the horse.[179]

18.1.4.2.12
Bicarbonate Overdose

The administration of bicarbonate (parenterally or orally) to horses has been a widespread practice among trainers, owners, and veterinarians. The excessive administration of bicarbonate results in alkalosis (decreasing Ca^{2+} concentrations) and in accumulation of bicarbonate ions in the distal nephron. In the presence of high anion concentrations, which alter the transepithelial voltage gradient, the kidney excretes cations in the urine, including Ca^{2+} and Mg^{2+}.

18.1.4.2.13

Cantharidiasis

Equine cantharidiasis (blister beetle toxicosis) is a condition reported in the southwestern and midwestern United States and is produced by the ingestion of alfalfa contaminated with beetles (*Epicauta* spp.), which produce cantharidin (cantharidic acid).[142,180] Horses are susceptible to cantharidin and develop clinical signs from the irritant effects that this toxin has on mucosal surfaces (gastrointestinal and urinary tracts). In addition, cantharidin causes acute hypocalcemia and hypomagnesemia. Synchronous diaphragmatic flutter and muscle fasciculation are frequent findings in horses with cantharidiasis. Because these horses may develop severe hypocalcemia, ataxia, dyspnea, laryngeal spasm, and cardiac arrhythmias may be present.[180] The reason that these horses develop acute hypocalcemia is unclear; however, the cause may be the combination of severe gastrointestinal disease, calcium losses, and sequestration in the gastrointestinal tract, associated with acute renal failure. Some horses with cantharidiasis develop myocardial necrosis, suggesting a direct effect of cantharidin in the myocardium. Possibly cantharidin increases intracellular calcium concentration in the myocardium, causing cell death. Therefore cardiac arrhythmias in these horses probably result from the direct effect of cantharidin in the myocardium and from hypocalcemia.

18.1.4.2.14

Acute Renal Failure

Hypocalcemia and hypomagnesemia are common findings in horses with acute renal failure and probably result from increased urinary losses of Ca^{2+} and Mg^{2+}. Reabsorption of Ca^{2+} and Mg^{2+} in the kidney depends highly on functional epithelial cells, and these cells are susceptible to various insults (hypoxia, toxins). The loss of the epithelial cells and their absorptive capacity results in decreased reabsorption and increased secretion of Ca^{2+} and Mg^{2+}.

18.1.4.2.15

Exertional Rhabdomyolysis

The pathogenesis of hypocalcemia in exertional rhabdomyolysis is unknown. Speculations are that muscle fiber damage during intense exercise results in Ca^{2+} influx and sequestration into the sarcoplasm and sarcoplasmic reticulum. Hypocalcemia has been reported in neonatal foals with severe rhabdomyolysis.[181] A severalfold increase in sarcoplasmic $Ca^{2+}{}_i$ concentrations have been reported in horses with exertional rhabdomyolysis,[182] indicating that elevations in $Ca^{2+}{}_i$ may be important in the pathogenesis of endoplasmic reticulum. Further discussion on exertional rhabdomyolysis can be found in Chapter 9.

18.1.4.2.16

Pancreatic Necrosis

The cause of hypocalcemia with pancreatic necrosis is unclear, but at least in human beings the cause is associated with deposition of calcium in regions of saponification of peripancreatic fat (fat necrosis). Pancreatitis principally causes colic in horses, although it may be associated with hypocalcemia.

18.1.4.3

HYPERCALCEMIC DISORDERS

Hypercalcemic disorders can be divided in two categories: (1) hypercalcemia associated with parathyroid gland dysfunction (parathyroid-dependent hypercalcemia), and (2) hypercalcemia independent of the parathyroid gland function (hypercalcemia develops despite parathyroid gland suppression). This distinction

is clinically important and emphasizes the use of specific diagnostic tests, including the determination of intact PTH, PTHrP, Ca^{2+}, phosphorus, and vitamin D_3 metabolites concentrations. Parathyroid-dependent hypercalcemia in the horse is limited to primary hyperparathyroidism, whereas parathyroid-independent hypercalcemia results from different conditions.

18.1.4.3.1 Primary Hyperparathyroidism

In primary hyperparathyroidism, an abnormality of the parathyroid gland chief cells results in excessive and autonomous synthesis and secretion of PTH, and the parathyroid gland does not respond to the negative feedback of Ca^{2+}. In the horse, primary hyperparathyroidism may result from a parathyroid adenoma or parathyroid hyperplasia. Parathyroid carcinoma has not been diagnosed in the horse. Elevated PTH concentrations result in increased renal Ca^{2+} reabsorption (hypocalciuria), hyperphosphaturia, 1318
increased bone resorption, and increased $1,25(OH)_2D_3$ synthesis. The end result is loss of cortical bone, 1319
and a condition known as osteodystrophia fibrosa. Primary hyperparathyroidism associated with osteodystrophia fibrosa has been reported in ponies and horses.[46,132,183–185] These horses may have enlargement of the facial bones, lameness, and a poor body condition. Radiographic findings reported include osseous proliferation of the maxilla and mandible, and loss of the lamina dura surrounding the molars.[132] Endoscopic examination may reveal narrowing of the nasal passages.

Hypercalcemia, hypophosphatemia, phosphaturia, and increased intact PTH concentrations are laboratory findings reported in horses with primary hyperparathyroidism. Additional tests to rule out other conditions associated with hypercalcemia in the horse may include measurement of PTHrP and vitamin D metabolite concentrations. Histologic examination of the parathyroid gland is important to confirm the diagnosis of primary hyperparathyroidism. However, finding the parathyroid glands may be a difficult task in some horses because of their small size and variable location (see anatomy section). Only in one case of primary hyperparathyroidism reported in the literature was an adenoma of the parathyroid gland found.[184] In human beings, primary hyperparathyroidism often results from one or more adenomas in previously normal parathyroid glands, and in some other cases the glands may be hyperplastic. Postmortem findings in horses affected with primary hyperparathyroidism include enlargement of the maxilla and mandible, stenosis of the nasal passages, and loosening of premolars and molars.

18.1.4.3.2 Secondary Hyperparathyroidism

In secondary hyperparathyroidism, excessive secretion of PTH is the response of the parathyroid gland to hypocalcemia, hyperphosphatemia, or hypovitaminosis D from renal dysfunction or from nutritional imbalances.

18.1.4.3.3 Renal Secondary Hyperparathyroidism

In the case of renal secondary hyperparathyroidism in small animals and human beings, phosphorus retention from renal disease (decreased glomerular filtration rate) can stimulate PTH secretion directly.[186] In addition, phosphorus lowers serum Ca^{2+} concentrations, which contributes to PTH stimulation. High phosphorus concentrations also inhibit 1α-hydroxylase, an important enzyme in the synthesis of $1,25(OH)_2D$, which has a negative effect on PTH gene expression. Therefore hyperphosphatemia indirectly stimulates PTH synthesis and secretion. Furthermore, the progressive destruction of the tubular

cells in the proximal convoluted tubule, which have 1α-hydroxylase activity, results in less $1,25(OH)_2D$ synthesis. In addition, decreased concentrations of $1,25(OH)_2D$ may result in impaired intestinal absorption of Ca^{2+}, contributing to hypocalcemia and PTH release.

In horses with chronic renal failure (CRF), hypophosphatemia and hypercalcemia are consistent findings, and hyperphosphatemia is uncommon. Horses with CRF have seen serum total and ionized calcium concentrations of up to 22 mg/dl and 12 mg/dl, respectively. The kidneys are important in eliminating calcium in the horse, and an abnormal renal function associated with normal intestinal calcium absorption may be an explanation for the hypercalcemia in these horses. Some horses with CRF have increased serum PTH concentrations, whereas others have serum PTH concentrations within or below the normal range, suggesting that hypercalcemia in most horses with CRF results from impaired renal calcium excretion. Therefore renal secondary hyperparathyroidism may not be an important cause of hypercalcemia in the horse.[130,187] The explanation for hyperphosphatemia in horses with CRF is unknown, although it possibly results from increased Ca^{2+} concentrations. PTHrP concentrations have not been measured in horses with CRF.

Calciphylaxis is a syndrome characterized by calcification of small blood vessels and necrosis of the skin, visceral organs, and subcutaneous fat.[188] These patients have chronic renal failure associated with hypercalcemia, hyperphosphatemia, or elevated calcium-phosphate complexes. This condition remains to be documented in the horse.

18.1.4.3.4 Nutritional Secondary Hyperparathyroidism

Horses that are fed a diet low in calcium, high in phosphorus, or both may develop a clinical condition known as nutritional secondary hyperparathyroidism (NSHPT). This disease also is known as bran disease, miller's disease, bighead, osteodystrophia fibrosa, osteitis fibrosa, and equine osteoporosis. The disease can affect one or many animals in one herd. Diets containing a phosphorus/calcium ratio of 3:1 or more may result in NSHPT.[133,189] Nutritional secondary hyperparathyroidism is uncommon in developed countries; however, occasionally a young horse may have clinical signs consistent with NSHPT, including enlargement of facial bones, upper respiratory noise, and lameness. In older horses, facial bone enlargement may not be evident. In the past, NSHPT commonly was associated with grain-rich diets, in particular with excessive amounts of wheat bran (miller's disease); however, with improvements in animal nutrition, NSHPT rarely is associated with excessive grain feeding. Grains with a high phosphorus content and a higher phosphorus/calcium ratio often result in excessive absorption of phosphorus with respect to calcium and decreased absorption of calcium. Various pastures contain a high content of oxalates (see Table 18.1-6), which bind dietary calcium to form calcium oxalate $(Ca[COO]_2)$ that is insoluble at high pH, predisposing horses to NSHPT.[190] 1319 1320

Excessive amounts of phosphorus in the intestinal tract reduce calcium absorption and may result in hyperphosphatemia. High-phosphorus and low-calcium diets induce parathyroid cell hyperplasia in the horse.[46] Hyperphosphatemia directly stimulates PTH secretion and inhibits $1,25(OH)_2D$ synthesis in the kidney. Because $1,25(OH)_2D$ inhibits PTH gene expression and synthesis, low $1,25(OH)_2D$ concentrations may result in parathyroid cell hyperplasia and increased secretion of PTH. One of the most important functions of $1,25(OH)_2D$ is to increase intestinal Ca^{2+} absorption by increasing the synthesis of Ca^{2+}-binding proteins (calbindin D_{9k}) in the small intestine. Therefore low $1,25(OH)_2D$ concentrations may

contribute to a calcium-deficient state. Although dietary deficiency of vitamin D may result in NSHPT in other species and has been proposed as a possible cause of NSHPT in the horse, little evidence exists that supports hypovitaminosis D as a cause of NSHPT in the horse. Hyperphosphatemia also may result in the formation of calcium phosphate, reducing blood Ca^{2+} concentrations, and stimulating PTH secretion. Parathyroid hormone increases osteoclastic activity, resulting in excessive bone resorption and bone loss. Horses given a diet high in phosphorus have increased bone resorption from increased osteoclastic activity.[191] Facial bone loss and excessive accumulation unmineralized bone matrix (osteodystrophia fibrosa) results in facial enlargement (bighead) (Figure 18.1-9).

Because NSHPT is a condition of slow progression, the homeostatic mechanisms that regulate extracellular Ca^{2+} concentrations (PTH, vitamin D, calcitonin) may be effective to maintain Ca^{2+} close to or within the normal range. These horses maintain normocalcemia at expense of the skeletal reserves, and do not develop clinical signs of hypocalcemia.

Clinical signs may include intermittent, shifting lameness and a stiff gait.[192,193] In many cases, swelling of the facial bones is typical and symmetric. Younger animals may develop physitis and limb deformities.[189] The facial changes and the bone resorption around the lamina dura of the molars and premolars may result in masticatory problems. These horses are physically weak and may be in poor body condition from the pain associated with lameness and mastication. In severe cases, teeth may become loosened and spontaneous fractures may occur. Upper airway obstruction and dyspnea may be present.[194,195]

Clinical laboratory findings may include mild hypocalcemia and hyperphosphatemia; however, these values may be within the reference ranges. Serum intact PTH concentrations are increased.[192] Fractional urinary clearance of calcium (FCa) is low and fractional urinary clearance of phosphorus is high. Serum alkaline phosphatase activity may be increased. Dietary corrections may result in rapid changes in FCa and fractional urinary clearance of phosphorus, and one must perform these tests before diet manipulation. The use of FCa to assess the calcium balance has been proposed,[30,31] and horses with a normal calcium balance are expected to have a FCa greater than 2.5%. The FCa in horses may vary from daily diet changes, and we have found a wide range of FCa in clinically healthy horses (0.2% to 6%), making the FCa alone not a good indicator of calcium balance in the horse.

Radiologic findings may include decreased bone density[192,196]; however, bone density must be decreased 30% before radiographic changes are evident.[197] Resorption of alveolar sockets and loss of the lamina dura dentes may be present before other radiographic changes are present, and long bones may be affected only in advanced cases.

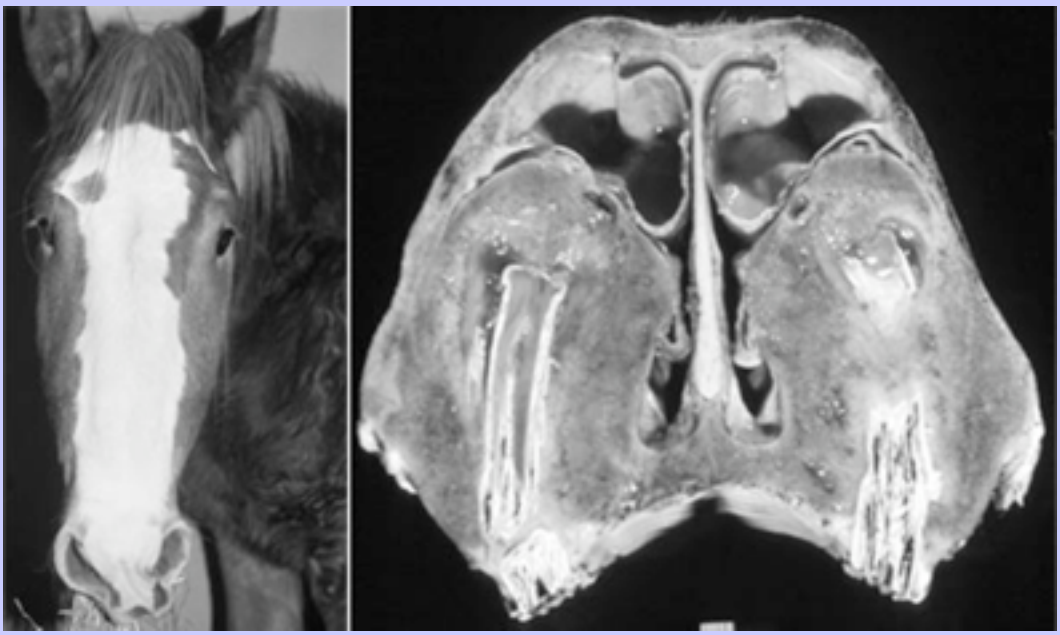
Supplementation with calcium carbonate (limestone) and dicalcium phosphate in the diet has resulted in improvement in horses treated for NSHPT.[133] For the first 2 to 3 months of calcium supplementation should be twice the daily requirements. The addition of alfalfa hay to the diet and decreasing the amount of grain may be helpful. Ground limestone, which contains no phosphorus, is recommended as a good source of calcium (35%). An affected animal may require a total of 100 to 300 g/day, and the diet should have a Ca:P ratio of 3:1 to 4:1. Limestone may decrease feed palatability, and adding molasses is recommended. Confinement of severely affected horses is advised. The use of NSAIDs may be indicated in horses with severe pain; however, one should use these drugs with caution because increased activity may result in fractures. Supplementation with vitamin D has been proposed. Horses required approximately 9 to 12 months for complete recovery and for the bony lesions to regress. Owners should

avoid using high-oxalate feeds; however, if this is not a practical option, one may increase calcium and phosphorus supplementation. For horses consuming feeds with high oxalate content, an additional 20 mg of calcium per kilogram and 10 mg of phosphorus per kilogram body mass per day may be necessary.[20]

18.1.4.3.5 Hypervitaminosis D

The accidental ingestion or administration of ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3) results in disturbances of the calcium and phosphorus metabolism in domestic animals.[134,198–205] In the horse, intoxication with ergocalciferol and 1,25-dihydroxycholecalciferol (1,25-dihydroxyvitamin D) has been reported.[134,199,205] Ingestion of plants containing $1,25(OH)_2D$-like compounds results in typical clinical signs of vitamin intoxication.[200,202–204] The ingestion of *Solanum malacoxylon* results in a 1320
condition known as "enteque seco" in Argentina and "espichamento" in Brazil.[198,204] In Hawaii the 1321
ingestion of *Solanum sodomaeum* results in hypercalcemia. The ingestion of jessamine *(Cestrum diurnum)*, a shrub widely distributed in the southern United States (from Florida to California) may cause hypervitaminosis D.[200] In Europe, the ingestion of *Trisetum flavescens* by horses results in a condition known as enzootic calcinosis. Hypervitaminosis D results in increased intestinal absorption of phosphorus and hyperphosphatemia. Hyperphosphatemia is the most consistent and early laboratory finding in horses with vitamin D intoxication.[199] Serum calcium concentrations may be within the normal range or increased.[134,199,203,205] Vitamin D is an important regulatory factor of parathyroid cell function and proliferation, and hypervitaminosis D may decrease PTH gene expression and secretion and also cause parathyroid cell atrophy.[201] In addition, hypercalcemia inhibits PTH secretion, lowering the bone turnover. Uremia and hyposthenuria may be present.[134]

Figure 18.1-9 Belgium yearling presented to the Ohio State University Veterinary Teaching Hospital with clinical signs consistent with nutritional secondary hyperparathyroidism, including enlargement of facial bones and upper respiratory noise. The horse was fed excessive amounts of grain. Narrowing of the nasal passages, loss of bone mass, and excessive accumulation of unmineralized bone matrix (osteodystrophia fibrosa) were evident.



Clinical signs result from hyperphosphatemia. Horses often have weight loss and poor appetite, develop lameness, painful stiffness, and are reluctant to move.[199,205] Acute death from severe cardiovascular mineralization has been reported.[134] Polyuria and polydipsia are frequent findings. In cases with hypercalcemia, the deposition of minerals in the kidneys may precede mineralization elsewhere, resulting in renal failure. In addition, hypercalcemia, by direct activation of the calcium receptor in the distal convoluted tubules of the nephron may induce diuresis. Lameness probably caused by calcification of ligaments and tendons.

Radiographically, these horses have increased bone density, decreased size of the medullary cavity, and increased calcification of soft tissues.

The prognosis for horses with hypervitaminosis D is poor. Treatment may include reducing dietary calcium intake. The use of calcium-binding agents such as sodium phytate, which is high in many cereals, has been proposed.[205] Glucocorticoids are used in human beings with hypervitaminosis D, with the rationale that they may inhibit the vitamin D–mediated calcium absorption in the intestine. Dexamethasone

administration to pony foals results in decreased intestinal absorption of calcium, decreased bone resorption, and increased urinary excretion of calcium.[25,26] Dexamethasone has been administered to horses with hypervitaminosis D with variable results.[205]

Postmortem examination may reveal mineralization of soft tissues. Extensive cardiovascular mineralization of the endothelium of the aorta and pulmonary vessels and of the endocardium of the atrium and ventricles is frequent. Mineralized plaques may be present in the endothelium and endocardium. Mineralization may be found in the kidney, liver, lymph nodes, lungs, ligaments, and tendons. 1321
Osteopetrosis of epiphyses and metaphyses may be present. Atrophy of the parathyroid chief cells may be 1322
severe.[201]

18.1.4.3.6 Pseudohyperparathyroidism

The term *pseudohyperparathyroidism* has been used to describe a paraneoplastic condition known as humoral hypercalcemia of malignancy, in which human beings and animals develop hypercalcemia associated with malignancies. Parathyroid hormone–related protein was discovered to be humoral factor responsible for hypercalcemia in human beings with malignancies.[34] Humoral hypercalcemia of malignancy has been described in several domestic animals. In the horse, HHM has been reported to be associated with gastric squamous cell carcinoma,[117] adrenocortical carcinoma,[206] squamous cell carcinoma of the vulva,[115] lymphosarcoma,[116,207] and ameloblastoma.[120]

18.1.4.3.7 Neonatal Hypercalcemia and Asphyxia

Clinical observations indicates that a number of critically ill newborn foals develop hypercalcemia associated with asphyxia (F.T. Bain, personal communication, 2002). These foals have severe hypotension with somnolence; however, they cannot be differentiated clinically from foals with severe asphyxia. The mechanisms underlying this problem are unknown.

18.1.4.3.8 Treatment of Hypercalcemia

Hypercalcemia rarely is an equine emergency; however, the differential diagnosis of hypercalcemia is important in the treatment of hypercalcemia. Few disorders in the horse are associated with hypercalcemia (hyperparathyroidism, CRF, HHM, hypervitaminosis D). Mild to moderate hypercalcemia in general is not a life-threatening condition, and one should direct treatment to the primary cause (primary hyperparathyroidism, chronic renal failure). Hypervitaminosis D can be fatal with mineralization of vital organs. Surgical removal of epithelial tumors may be a successful treatment in some patients. In cases of severe hypercalcemia that may require treatment, initial therapy should include the administration of 0.9% saline solution and loop diuretics. Furosemide is the diuretic of choice because it inhibits the $Na^+/K^+/2Cl^-$ contransporter in the distal tubules, inducing urinary calcium excretion. Thiazide diuretics are contraindicated because they stimulate calcium reabsorption. One should consider glucocorticoid administration.

18.1.5

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18.2

18.2—Pars Intermedia Dysfunction (Equine Cushing's Disease)

Ramiro E. Toribio

A clinical syndrome associated with hirsutism, laminitis, polyuria and polydipsia, and weight loss has been described in aged horses.[1–5] This condition results from dysfunction of the pituitary gland, progresses slowly, and affects horses and ponies.

The equine syndrome is similar to Cushing's disease in human beings, in which occurs excessive and autonomous secretion of proopiomelanocortin-derived peptides, including adrenocorticotropic hormone (ACTH, corticotropin), resulting in secondary hyperadrenocorticism. However, in horses, pituitary adenomas arise from the pars intermedia of the pituitary gland and rarely arise from the anterior lobe (pars distalis, adenohypophysis) as occurs in human beings and dogs. Although many of the clinical signs associated with equine Cushing's disease are frequent among affected horses, some clinical signs may vary, perhaps from variable secretion of other proopiomelanocortin-derived peptides. Compared with other equine endocrine disorders, equine Cushing's disease is a frequent clinical syndrome, and pars intermedia adenomas often are identified at postmortem examination in elderly horses with no previous history or reported clinical signs of this condition. The underlying cause of equine Cushing's disease remains unclear, although some evidence suggests that the disease results from lack of inhibitory control on the pars intermedia, as will be discussed later in this section.

Equine Cushing's disease has been described as a equine Cushing's syndrome, pituitary adenoma, hypophyseal adenoma, chromophobe adenoma, pars intermedia adenoma, diffuse adenomatous hyperplasia of the pituitary, pituitary-dependent hyperadrenocorticism, pars intermedia hyperplasia, and pars intermedia dysfunction.[1,3,6–21] The latter two terms seem to be more appropriate to describe equine Cushing's disease because, unlike human beings, adrenocortical hyperplasia and hypercortisolemia are not consistent findings in affected horses. Pars intermedia dysfunction is used in this section to refer to equine Cushing's disease.

18.2.1 Clinical Signs

Pars intermedia dysfunction (PID) is a clinical syndrome of aged horses associated with hirsutism, poor hair 1327
coat, laminitis, lethargy, narcolepsy, polyuria and polydipsia, muscle wasting, weight loss, docility, decreased 1328
responsiveness to painful stimuli, increased appetite, and recurrent infections.[1,2,15,18,20] Generally, PID is diagnosed in horses older than 7-years of age (range 7 to 40 year; average 19 to 21 years)[1,2,5,22,23] with no breed predilection, but PID seems to be more prevalent in ponies than in horses.[2] No sex preference exists,[2,5,23] although in several studies PID appeared to be more prevalent in females,[1,11,13,24] and one report found that Cushing's disease was more common in males.[2]

Figure 18.2-1 Horse diagnosed with pars intermedia dysfunction. The horse was treated with a combination of pergolide and cyproheptadine, and clinical signs improved by 8 weeks of treatment. (Courtesy Tracy Miesner, Columbus, The Ohio State University.)

Hirsutism (55% to 80%) is the most frequent clinical sign in affected horses[1,2,5,20,25] (Figure 18.2-1). These horses have a long and thick hair coat, in many cases long and curly hair, and abnormal hair coat shedding; some horses shed in late spring and grow a winter coat early in the fall, and many horses develop a patchy coat with areas of alopecia. Retention of long hair in the jugular grove, on the legs, and along the ventral abdomen are also frequent findings. The mechanisms underlying the abnormal hair coat and shedding process are not understood. Horses with adrenal exhaustion or insufficiency may also have an abnormal hair coat.[26]

Weight loss, lethargy, laminitis, and polyuria and polydipsia are also consistent clinical findings.[2,23,25] Skin infections (dermatophilosis), sinusitis, pneumonia, tachypnea (in particular in horses with long hair coat during warm weather), dental disease, abnormal estrus cycles, neurologic signs (seizures, narcolepsy), colic, abnormal vision, and bulging of supraorbital fat pads are among other clinical signs or conditions reported concomitantly with PID.[*]

Chronic laminitis is a consistent finding in these horses,[25] and laminitis is speculated to result from increased concentrations of glucocorticoids. Regardless of the underlying cause of laminitis, one should include PID in the differential diagnoses in any elderly horse that has chronic laminitis, even if other clinical signs of PID are not present.

Polyuria and polydipsia have been reported in up to 76% of horses diagnosed with PID[2]; however, the pathogenesis behind the development of polyuria and polydipsia is unclear. Compression of the pars nervosa by a pars intermedia adenoma has been speculated to result in decreased secretion of vasopressin (arginine vasopressin, antidiuretic hormone).[1,6] Hyperglycemia, which is a frequent laboratory finding in these horses, may result in osmotic diuresis. Glucocorticoids can increase glomerular filtration rate and may contribute to diuresis. One must rule out other differential diagnoses for polyuria and polydipsia, including neurogenic diabetes insipidus, nephrogenic diabetes insipidus, psychogenic polydipsia, and hyperglycemia of various origins (pancreatic disease, pheochromocytoma). Horses that are stalled are most likely to have obvious polyuria and polydipsia.

Increased docility in affected horses often is reported by owners and clinicians. Horses with PID have increased plasma and cerebral spinal fluid concentrations of β-endorphin,[12] which may explain the docility and lethargy in some of these animals. Increased β-endorphin concentrations also may explain the decreased responsiveness to painful stimuli.

The author has been recognizing narcolepsy as a presenting complaint in some horses diagnosed with PID. 1328

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Excessive sweating (hyperhidrosis) and, less commonly, lack of sweating (anhidrosis) are reported in horses with PID, although the mechanisms underlying abnormal sweating have not been determined.

The majority of horses with PID (up to 88%) have weight loss[2]; however, some horses may be obese or in good body condition.[23] Affected horses often have a weak and pendulous abdomen, a sway back, and lose muscle mass along the dorsum of the back. The pot-bellied appearance probably results from lack of tone in the abdominal musculature. The muscle wasting along the dorsal midline and elsewhere results in prominence of the croup, tuber coxae, and tuber sacrale regions. What the effect of different proopiomelanocortin-derived peptides is on the equine energy metabolism is unclear. Weight loss in some horses may result from other conditions associated with aging (dental problems, immunosuppression and disease, parasites) rather than the direct effect of an abnormally regulated hypothalamus-pituitary-adrenal (HPA) axis on body condition. The role

of increased concentrations of cortisol on weight loss is unclear; glucocorticoids, in addition to being immunosuppressive, have catabolic effects on skeletal muscle, and in some horses increased cortisol concentrations may be responsible in part for muscle wasting and weight loss. In horses with normal or low cortisol concentrations, other causes may be responsible for the weight loss.

The bulging supraorbital fat pads often observed in horses with PID result from fat redistribution.[25] Likewise, some horses in good body condition may have a “cresty neck” and fat accumulation around their tails.

* References 1, 2, 18, 21, 25, 27.

18.2.2 Pathogenesis

Despite several excellent studies trying to elucidate the pathogenesis of PID in horses,[8,9,12,28] several questions still remain unanswered; for example, why a number of horses with PID have low cortisol concentrations in spite of high ACTH concentrations. Cortisol may be responsible for some of the clinical signs associated with PID; however, many studies have found that cortisol concentrations in horses with PID may be within or below the normal range, suggesting that unidentified factors may be as or more important than hypercortisolemia in the pathogenesis of this condition. Furthermore, these findings are consistent with the low frequency (20%) of adrenocortical hyperplasia in horses diagnosed with Cushing's disease.[1,13] Another explanation for the low cortisol concentration in some affected horses may be that glucocorticoid synthesis and secretion from the adrenal gland is downregulated from excessive and prolonged exposure to ACTH.

An important aspect to consider with this syndrome is the considerable number of differences in clinical signs and laboratory testing among horses with Cushing's disease; probably, veterinarians are dealing with two different pathologic entities, and their diagnostic and therapeutic approaches may be different.

As previously mentioned, based on histologic and pathologic findings, *pars intermedia dysfunction* and *hyperplasia* are considered to be more appropriate terms for equine Cushing's disease,[15,21] in which the lack of inhibitory control on pars intermedia cell function appears to be the trigging mechanism for excessive cell proliferation and the development of adenomas. Human beings and dogs with Cushing's disease have increased plasma cortisol concentrations, and plasma ACTH concentrations are within or above the normal range, whereas in horses diagnosed with Cushing's disease, cortisol concentrations vary.

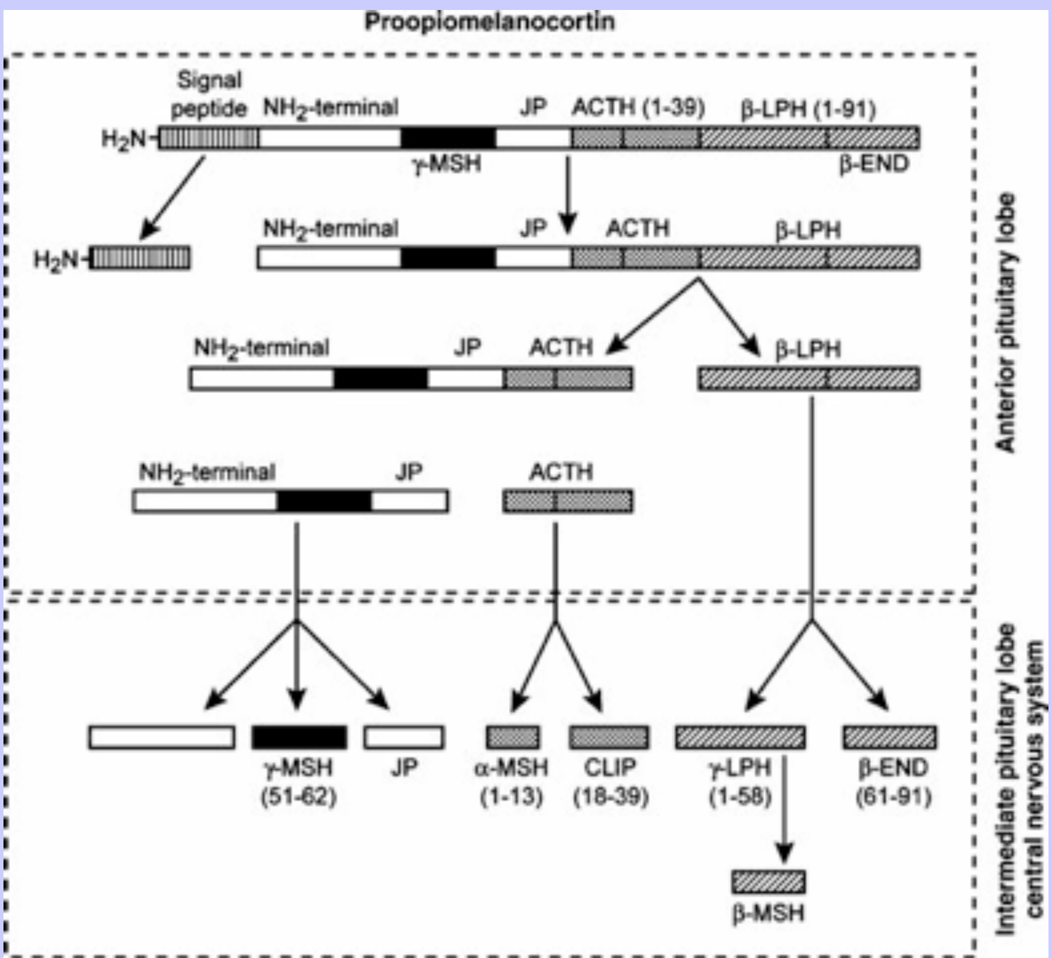
Pars intermedia dysfunction has been suggested to result from hypothalamic dysfunction associated with pituitary adenoma invasion because horses have an incomplete diaphragm of the sella turcica, and pars intermedia adenoma may extend out of the sella turcica and compress the hypothalamo-neurohypophyseal tract.[1,6,29] Compression of the pars nervosa by a pars intermedia adenoma also has been speculated to result in decreased secretion of antidiuretic hormone, resulting in polyuria and polydipsia. However, many of the clinical signs of the PID apparently result from excessive production and secretion of proopiomelanocortin-derived peptides such as ACTH, melanocyte-stimulating hormone (MSH), corticotropin-like intermediate lobe peptide (CLIP) and β-endorphin,[8,12] as well as from increased serum concentrations of cortisol. The importance of the hypothalamus (hypothalamic dysfunction) in the pathogenesis of PID remains unclear and deserves additional investigation.

Hypothalamic factors such as serotonin, dopamine, and γ-aminobutyric acid are important in regulating melanotrope function in the pars intermedia of different species, including the horse.[12,30] Low concentrations of dopamine were found in the pars intermedia of horses with PID.[12] Proopiomelanocortin synthesis is regulated differently between the anterior and intermediate lobes; in the intermediate lobe, for example, serotonine and

dopamine regulate proopiomelanocortin synthesis,[5,8,31] whereas in the anterior lobe, glucocorticoids, corticotropin-releasing hormone, and arginine vasopressin regulate proopiomelanocortin synthesis.[8,32–35]

In the anterior lobe (corticotropes) and in the intermediate lobe (melanotropes), the cleavage of proopiomelanocortin results in the same products[9]; however, in the intermediate lobe the posttranslational processing of proopiomelanocortin is different than in the anterior lobe.[12,28] In the anterior lobe the major 1329
products of proopiomelanocortin are ACTH, β-lipotropin, γ-lipotropin, and β-endorphin (Figure 18.2-2). In the 1330
intermediate lobe, ACTH is cleaved to produce α-MSH and CLIP, and the major products are α-MSH, β-MSH, β-endorphin, γ-MSH, and CLIP, and more β-lipotropin is processed to γ-lipotropin.[28,36] Therefore in the pars intermedia, ACTH is a minor (2%) posttranslational product of proopiomelanocortin.[9] Little difference also is apparent in the processing pathway of proopiomelanocortin between normal pars intermedia and pars intermedia adenomas.[9,12] Therefore proopiomelanocortin peptides (β-endorphin, α-MSH, β-MSH, CLIP) also are secreted, and their plasma concentrations are disproportionately higher than ACTH concentrations.[8,9,12] Orth and Nicholson[28] found that the pars intermedia of horses with PID had concentrations of bioactive ACTH similar to pars intermedia of healthy horses; however, the total adenoma content of bioactive ACTH was higher than the total pituitary gland ACTH content in healthy horses. Horses with PID had lower concentrations of β-endorphin in the pars distalis than control horses[12]; however, immunoreactive β-endorphin concentrations in the pars intermedia of affected horses were not different than those of control horses. Millington, Dybdal, Dawson, et al. found that the posttranslational processing of β-endorphin in normal tissues was different to that in equine pituitary adenomas, and this difference was associated with decreased concentrations of dopamine in the pars intermedia of horses with PID.[12] Alexander and Irvine[37] concluded that endogenous opioids inhibit the HPA axis and decrease ACTH secretion from the pars distalis of the horse. Therefore a decrease in anterior lobe β-endorphin concentrations, as occurs in horses with PID, probably may lead to additional ACTH secretion from the anterior lobe.

Figure 18.2-2 Processing of proopiomelanocortin in the anterior pituitary lobe, the intermediate pituitary lobe, and the central nervous system. In the anterior lobe, adrenocorticotropic hormone *(ACTH)* and β-lipotropin *(β-LPH)* are the major products of proopiomelanocortin. In the intermediate lobe and in the central nervous system, adrenocorticotropic hormone is cleaved to produce α-melanocyte-stimulating hormone *(α-MSH)* and corticotropin-like intermediate lobe peptide *(CLIP)*, and the major products are α-MSH, β-MSH, CLIP, γ-lipotropin, β-endorphin *(β-END)*, and γ-MSH. *JP*, joining peptide. (Adapted from Castro MG, Morrison E: Post-translational processing of proopiomelanocortin in the pituitary and in the brain, *Crit Rev Neurobiol* 11:35-57, 1997.)



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In horses with PID, plasma concentrations of proopiomelanocortin peptides and cortisol do not seem to have a diurnal rhythm.[8,13,15,38] Dopamine has been identified as an important hypothalamic regulatory factor of pituitary gland function.[8,12,39] The arcuate nucleus tuberoinfundibular dopaminergic system of the hypothalamus is important in modulating growth hormone, luteinizing hormone, and thyroid-stimulating hormone secretion from the adenohypophysis. Dopamine regulates the synthesis and posttranslational processing of proopiomelanocortin-derived peptides by the melanotropes in the intermediate lobe.[40] Through the tuberoinfundibular dopaminergic system, dopaminergic neurons from the arcuate nucleus release dopamine in the median eminence, and these neurons also make direct synaptic contact with melanotropes in the intermediate lobe.[41] The effects of dopamine on the anterior and intermediate lobes are mediated predominantly by dopamine D_2-type receptors.[42] Orth, Holscher, Wilson, et al.[8] found that D_2-receptor dopaminergic agonists (bromocriptine, pergolide) decreased proopiomelanocortin-derived peptide concentrations in horses with PID, suggesting that the pars intermedia function in horses with PID is under a control similar to that of the pars intermedia function of other species. Dexamethasone administration to horses with PID resulted in an additional decrease in ACTH plasma concentrations, but in many cases not less than normal concentrations, indicating that dexamethasone is suppressing residual pars distalis secretion. In contrast to their effect on proopiomelanocortin-derived peptides from the pars distalis, glucocorticoids have minimal inhibitory, and arginine vasopressin has minimal stimulatory effect on proopiomelanocortin-peptide secretion by the equine pars intermedia. Dopamine agonists inhibited proopiomelanocortin-secretion from the pars intermedia but had no effect on pars distalis secretion of proopiomelanocortin-derived peptides. This understanding on the pathophysiology of pituitary adenomas has made it possible to recommend dopamine agonist in the palliative treatment of PID.

Pars intermedia cell function in other species is also under positive serotoninergic influence; however, the role of serotonin on equine pars intermedia function is unclear. Millington, Dybdal, Dawson, et al.[12] found no difference in serotonin concentrations in the pars intermedia of affected versus control horses. γ-Aminobutyric acid has a tonic inhibitory effect on proopiomelanocortin synthesis in the pars intermedia of other species[30]; however, the role of γ-aminobutyric acid in the pathogenesis of equine PID is unknown.

Horses with PID have higher plasma concentrations of proopiomelanocortin-derived peptides, including immunoreactive ACTH (which includes bioactive ACTH), than do healthy horses,[*] and are not responsive to the negative feedback effect of glucocorticoids on ACTH secretion.[4,5,8,9,12]

The pathogenesis of polyuria and polydipsia is considered to be multifactorial. Some believe that compression of the pars nervosa by a pituitary adenoma results in decreased secretion of antidiuretic hormone.[1] Furthermore, hyperglycemia in these horses may result in osmotic diuresis. Glucocorticoids increase glomerular filtration rate and also may contribute to diuresis. Hypothalamic and neurohypophysis dysfunction in horses with PID deserve additional studies. Determination of plasma, pituitary, and hypothalamic concentration of neuropeptides such as arginine vasopressin and oxytocin and their hypothalamic messenger RNA expression in healthy and affected horses will provide a better understanding on the pathogenesis of this syndrome in the horse, in particular regarding polyuria and polydipsia.

Hyperphagia is believed to result from hypothalamic dysfunction and insulin resistance, and hyperinsulinemia may result from increased circulating concentrations of proopiomelanocortin peptides (in particular CLIP) and cortisol.[13] Cortisol stimulates proteolysis, glycogen formation, and gluconeogenesis. Glucocorticoids also increase glycemia by decreasing the skeletal muscle and adipocyte response to insulin-stimulated glucose

uptake. The insulin-antagonistic effect of glucocorticoids on the energy metabolism results in hyperglycemia and hyperinsulinemia (insulin resistance).[43]

Cortisol concentrations vary in affected horses,[13,15,27,44] and adrenocortical hyperplasia is not a consistent finding[1,13]; therefore to explain hyperglycemia, neutrophilia, lymphopenia, and other clinical signs such laminitis and predisposition to infections caused by glucocorticoid excess is difficult.

The pathogenesis of hirsutism is unknown. Holscher, Linnabary, and Netsky et al.[7] suggested that hirsutism in horses with PID may result from increased adrenal gland secretion of androgens, as occurs in women with Cushing's disease. Hirsutism and hyperhydrosis also have been suggested to result from dysregulation of the thermoregulatory center of the hypothalamus following pituitary compression.[6]

The underlying mechanisms responsible for the development of laminitis remain unclear. One proposal is that horses with PID develop laminitis from increased blood concentrations of glucocorticoids. However, the role of glucocorticoids in the pathogenesis of laminitis remains unclear; many horses with PID have cortisol concentrations within or below the normal range[13,15] to explain steroid-induced laminitis, suggesting that other 1331
unidentified factors may be as or more important than cortisol in the pathogenesis of laminitis in these horses. 1332
The pathogenesis of laminitis is described in other sections.

The increased docility in some horses with PID may result from increased plasma and cerebral spinal fluid concentrations of β-endorphin[12] and also may explain the decreased responsiveness to painful stimuli. The reason that some of these horses develop narcolepsy is unknown. One can speculate that the lack of dopaminergic control may be altering the sleep-wake cycle as occurs in human beings. Narcolepsy also may result from decreased orexin activity in the hypothalamus. Orexins (hypocretins) are recently discovered peptide neurotransmitters expressed in the lateral hypothalamus (orexinergic neurons) that are important in the sleep-wake cycle, and the absence of the effects of orexin results in narcolepsy.[45]

* References 8, 9, 12, 18, 20, 28, 31.

18.2.3 Pathologic Findings

Adenomas of the pars intermedia are white to yellow nodular or multinodular masses that can incorporate or compress the pars distalis and pars nervosa.[1,13] In some horses, pituitary microadenomas may be evident. Microscopically, these adenomas have a uniform cellular pattern with large columnar, spindle-shaped, or polyhedral cells that form palisades and pseudoacini. The tumor cells are slightly acidophilic with hematoxylin and eosin staining and are chromophobic with trichrome stain.[1] Pars intermedia adenomas may compress the overlying hypothalamus, resulting in necrosis of nuclei that are important in regulating body temperature, hair cyclic shedding, and endocrine and metabolic functions.

Approximately 50% of horses with PID have corticotrope hyperplasia in the pars distalis[1]; however, the explanation for this hyperplasia is unclear. One explanation may be that corticotrope hyperplasia in horses with PID results from less negative feedback from decreased plasma cortisol concentrations. Another possibility is that corticotrope hyperplasia results from decreased concentrations of endogenous opioids in the anterior lobe.[12,37]

Most cells of the pars intermedia of healthy horses are positive to immunostaining to ACTH and proopiomelanocortin, whereas approximately 10% of cells in the pars distalis are immunopositive to

proopiomelanocortin and ACTH.[1,46,47] Immunocytochemical evaluation of pars intermedia adenomas revealed that the cells have a uniform immunostaining for proopiomelanocortin; a moderate to strong staining for α-MSH, β-endorphin, β-lipotropin, β-MSH[1,13]; and a weak and patchy staining for ACTH.[1] These results agree with other studies that found increased concentrations of immunoreactive proopiomelanocortin, α-MSH, β-MSH, CLIP, and β-endorphin relative to ACTH.[8,9,12]

Adrenal cortex hyperplasia is not a consistent finding; one fifth of horses in one study[13] and 4 of 19 horses in another study[1] had adrenal cortex hyperplasia. The lack of cortical hyperplasia in most horses with PID may indicate that downregulation of ACTH receptors occurs in the adrenocortical cells or that the clinical signs in these horses do not result from hyperadrenocorticism. The variability among affected horses to the ACTH stimulation test seems to support this.

Skin biopsies reveal normal hair follicles in anagen phase, normal epidermis and dermal collagen, and a lack of the characteristic lesions present in dogs with Cushing's disease from cortisol excess (C.C. Capen, personal communication, 2002).

Laminitis is a consistent finding in horses with PID; however, the pathologic features in the lamina of these horses are nonspecific.

18.2.4 Laboratory Testing

The diagnosis of PID in many cases can be suggested by history, clinical signs, and preliminary laboratory results. Initial testing should include a complete blood count, a serum chemistry profile, and urinalysis. Horses with PID may have a normal complete blood cell count and serum chemistry profile. Clinicopathologic abnormalities reported in horses with PID include anemia, neutrophilia, lymphopenia, eosinopenia, hyperglycemia, hyperlipemia, increased liver enzymes, and glucosuria.[*] Persistent hyperglycemia is frequent in affected horses; more than 45% (94% in one study) of horses with PID had hyperglycemia.[2,5,13,20,24] Many horses with PID and hyperglycemia have insulin resistance (hyperglycemia and hyperinsulinemia).[10] Although insulin-resistant hyperglycemia can be present, another possibility is that hyperglycemia in some horses may result from stressing conditions. Glucosuria is a frequent finding (up to 77% in one study) in horses with PID and hyperglycemia.[2] Hyperlipemia may be present in horses but is more frequent in ponies.[19,49]

Additional testing to help confirm the diagnosis might include determination of resting plasma cortisol and ACTH concentrations. Significant variability exists in plasma cortisol concentrations among horses with PID,[13] and cortisol concentrations are affected by stress, exercise, and serum glucose concentrations. Therefore measurement of resting plasma cortisol concentrations is not diagnostic for PID. Resting ACTH concentrations are considered to be more reliable in the diagnosis of PID than resting cortisol concentrations.

* References 2, 5, 11, 13, 22, 24, 48.

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18.2.5 Diagnosis

Diagnostic testing for horses suspected of having PID is based on history, clinical signs, and laboratory findings. Many diagnostic tests for the diagnosis of PID have been evaluated; however, based on the limited number of horses studied and the variable results from some of these studies, to recommend them without additional validation is difficult. One limitation in the diagnosis of PID is establishing a gold standard, and

because opinions differ among clinicians, perhaps the only well-accepted gold standard is postmortem examination.

18.2.5.1 BASELINE CORTISOL CONCENTRATIONS

Measurement of baseline plasma concentrations of cortisol has been proposed in the diagnosis of PID. Healthy horses have diurnal variations in plasma cortisol concentrations, with significantly higher concentrations in the morning than in the evening.[50,51] No diurnal variations in cortisol concentrations are reported in horses with PID.[15] In horses with PID, resting plasma concentrations of cortisol may be within the normal range,[13] mildly increased,[15] or mildly decreased,[27,44] and therefore measuring baseline plasma cortisol concentrations to diagnose PID is not recommended.

In addition to plasma, cortisol concentrations have been determined in saliva and urine of healthy horses and horses with PID.[16,49,51] Van der Kolk, Nachreiner, Schott, et al.[51] measured salivary cortisol concentrations in healthy horses and found a trend for higher salivary cortisol concentrations in the morning than in the evening; however, the differences were not statistically significant. They also found that plasma and salivary cortisol concentrations were correlated and that administration of dexamethasone decreased and administration of ACTH increased plasma and salivary cortisol concentrations.

18.2.5.2 URINARY CORTICOID/CREATININE RATIO

The urinary corticoid/creatinine ratio has been determined in ponies and horses suffering of PID.[16,49] Van der Kolk, Kalsbeek, Wensing, et al.[16] found that horses with PID had higher urinary concentrations of corticoids and a higher corticoid/creatinine ratio than healthy horses; however, in their study the corticoid/creatinine ratio overlapped between healthy and diseased horses and a number of results were false positive and false negative, limiting the value of this test alone for the diagnosis of PID. In addition, horses were included in that study based on the ACTH stimulation test, which itself has significant variability among horses with PID and between healthy ponies and horses.[20] No ponies were included in the control group, and most of the affected animals were ponies. Additional studies comparing the corticoid/creatinine ratio to well-accepted and validated diagnostic procedures are necessary to recommend this test over other tests.

18.2.5.3 DEXAMETHASONE SUPPRESSION TEST

The dexamethasone suppression test (DST) has been a well-accepted and recommended test for the diagnosis of PID in horses.[15,38,52] This test is based on the lack of suppression of plasma cortisol concentrations in horses with PID at 19 to 24 hours after dexamethasone administration.[15] The suggestion is that in horses with PID, plasma cortisol concentrations are not suppressed by dexamethasone administration[15]; however, in a recent study,[44] cortisol concentrations were suppressed for up to 3 hours after dexamethasone administration in horses with PID. The lack of suppression in some horses with PID therefore possibly results from infrequent sampling protocols rather than from lack of suppression. Furthermore, the suppression of plasma cortisol concentration after dexamethasone administration in horses with PID does not appear to last as long as in clinically healthy horses[15,44] and is a good reason for a long sampling interval as described in the DST.[15] Two modifications of this protocol are available.

18.2.5.3.1 The 24-Hour Dexamethasone Suppression Test: Standard Protocol

One should begin the test at midnight and should collect a baseline blood sample in a heparinized tube and administer 40 μg/kg of dexamethasone intramuscularly. One should draw blood samples at 8 am, noon, 4 pm, 8 pm, and midnight and should expect plasma cortisol concentrations in healthy horses to be less than 1 μg/dl 24 hours after dexamethasone administration or to find a significant decrease in plasma cortisol concentrations compared with baseline concentrations.[15]

18.2.5.3.2 Overnight Dexamethasone Suppression Test

The overnight dexamethasone suppression test is a simplified modification of the 24-hour DST[15] and is the test the author routinely uses at the Ohio State University. One draws a baseline blood sample in heparinized tubes and administers dexamethasone (40 μg/kg intramuscularly) at 5 pm (4 to 6 pm). One takes blood samples at 15 and 19 hours (8 am and noon the next day) after dexamethasone administration. One should expect plasma cortisol concentrations to be less than 1 μg/dl in horses with an intact HPA axis. This test provides simplicity, is less expensive, is well accepted by clinicians, and is useful for distinguishing horses with PID from horses with an apparently functional HPA axis.

The 24-hour DST is useful for assessing the severity of pituitary dysfunction or for evaluating the response to treatment. Although many clinicians are concerned with steroid-induced laminitis resulting from the 1333
DST, no adverse effects of this test were reported when a large number of horses with PID were evaluated. 1334
[15]

18.2.5.4 RESTING ADRENOCORTICOTROPIC HORMONE CONCENTRATIONS

Increased plasma concentrations of ACTH and proopiomelanocortin-derived peptides have been reported in horses with PID.[*] Couetil, Paradis, and Knoll found that horses and ponies with PID had significantly higher resting plasma concentrations of ACTH compared with healthy horses and ponies. Plasma ACTH concentrations higher than 50 pg/ml in horses and 27 pg/ml in ponies strongly supported the diagnosis of PID. In this study, plasma ACTH concentrations for the diagnosis of PID had a sensitivity of 90.9% in horses and 81.8% in ponies and a specificity of 100%.[20] Based on the result of this and other studies,[18,31] plasma ACTH concentrations are considered a better diagnostic test for equine PID than the ACTH stimulation test. Some clinicians consider increased ACTH concentrations (>200 pg/ml) as the gold standard for the diagnosis of PID. However, others have claimed that resting ACTH concentrations have little value in the diagnosis of PID.[23] Measurement of plasma resting ACTH concentrations in a reliable laboratory together with the DST is reasonable combination to confirm the diagnosis of PID. Determination of resting ACTH concentrations is a good alternative when the results of the DST are nondiagnostic or when the DST is contraindicated. Compared with the DST and the thyrotropin-releasing hormone (TRH) stimulation test, resting ACTH concentrations have been reported to produce many false-positive and false-negative results.[23] One must consider when determining baseline ACTH concentrations that stressing conditions in addition to increase cortisol concentrations also increase ACTH plasma concentrations. One should collect blood samples to determine ACTH concentrations in cold EDTA tubes, kept at 4° C, centrifuge them immediately, and store them at −70° C until analysis.

* References 8, 9, 12, 18, 20, 31.

18.2.5.5 ADRENOCORTICOTROPIC HORMONE STIMULATION TEST

The administration of ACTH (1 IU/kg of natural ACTH gel intramuscularly; 25 IU ACTH$_{1-24}$ intravenously; or 100 IU of synthetic ACTH [cosyntropin] intravenously) has been used in different studies with different results.[†] A horse with PID is expected to have an exaggerated (at least fourfold) cortisol response to ACTH stimulation, and when an increased response is present in a horse with elevated resting ACTH concentrations, one can make a more accurate diagnosis of PID.[31] Dybdal, Hargreaves, Madigan, et al.[15] failed to detect a statistical difference in cortisol concentrations between control horses and horses with PID after administration of 1 IU/kg of natural ACTH gel intramuscularly between 8 and 10 am, with samples being collected in heparinized tubes before and 2, 4, 8, and 12 hours after ACTH administration. Van der Kolk, Wensing, Kalsbeek, et al.[18] found a significantly higher increase in cortisol concentrations in horses with PID after administration of 25 IU of synthetic ACTH$_{1-24}$ intravenously at 9 am, with samples being collected in EDTA tubes just before and 2 hours after ACTH administration. The ACTH stimulation test is more useful in assessing adrenal gland function or exhaustion and does not seem to be the test of choice to assess HPA axis function in horses suspected of having PID.

† References 5, 15, 16, 18, 31, 53.

18.2.5.6 COMBINED DEXAMETHASONE SUPPRESSION TEST AND ADRENOCORTICOTROPIC HORMONE STIMULATION TEST

One takes a blood baseline sample in a heparinized tube (9 am) and administers dexamethasone (10 mg intramuscularly)[54]; takes a blood sample 3 hours after dexamethasone injection and administers 100 IU of synthetic ACTH intravenously; and takes a blood sample 2 hours after ACTH administration (2 pm). This test failed to distinguish horses with PID from control horses.[15]

18.2.5.7 THYROTROPIN-RELEASING HORMONE STIMULATION TEST

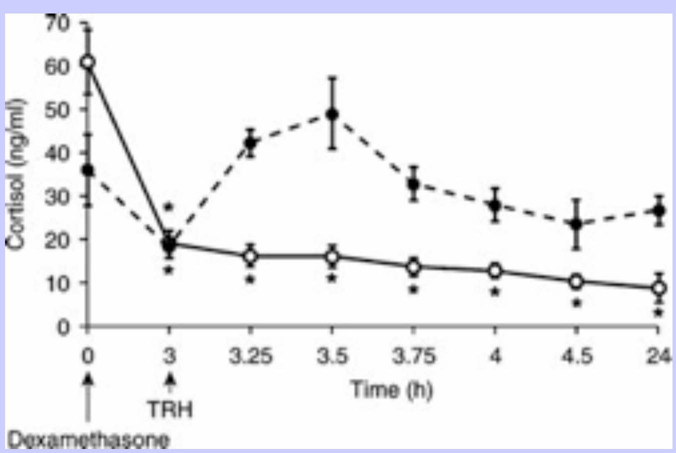
Beech and Garcia[27] evaluated the effect of TRH administration on plasma cortisol concentrations in healthy horses and in horses with PID. They found that TRH administration (1 mg intravenously) to horses with PID, in addition to increasing triiodothyronine and thyroxine concentrations, also resulted in a significant increase in plasma concentrations of cortisol within 15 minutes after TRH administration, and cortisol concentrations remained elevated for 90 minutes. No significant increases in plasma cortisol concentrations were present in healthy horses. Eiler, Oliver, Andrews, et al.[44] evaluated the TRH stimulation test in two horses suspected of having PID and three healthy horses and found that maximal cortisol concentrations overall were not statistically different between groups; however, too few horses were included to make any conclusion. Another study found increases in plasma cortisol concentrations after TRH administration to healthy horses. [17] The reason for the increase in cortisol concentrations after TRH administration is unclear, although speculation is that it results from increased blood concentrations of ACTH or CLIP. The TRH stimulation 1334
test has been advocated by some clinicians as an alternative method to the DST in horses with laminitis or at 1335
greater risk for developing steroid-induced laminitis.

18.2.5.8

COMBINED DEXAMETHASONE SUPPRESSION TEST AND THYROTROPIN-RELEASING HORMONE STIMULATION TEST

The combination of a DST with the TRH stimulation test was evaluated as a diagnostic method for PID. After baseline sampling in EDTA-containing tubes, dexamethasone (40 µg/kg intravenously) was administered at 8:30 am. A second sample was collected 3 hours later, and then TRH (1.1 mg intravenously) was administered. Serial blood samples (15, 30, 45, 60, 90 minutes, and 21 hours after TRH administration [24 hours after dexamethasone]) were collected. In this study, 3 hours after dexamethasone administration, plasma concentrations of cortisol decreased in healthy horses and in horses with PID (Figure 18.2-3). TRH administration resulted in a significant increase in cortisol concentrations in horses with PID but not in healthy horses, and 21 hours after TRH administration, cortisol concentrations were still less than baseline in healthy horses but were higher in horses with PID.[44] Based on the results of this study, the combined DST/TRH stimulation test seems to be a promising method for the diagnosis of PID.

Figure 18.2-3 Combined dexamethasone suppression/thyrotropin-releasing hormone *(TRH)* stimulation test in healthy horses (*n* = 7; ○) and in horses with pars intermedia dysfunction (*n* = 5; •). Plasma cortisol concentrations decreased 3 hours after dexamethasone administration in both groups; however, thyrotropin-releasing hormone administration resulted in a significant increase in cortisol concentrations in horses with pars intermedia dysfunction but not in healthy horses, and 21 hours after thyrotropin-releasing hormone administration, cortisol concentrations were still less than baseline in healthy horses but were higher in horses with pars intermedia dysfunction. *Significant difference from baseline value at time 0.



18.2.5.9

RESTING INSULIN CONCENTRATIONS

In horses with hyperglycemia, measurement of blood insulin concentrations together with glucose concentrations may be useful. Increased insulin concentrations in the presence of persistent hyperglycemia are highly suggestive of PID. However, if insulin concentrations are within the normal range, one should not rule out PID. Resting glucose and insulin concentrations are reported to have a sensitivity of 58% and 92% for the diagnosis of PID, respectively.[18] Baseline serum insulin concentrations were increased in ponies that had laminitis but had a normal combined DST, suggesting that insulin concentrations may not be a reliable indicator of PID.[55] When hyperglycemia and low insulin concentrations are persistent, one should consider pancreatic disease as a differential diagnosis.

18.2.5.10

GLUCOSE TOLERANCE TEST

The glucose tolerance test may be helpful in confirming the diagnosis of PID and is based on the finding that a number of horses with PID have hyperglycemia and hyperinsulinemia. When horses with PID are given dextrose (0.5 g/kg, 50% solution, intravenously) insulin concentrations do not peak as rapid as in healthy horses, and glucose concentration return to baseline values (3 hours versus 1 hour in controls) is delayed despite hyperinsulinemia, suggesting insulin resistance.[10] After 12 to 18 hours of fasting, one also can administer glucose via nasogastric tube (1 g/kg, 20% solution). In intravenous and oral glucose administration, one should take blood samples every 30 minutes for 4 hours. This test is more useful for evaluating horses known to have PID and hyperglycemia (prognosis), and in horses with hyperglycemia and possible pancreatic disease.

18.2.5.11

INSULIN TOLERANCE TEST

The rationale of this test is that many horses with PID are insulin resistant, and the administration of exogenous insulin will not result in a significant decrease in glucose serum concentrations as occurs in healthy horses.[8,22] One administers 0.05 U/kg of crystalline insulin intravenously and takes blood samples every 15 minutes for 3 hours. In healthy horses one can expect a 30% to 50% decrease in glucose concentrations at 15 minutes, 60% at 30 minutes, and normal glucose concentrations at 2 hours. Alternatively, one can administer 0.4 to 0.8 IU/kg of regular insulin intravenously and can expect a more than 50% decrease in glucose concentrations at 30 minutes. This test proves insulin resistance but is not diagnostic, and therefore one must take its validity together with other clinical and laboratory findings. When performing this test, one must have dextrose ready for parenteral use because insulin administration may result in hypoglycemic shock.[56,57] 1335

1336

18.2.5.12

DETERMINATION OF PROOPIOMELANOCORTIN PEPTIDES

Based on current knowledge of equine pars intermedia dysfunction, determination of plasma concentrations of proopiomelanocortin-derived peptides (ACTH, α-MSH, β-endorphin, β-lipotropin, CLIP) in horses suspected of having PID would be reasonable. These peptides were found to be increased in horses with PID.[*] Determination of proopiomelanocortin-derived peptides in the clinical scenario has been limited to ACTH plasma concentrations, and the use of human immunoassays to determine equine ACTH concentrations has been validated.[20]

Severalfold increases in plasma and cerebral spinal fluid β-endorphin concentrations were found in horses with PID.[12] Furthermore, proopiomelanocortin peptides were disproportionally higher compared with ACTH plasma concentrations in affected horses.[8,9]

* References 4, 8, 9, 12, 18, 20.

18.2.5.13 RADIOGRAPHIC METHODS

Allen, Barbee, and Crisman[58] used computed tomography to diagnose PID in horses. Levy, Blevins, and Janovitz[59] used ventral radiography and contrast venography successfully to diagnose PID in horses and ponies. These methods are far from being practical in the diagnosis of PID; they require anesthesia and expensive equipment and are not cost-effective compared with other diagnostic methods.

18.2.6 Treatment

Horses with pituitary tumors are difficult not only to diagnose but also to treat. The clinical signs of PID vary and, because this disease occurs primarily in older horses, one must treat the dysfunctional HPA axis and many of the pathologic conditions associated with aging. One should give special attention to good-quality feed, to deworming, to dental care, and to the hooves (because many affected horses have or may develop laminitis). One also must pay particular attention to the skin and hair coat, for many horses cannot shed their winter coat properly, may develop hyperthermia, and are prone to skin infections. The most difficult complication to manage is the laminitis, which may be complicated by overfeeding of high-energy hay or grain and by the high concentrations of cortisol and of blood glucose in affected horses. Therefore reducing the amount of soluble carbohydrates is advisable. In addition, in mildly affected horses exercise is recommended.

As discussed previously, the regulation of proopiomelanocortin peptide synthesis and secretion is under the influence of the hypothalamic tuberoinfundibular dopaminergic and serotoninergic systems. Proopiomelanocortin synthesis and secretion by the pars intermedia of horses with PID is under control similar to the pars intermedia from other species.[8] Based on this information, the medical treatment of PID involves the use of dopamine agonists or serotonin antagonists.

Bromocriptine and pergolide are the two most commonly used dopamine D_2 receptor agonists.[14,60–63] One can give bromocriptine orally or by subcutaneous injection (0.03 to 0.09 mg/kg b.i.d.),[8,60,64] but its use for treating PID is limited. Oral administration of pergolide is used and widely has been shown to be beneficial for treating horses and ponies with PID.[23,25,62] Doses of pergolide from 0.2 mg up to 5 mg/horse per day, once a day or divided twice daily, have been used in ponies and horses. Starting with a total daily dose of 0.5 mg/day is recommended, and if the horse shows no clinical evidence of improvement by 4 to 6 weeks, one should gradually increase the dose by 0.25 mg every 3 to 4 weeks. In horses that are hyperglycemic, one may assess the success of treatment by regularly determining glucose concentrations. In horses that are normoglycemic, one may need to repeat the DST. Most horses show improvement with doses of 0.75 to 1.5 mg of pergolide per day. Improvement is evident by 6 weeks of treatment, and includes a decrease in frequency of polyuria and polydipsia, shedding of the hair coat, a decrease in plasma ACTH concentrations, and a decrease in serum glucose concentrations. Measuring ACTH concentrations has been recommended in horses in which they are initially high.[25] Horses with PID treated with pergolide showed a better response to treatment than horses

treated with cyproheptadine.[23,25] Some horses treated with pergolide may develop anorexia, which one may resolve by reducing the dose.[23]

Cyproheptadine is a nonselective 5-hydroxytryptamine receptor blocking agent (serotonin antagonist) that reduces ACTH and β-endorphin secretion from ACTH-producing tumors in human beings.[65] Information on the serotonin functions in the hypothalamus and pituitary gland of the horse is limited, and no difference in serotonin concentration was found between the pars intermedia of horses with PID and control horses.[12] Cyproheptadine has been used for many years to treat horses with PID with variable results, and one third of horses with PID are estimated to show improvement when treated with cyproheptadine. This drug is recommended at 0.25 mg/kg orally once a day for 4 to 8 weeks. If the horse shows no clinical evidence of improvement and no reduction in plasma ACTH concentrations, one should increase the dosage frequency to twice a day for 1 month, at which time one can determine ACTH concentrations or repeat the DST. One notes 1336
response to treatment by a decrease in frequency of the polyuria and polydipsia 1 to 2 weeks after the initiation 1337
of treatment. In horses that fail to respond to high doses of cyproheptadine, switching to pergolide is recommended. Horses that respond to any of these drugs may require treatment for life. A combination of cyproheptadine and pergolide has been evaluated at the Ohio State University with promising results (T. Miesner, personal communication, 2002).

One key element in the decision to treat horses with PID is economic. Long-term treatment with pergolide is expensive, and in horses that respond well to pergolide therapy, one should consider decreasing the dose. Cyproheptadine therapy is inexpensive compared with pergolide and also should be considered when the budget is tight.

Alternative therapeutic compounds have been suggested or are being evaluated currently to treat horses with PID (*www.laminitis.org*). Aqueous extracts of *Vitex agnus-castus* (chasteberry, chaste tree) have been used to treat horses with PID. *V. agnus-castus* extracts contain compounds (diterpenoids) that stimulate dopamine D_2 receptor activity and inhibit different opioid receptors.[66] Because of its dopaminergic effects, *Vitex* has been recommended as an alternative phytotherapeutic agent for treating hyperprolactinemia and premenstrual syndrome in women.[67] A physiologic and pharmacologic rationale exists for the possible use of these extracts to increase dopaminergic activity in the equine pituitary; however, appropriate studies are necessary before *Vitex* can be recommended to treat horses with PID. Trilostane is an enzymatic inhibitor of 3-β-hydroxysteroid dehydrogenase, an important enzyme in steroid synthesis. Trilostane has been proposed for treating equine Cushing's disease and for treating the recently described "equine peripheral cushingoid syndrome."[68] However, little information is available on the effects of trilostane in the equine steroid metabolism.[69] Until the mid-1980s trilostane was recommended to treat Cushing's disease in human beings; however, because of its lack of potency in blocking cortisol synthesis, trilostane is no longer recommended to treat this condition.[70] Instead, more potent compounds (i.e., ketoconazole) currently are being used in small animals and human beings.

18.2.7 Pituitary-Independent Cushing's Syndrome

Primary adrenocortical tumors are rare in horses,[5,64,71,72] and the few cases reported in the literature were nonfunctional tumors. Only one case of a functional adrenocortical tumor associated with clinical signs consistent with Cushing's disease has been reported.[64] A 12-year-old Dutch Warmblood gelding had a history of polyphagia, polydipsia, reduced muscle mass, hyperhydrosis, lethargy, and delayed shedding of the hair coat. The horse had persistent hyperglycemic and normal adrenocortical response to ACTH stimulation. After

unsuccessful treatment with bromocriptine the horse was euthanized, and a unilateral adrenocortical adenoma was found.

18.2.8 Ectopic Cushing's Syndrome

In human beings and small animals, Cushing's disease results from excessive secretion of ACTH from the pituitary gland, ectopic secretion of ACTH by nonpituitary tumors, ectopic secretion of corticotropin-releasing hormone, or excessive and autonomous secretion of cortisol from adrenocortical tumors. The secretion of ACTH or corticotropin-releasing hormone from nonpituitary tumors results in a condition known as ectopic Cushing's syndrome or ectopic ACTH syndrome. Ectopic production of ACTH or corticotropin-releasing hormone has not been demonstrated in the horse.

18.2.9 Peripheral Cushingoid Syndrome

A clinical syndrome of laminitis, hypothyroidism, and obesity without hirsutism has been described recently in horses.[68] These horses have hyperinsulinemia and normal DST and TRH stimulation test results. Treatment with pergolide does not improve clinical signs. The term *peripheral cushingoid syndrome* has been proposed to separate these animals from horses with pars intermedia dysfunction. An important item to mention, however, is that in human medicine the term *cushingoid* is considered a phenotype more than a syndrome and is linked to the ectopic production of ACTH. No information is available on the pathogenesis of this condition in the horse. As in human beings, this syndrome seems to be a pseudo-Cushing's condition (typically obesity and hypothyroidism) that may be caused by different processes and should not be confused with pituitary-dependent and pituiatry-independent hyperadrenocorticism. The clinical appearance of horses with peripheral cushingoid syndrome probably results from abnormal energy metabolism, as occurs in human beings with central obesity, in which decreased concentrations of hormones important in cortisol metabolism (e.g., growth hormone and insulin-like growth factor I) result in increased cortisol production, omental adipocyte differentiation, and insulin resistance. Similar features are reported in the metabolic syndrome X or the insulin-resistance syndrome in human beings.[73] 11β-Hydrosteroid dehydrogenases (11β-HSDs) are important enzymes in regulating the interaction of glucocorticoids with their cellular receptors; they control the activation and inactivation of 1337
cortisol and cortisone (the cortisol-cortisone shuttle). 11β-HSD1 converts inactive cortisone to cortisol in the 1338
liver and adipose tissue, whereas 11β-HSD2 converts the active cortisol to inactive cortisone in the kidney.[68,74] Overexpression of 11β-HSD1 in adipose tissue may result in adipocyte differentiation and obesity. Growth hormone (and insulin-like growth factor 1) inhibits 11β-HSD1 and therefore decreases cortisol production; many human patients with hypothyroidism and insulin resistance have decreased concentrations of growth hormone and increased 11β-HSD1 activity, which promotes cortisol production, insulin insensitivity, and visceral adiposity.[73,74] This condition is known as central obesity or omental Cushing's disease. Johnson and Ganjam[68] found that 11β-HSD1 was expressed in the equine skin and lamellar tissue and that integumentary 11β-HSD expression was increased in alimentary-induced laminitis. They proposed that increased expression of this enzyme in the equine digit may magnify the biologic effects that glucocorticoids may have in the equine lamellae during laminitis.

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18.3

18.3—Thyroid Gland

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The thyroid glands arise from two different embryologic origins: the thyroid bud and the ultimobranchial body. The thyroid bud originates from the endoderm of the primitive larynx and is the origin of the follicular cells, which are responsible for the synthesis of thyroglobulin and thyroid hormones (THs). The ultimobranchial body originates from the fourth pharyngeal pouch, contains cells from the neural crest, and gives rise to calcitonin-producing cells (parafollicular or C cells). In mammals the ultimobranchial body is a transient organ destined to join the thyroid bud. Immediately after thyroid ontogeny, thyroid function begins but remains basal until the hypothalamic structures and the pituitary portal system are organized to maintain a functional hypothalamus-pituitary-thyroid (HPT) axis.[1]

In the horse the thyroid glands are two discrete, firm lobes located in the dorsolateral aspect of the third to sixth tracheal rings.[2,3] The gland has two lobes connected by a narrow isthmus that contains fibrous tissue. In most healthy horses the thyroid glands are not visible but can be palpated as firm and movable structures. The weight of the glands as a ratio of grams of thyroid tissue per kilogram of body mass is largest for fetuses and foals (mean, 0.28 g/kg; range, 0.12 to 0.67 g/kg) and diminishes progressively with age. The mass ratio for adults is on average 0.08 g/kg (range, 0.01 to 0.15 g/kg).[4] The normal total gland mass for newborn foals is around 15 g.[5] The glands are vascular, being supplied by two major arteries arising from the external carotid and subclavian arteries, supplying the cranial and caudal poles, respectively. Gland size does not parallel function, and one must use other tests to establish an accurate diagnosis.

18.3.1 Thyroid Function

Thyroid hormones (triiodothyronine [T_3] and thyroxine [T_4]) are produced by the thyroid glands and play critical functions in nearly all tissues, most of which are related to cell growth and differentiation and to cell metabolism. An understanding of the function of the thyroid gland and physiologic effects of THs is important in recognizing, diagnosing, and managing thyroid gland dysfunction. The relative importance of the thyroid glands is reflected in the large volume of blood flow: 4 to 6 ml/min/g of tissue, which is greater than the blood flow to the kidneys.

The synthesis and secretion of TH is regulated by a negative feedback system that includes the hypothalamus, the pituitary gland, and the thyroid gland (HPT axis). Thyroid hormone synthesis and secretion is stimulated by thyroid-stimulating hormone (TSH, thyrotropin), which is a glycoprotein (28 kd) composed of two subunits (α and β) and is secreted by the thyrotropes of the pituitary gland. The α-subunit is shared with other hormones (luteinizing hormone, follicle-stimulating hormone). Thyroid-stimulating hormone secretion from the anterior pituitary (adenohypophysis) is controlled by the effects of hypothalamic neurons that release a tripeptide, thyrotropin-releasing hormone (TRH).[6] Thyrotropin synthesis and secretion is also under the control of the negative feedback (long-term inhibition) effect of THs.[7] Thyroid-stimulating hormone concentrations depend on the rate of disappearance of THs and TSH from circulation and on the rate that T_4 is converted to T_3. The hypothalamus also can inhibit (short-term inhibition) TSH secretion through dopamine and somatostatin release in the median eminence.[8]

Thyrotropin-releasing hormone–positive neurons are present in different areas of the brain. Within the hypothalamus the parvocellular division of the paraventricular nucleus contains most of the TRH-positive neurons, and their projections end in the zona externa of the median eminence. Thyrotropin-releasing hormone–positive neurons are also present in the preoptic area, suprachiasmatic nucleus, ventromedial nucleus, and dorsomedial nucleus.[9] In the pituitary gland, T_3 decreases the thyrotrope response (TSH secretion) to TRH

stimulation, and in the hypothalamus, T_3 decreases TRH messenger RNA expression and TRH secretion.[10–12] In the pituitary, T_4 is converted to T_3 by a type II deiodinase. For T_4 to gain access to hypothalamic neurons, it must be transported through the choroid plexus to the lateral ventricles bound to transthyretin (T_4-binding protein). Once within the brain, T_4 is converted to T_3 by the type II deiodinase.[13]

Thyrotropin-releasing hormone is released in the median eminence of the hypothalamus and through the pituitary portal system reaches the anterior pituitary. In the pituitary, TRH interacts with specific receptors in the thyrotropes, inducing TSH release, and also induces prolactin release; however, its role in prolactin secretion remains unclear. Injections of TRH have minimal or no effect stimulating prolactin release in mares.[14–16]

Cold temperatures stimulate and high temperatures inhibit the HPT axis. Low temperatures stimulate peripheral cold receptors and through noradrenergic pathways stimulate neurons in the paraventricular nucleus to release TRH.[7] Stress inhibits TSH release through the hypothalamic release of somatostatin.[17] Inflammation 1340
suppresses TSH secretion. Cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor α inhibit TSH 1341
secretion and stimulate somatostatin secretion.[18–21]

18.3.1.1 IODINE AND HORMONE METABOLISM

One major function of the thyroid is actively to trap and conserve iodine against a large concentration gradient.[2,22,23] Iodine can be absorbed in its soluble form, iodide (I^-), via the intestinal mucosa or any moist body surface such as other mucosa or broken skin.[2] Iodide actively is transported and concentrated into the thyroid by a Na^+/I^- symporter.[24] The trapped iodide is oxidized by a thyroid peroxidase in the presence of hydrogen peroxide and incorporated into the tyrosine residues of thyroglobulin (660 kd) to form the inactive precursors monoiodotyrosine and diiodotyrosine. Coupling of these precursors results in synthesis of the active iodothyronines T_4 and T_3. The iodinated thyroglobulin containing monoiodotyrosine, diiodotyrosine, T_3, and T_4 is stored as an extracellular polypeptide in the colloid within the lumen of the thyroid follicles.[25] When needed, T_4 and T_3 are cleaved from the thyroglobulin, picked up by the follicular cells by a process of endocytosis, and transported into the circulation.[2,23] Most of the TH released into circulation is T_4, for total serum T_4 concentrations are 20 times higher than serum T_3 concentrations. Hormone synthesis and release are controlled by iodine availability and by TSH. The feedback regulation of TSH secretion is related closely to the circulating concentrations of unbound (free) thyroid hormones (fT_4 and fT_3).[2,23]

The circulating THs are T_4, T_3, and reverse T_3 (rT_3). Free T_4 and T_3 are the biologically active hormones, whereas T_3 and rT_3 are deiodination products of T_4. All of the circulating T_4 is derived directly from the thyroid gland. Only 10% to 20% of the circulating T_3 is secreted directly from the thyroid gland. The major pathway in the production of T_3 is the 5′-monodeiodination of T_4 by a type I deiodinase (which is a selenoprotein) in peripheral tissues, which is referred to as T_3 neogenesis. In the fetus, type I deiodinase activity is delayed so that T_3 neogenesis is low, and most deiodinase activity results in high concentrations of rT_3. A switch in enzymatic activity occurs at birth, partly because of the influence of glucocorticoids.[23] THs circulate in the blood bound to transport plasma proteins (thyroid-binding proteins).

Thyroxine-binding globulin is the major protein (70% of T_4 and T_3 is bound to thyroxine-binding globulin); transthyretin (thyroid-binding prealbumin) and albumin bind to a lesser degree. In horses the percentages of circulating T_4 bound to thyroxine-binding globulin, thyroid-binding prealbumin, and albumin were found to be 61%, 22%, and 17%, respectively.[26] T_3 is bound to thyroxine-binding globulin and albumin but not to thyroid-binding prealbumin. Transthyretin is important in transporting T_4 into the brain. These hormones are bound reversibly to these transport proteins, which act as TH reservoirs. The hormones in their unbound or free state readily can traverse capillary endothelium and are available to exert their biologic effects at the peripheral tissues.[27] The protein-binding properties thus in part determine the biologic activity of each hormone. Triiodothyronine is much more potent than T_4 and has a shorter half-life. Thyroxine has much greater protein-binding affinity; T_3 is therefore more readily available to interact with receptors at peripheral tissues. Thyroxine sometimes is thought of in terms of a prohormone. The half-life of T_4 in horses is approximately 50 hours.[28] Because a large fraction of THs is bound to plasma proteins, changes in protein binding may result in changes in total TH concentrations.

Metabolic degradation of the thyronine ring after deiodination includes deamination, decarboxylation, and conjugation. The enzymes responsible for the production of T_3 and rT_3 are also responsible for their destruction. The three classes of 5′-deiodoninases are as follows: Type I deiodinase is found predominantly in peripheral tissues such as thyroid, kidney, and liver; is responsible for the conversion of most of T_4 to T_3 and rT_3; and is inhibited noncompetitively by propylthiouracil. Type I deiodinase activity is increased by TSH and T_3 and is decreased in hypothyroidism. Type II deiodinase is found in brown fat tissue, the brain, and the pituitary gland; is not affected by propylthiouracil[23]; and its primary function is to convert T_4 to T_3 for intracellular use.[25] Type II deiodinase activity is increased in hypothyroidism, probably to sustain intracellular T_3 concentrations despite low peripheral T_4 concentrations, especially in the brain. Type III deiodinase is found in the placenta, developing brain, and skin, and its primary function is to inactivate T_4 and T_3 (converts T_4 to rT_3 and inactivates T_3). Type III deiodinase may be important regulating T_3 availability during development. The generation of rT_3 by type I and III deiodinases is a key step in the inactivation of THs. Metabolites are excreted via the urine, and some are conjugated and enter the enterohepatic circulation. Most iodide is returned to the thyroid gland.

The thyroid is also the site of production of calcitonin, secreted by the C cells, which are interspersed among the thyroid follicles. Calcitonin is involved in calcium homeostasis.[2,23,29]

18.3.1.2 MOLECULAR ACTIONS OF THYROID HORMONES

The TH receptors belong to the superfamily of nuclear receptors that work as transcription factors. The two
types of TH receptors are TR-α and TR-β. Triiodothyronine, whether directly transported into the cell or 1341
derived intercellularly from T_4, is considered to be the effector hormone in target cells. The thyroid receptors 1342
interact with specific DNA sequences (T_3-response elements), regulating gene expression. Growth and thermogenesis depend on the presence of THs. Triiodothyronine stimulates thermogenesis by increasing the expression of proteins associated with uncoupling oxidative phosphorylation. Thyroid hormones decrease the expression of the α- and β-subunit genes of TSH and the TRH gene. From its effects on gene expression, T_3 actions result in thermogenesis; increased oxygen consumption; increased protein synthesis; increased metabolic rate; increased carbohydrate absorption and glucose metabolism; stimulation of growth and

maturation and erythropoiesis; increased lipid metabolism and conversion of cholesterol into bile salts; activation of lipoprotein lipase; increased sensitivity of adipose tissue to lipolysis; stimulation of heart rate, cardiac output, and blood flow; increased neural transmission; and cerebral and neuronal development in young animals.[2,22]

18.3.2 Extrathyroid Factors That Affect Thyroid Function and Testing

Many endogenous and exogenous factors can affect thyroid function and sometimes test results. Awareness of these factors and interactions can help one interpret tests and establish an accurate diagnosis.

18.3.2.1 AGE

Age-related differences in TH concentrations occur in horses. Foals have high concentrations of T_3 and T_4 at birth that decrease with age.[30–36] A recent study[35] found that plasma T_3 concentrations decreased from 7.9 ng/ml at birth to 0.9 ng/ml at 6 months of age and to 0.7 ng/ml at 9 months of age. Plasma T_3 concentration in mares was 0.5 ng/ml. In the same study, T_4 concentrations decreased from 233 ng/ml at birth to 49 ng/ml at 14 days and to 35 ng/ml at 6 months.

18.3.2.2 GENDER

The correlation of gender and TH concentrations appears to vary. In one study, T_4 concentrations were not influenced significantly by sex: males had slightly higher T_3 values than females.[30] Serum TH concentrations may be lower for stallions and geldings than for females,[36,37] but others report no gender differences.[38–41] Lower TH concentrations have been reported in mares than in stallions.[42] The concentration of free T_4 was found to be higher in stallions than in other horses.[43] Mares tested at all stages of the estrous cycle were found to show no statistically significant differences according to stage of the cycle. Thyroxine concentrations, however, were decreased following ovulation, which is the converse of the hormone pattern in other species following ovulation. Individual variations were found to be greater than variations within the estrous cycle.[39] Higher than normal serum THs were reported during pregnancy in mares,[44] which is similar to findings in other species and may be due in part to increased thyroxine-binding globulin concentrations during pregnancy.[23] Furthermore, TH concentrations were found to vary with stage of gestation. Thyroxine concentrations were statistically significantly higher in the mares between days 49 to 55 of gestation compared with those in advanced stages. Triiodothyronine did not vary statistically significantly with gestational stage in mares.[45] One investigator found that hormone concentrations in mares at midgestation were no different from normal.[37] The high hormone concentrations were believed to be due more to the effect of the cold.[46] No significant differences in stages of gestation or in pregnant versus nonpregnant mares was found in one study.[28]

18.3.2.3 OTHER HORMONES

Glucocorticoids inhibit endogenous TRH secretion and decrease the response of TSH to TRH. Glucocorticoids also inhibit peripheral deiodination in adults. In neonates, glucocorticoids enhance conversion of T_4 to T_3. Glucocorticoids decrease serum thyroxine-binding globulin, increase thyroid-binding

prealbumin,[23] decrease total T_4, and increase fT_4. Catecholamines enhance the rate of deiodination of T_4 to T_3 and have a potentiating effect.[47] Estrogen enhances thyroxine-binding globulin production, and androgens tend to suppress it.[23]

18.3.2.4 BREED

No evidence indicates a difference in TH concentrations among breeds.[30,41]

18.3.2.5 DAILY RHYTHM

A daily rhythm in equine TH concentrations has been identified.[48,49] In adult geldings, T_4 was found to peak around 4 pm (mean, 2.43 ± 0.81 µg/dl) at concentrations significantly higher than the lowest T_4 values around 4 am (mean, 1.79 ± 0.63 µg/dl). Triiodothyronine was shown to peak between 8 am and 4 pm (~54 ± 13 ng/dl) at concentrations significantly different than the lowest T_3 around midnight (mean, 38.71 ± 10.81 ng/dl).[48] Higher concentrations of T_4 between 5 and 8 pm than at 8 am also have been reported.[49]

18.3.2.6 DISEASES: NONTHYROIDAL ILLNESS SYNDROME

Thyroid hormone concentrations are known to decrease during starvation and illness. First, T_3 concentrations decrease, and as the severity of the disease progresses, T_4 concentrations also decrease.[50] Because no clinical 1342
evidence of hypothyroidism exists despite decreased TH concentrations, this condition has been called the 1343
low T_3 syndrome or the euthyroid sick syndrome. A new designation, the nonthyroidal illness syndrome (NTIS) has been proposed because it does not presume the metabolic status of the patient, and it seems more appropriate based on current knowledge on thyroid gland function during disease. In this syndrome, T_3 concentrations are decreased, whereas T_4 concentrations can be low, normal, or elevated. In critically ill human patients, a significant decrease in T_4 and T_3 or an increase in rT_3 concentrations is associated with increased mortality rate.[51]

Starvation in different species, including the horse, results in decreased concentrations of T_3 and fT_3.[51–53] Carbohydrate deprivation inhibits the deiodination of T_4 to T_3 by the type I deiodinase, resulting in decreased concentrations of T_3 and increased concentrations of rT_3. Because starvation decreases the basal metabolic rate, the decrease in TH concentrations has been proposed to be an adaptive process of the organism to conserve calories by inducing a hypothyroid state. Concentrations of TSH in general are low or within the normal range despite the low TH concentrations. TSH has been proposed to decrease biologic activity from decreased TSH glycosylation. Recent evidence suggests that hypothalamic and pituitary dysfunctions are the proximal causes of NTIS.[51] Low TRH mRNA expression has been detected in the hypothalamus of starved rats and in human patients with NTIS.[54,55] Furthermore, administration of TRH to patients with NTIS results in increased TSH, T_4, and T_3 concentrations, supporting hypothalamic dysfunction as the most probable cause of NTIS.[56]

Cytokines, glucocorticoids, and more recently leptin (a 16-kd protein) are the most important candidates in the pathogenesis of NTIS. The diurnal variations in glucocorticoid concentrations control some of the diurnal

variations in THs by suppressing the pituitary response to TRH.[57] This is evident in human patients with Cushing's disease, in which increased glucocorticoid concentrations suppress TSH secretion. Increased cortisol concentrations may be present in horses under different conditions, including stress, sepsis, and pituitary gland dysfunction (equine Cushing's disease), and probably decreased TH concentrations are present in the same horses. Cytokines have received considerable attention in the pathogenesis of NTIS; IL-1, IL-6, and tumor necrosis factor α suppress TSH, T_4, and T_3 synthesis and secretion. Many of the effects of these cytokines on the HPT axis are mediated at the hypothalamic level, although some evidence suggests peripheral effects of these cytokines. Interleukin-1 can impair TH synthesis by the thyrocytes, whereas IL-6 and tumor necrosis factor α can decrease T_4 and T_3 concentrations and increase rT_3 concentrations. Therefore these cytokines can create a stage of low supply of THs to tissues. The role of leptin in the pathogenesis of NTIS may be associated with a decreased caloric intake during disease, which results in decreased concentrations of leptin, which then modulates hypothalamic functions (at the arcuate and paraventricular nuclei) and alters TRH mRNA expression.[58] Information on leptin in the horse is limited.[59,60]

Although NTIS is a hypothyroid state, evidence to suggest that the tissues are chemically hypothyroid is minimal[51]; however, decreased TH concentrations are accepted widely to be beneficial, and a process of maladaptation occurs in those cases in which low T_4 concentrations are associated with increased mortality.

In cases of low TH concentrations associated with nonthyroid illness, stimulation tests should elicit a normal response. In NTIS a reciprocal increase in rT_3 occurs because the suppressed enzyme—type I deiodinase, which is responsible for T_3 neogenesis—is also the enzyme that degrades rT_3.[23]

Based on the number of studies in human beings and animals, no clear evidence is available that TH replacement therapy is beneficial or unbeneficial to NTIS. Some have suggested that replacement therapy may be more harmful because exogenous TH may inhibit the HPT axis in a patient with decreased endogenous TH concentrations.

18.3.2.7 SEASON

Thyroid activity increases while horses are becoming acclimatized to colder temperatures.[28,61,62]

18.3.2.8 WEANING

In a study to evaluate the effect of weaning on thyroid function, no difference in hormone concentrations was found between abrupt and gradual weaning methods.[40]

18.3.2.9 ACTIVITY

Interest in the relationship of thyroid function and muscle metabolism has prompted investigation in racehorses. Irvine[31,63] found the T_4 secretion rate to be increased in horses in full training, whereas protein-bound iodine was decreased. This decrease in protein-bound iodine was not consistent with the finding of increased protein-bound iodine in human athletes.[37] Thyroxine concentrations were found to be greatly increased in the latter stages of training, and the proposal was made that T_4 measurement might be used to

monitor the training reserves in racehorses.[64] Other investigators found low T_4 concentrations in healthy Thoroughbreds and concluded that serum T_4 values were an unreliable indicator of thyroid function and that T_3 was a more accurate one.[65] They found no correlation between T_4 and T_3 concentrations and racing 1343
performance. Another investigator documented substantial increases in T_4 within 30 minutes after exercise 1344
but no differences in T_3.[38,44] Five minutes after a maximal speed race (1200 m), serum T_3 concentrations were increased in Thoroughbred racehorses (from 55.6 ± 2.9 to 81 ± 3.7 ng/dl); however, T_4 concentrations did not change (0.67 ± 0.04 to 0.70 ± 0.05 μg/dl).[66] Significant increases in T_3 and T_4 concentrations were found 1 hour after swimming.[67]

18.3.2.10 FEEDING

Feeding a high-energy, high-protein diet to weanlings 6 to 8 months of age was found to stimulate thyroid gland secretion initially, and then peripheral conversion of T_4 to T_3 resulted in a relative T_4 decrease 3 hours after a meal.[68] The soluble carbohydrate (glucose) rather than protein portion of the diet was found to increase thyroid hormone response in weanlings.[69] The accelerated T_4 to T_3 conversion following the high-carbohydrate meal was found to correlate with the increased insulin secretion that followed the glucose rise.[70] Insulin also accelerates deiodination of T_3, thus shortening its activity.[71] Furthermore, this response was found to be age related. By the time the animals were 12 to 14 months of age, feeding the high-carbohydrate and high-protein diet no longer affected TH concentrations.[70] One study on the effects of meal frequency and T_3 concentrations observed a hormone rise after meals, the rise being greater when animals were given one feeding a day than after six feedings a day.[72]

When the effect of food deprivation on baseline TH concentrations was evaluated in euthyroid adult horses,[53] serum T_3, fT_3, and fT_4 concentrations were found to decrease and rT_3 concentrations to increase after 1 day of food deprivation. Serum T_3 concentrations were lowest after 2 days of deprivation, whereas T_4, fT_3, and fT_4 reached their lowest concentrations on day 4 of food deprivation. Three days after the horses were placed on their normal ration, serum T_4 and fT_4 concentrations significantly increased and rT_3 concentrations decreased. This study provided valuable information in the interpretation of thyroid hormone tests in anorectic and sick horses. If a significant decrease (>50%) in serum T_3 and T_4 concentrations can be present in just 1 to 2 days of food deprivation, then the validity of measuring TH in diseased horses is questionable. Prolonged food restriction to Shetland ponies also resulted in decreased serum T_3 and fT_3 concentrations.[73]

The mechanisms that regulate thyroid gland function during starvation remain unclear. A decrease in caloric intake in known to result in decreased expression of TRH mRNA in the hypothalamus, but not in other areas of the brain. A reduction in TRH secretion subsequently results in decreased secretion and glycosylation of TSH. The picture on how the thyroid gland function is regulated at the hypothalamic level is becoming clearer with recent findings. Perhaps the most important signal in the central nervous system to suppress TRH expression in the paraventricular nucleus is a decrease in hypothalamic concentrations of leptin. Leptin is expressed in adipocytes, and a fall in leptin signals the hypothalamus to conserve energy, to increase appetite, and to modify the neuroendocrine system to favor survival. Thus a decrease in leptin suppresses the HPT axis, the neuroreproductive pathways, and growth and activates the hypothalamus-pituitary-adrenal axis. The effects of leptin on hypothalamic TRH expression are mediated primarily through the arcuate nucleus, although leptin also has direct effect in the paraventricular nucleus. Through the hypothalamic

melanocortin system, leptin increases proopiomelanocortin synthesis in the arcuate nucleus, which then is cleaved to produce α-melanocyte-stimulating hormone that stimulates TRH secretion from the paraventricular nucleus nucleus. A decrease in leptin results in decreased hypothalamic α-melanocyte-stimulating hormone concentrations, reducing TRH synthesis.[58] The importance of leptin in the equine HPT axis has not been investigated; however, feed restriction led to a significant decrease in leptin concentrations in mares,[60] indicating that leptin and its relationship to the HPT axis in the horse deserves attention.

18.3.2.11 EXOGENOUS COMPOUNDS

Substances that exert their goitrogenic effect by blocking or competing with iodide uptake are thiocyanate and perchlorate. Iodide oxidation and coupling of iodotyrosines are inhibited by drugs such as sulfonamides, phenylbutazone, phenothiazines, thiouracils, thiopental, and methimazole. Soybean meal, linseed meal, and plants of the *Brassica* genus (i.e., rapeseed) contain goitrogenic substances.[2,22] Phenylbutazone administration decreases TH concentrations in horses,[49,74] and this effect can last up to 10 days after discontinuation of phenylbutazone administration.[74] The thyroid-stimulating hormone stimulation test may be normal or exacerbated in horses receiving phenylbutazone.[49] Exogenous and endogenous glucocorticoids have the same effects: suppressing pituitary TSH secretion, decreasing the response of TSH to TRH, inhibiting monodeionination (except in the perinatal period), inhibiting thyroxine-binding globulin, and decreasing release of TH from the gland.[23,75] Glucocorticoid administration decreases serum T_4 and T_3 concentrations, and the effect is more profound on T_3 concentrations from decreased conversion of T_4 in T_3 in peripheral tissues. The decreased uptake and release of iodine by the thyroid gland during glucocorticoid treatment results from decreased TSH secretion.[76] One study in healthy horses found that dexamethasone administration for 5 days resulted in a significant increase in serum baseline rT_3 and fT_3 concentrations[77]; 1344
however, total T_3 and total T_4 concentrations did not change. In that study the fT_3 response to TSH 1345
stimulation was decreased after dexamethasone administration.

High levels of nitrates in the water may result in thyroid gland hypertrophy,[78] and nitrates can reduce the active transport of iodine into the thyroid gland.

Propylthiouracil, in addition to affecting iodine metabolism, inhibits peripheral conversion of T_4 to T_3. In human beings, aspirin competes for TH binding sites on transthyretin and thyroxine-binding globulin, decreasing T_4 and T_3 and increasing fT_4 and fT_3. This competition results in a hypermetabolic state from increased free fractions of T_4 and T_3 and also may lower the TSH response to TRH stimulation.[75] Phenobarbital increases TH binding to liver microsomes and increases deiodinating activity.[79] Reserpine, a catecholamine-depleting agent, inhibits secretion of TSH and inhibits deiodination.[47] Antipyrine has antithyroid activity.[23] Anabolic steroids can have an inhibitory effect on thyroxine-binding globulin concentrations[23]; however, in one study, short-term anabolic steroid use was found not to affect thyroid function tests of horses significantly.[80] Exogenous compounds containing iodine or iodides are of particular interest. Iodine excess, as well as deficiency, can cause thyroid dysfunction and interfere with thyroid tests. Horses are exposed to iodine or iodide compounds in the form of feedstuffs, expectorants, leg paints, shampoos, injectable counterirritants, radiographic contrast media, and the antiprotozoal drug iodochlorhydroxyquin. Individuals can respond to excessive amounts of iodine by suppressing or accelerating hormone production. Iatrogenic hypothyroidism following cleansing of an open wound with

povidone-iodine is documented in human beings.[81] Iodine excess reduces iodine uptake and organification, decreases TH concentrations, and decreases the response to the TSH stimulation test; this phenomenon is known as the Wolff-Chaikoff effect.

Iodine excess also may inhibit the cleavage of T_4 and T_3. The end result of iodine excess may be hypothyroidism. The second way an individual can respond to large amounts of iodide is by accelerated production, which produces elevated concentrations of THs and is referred to as Jod-Basedow phenomenom. In general the phenomenon occurs in patients with underlying thyroid disease, in particular those with iodine deficiency.[23]

Boosinger, Brendemuehl, Bransby, et al.[82] found that newborn foals of mares grazing *Neothyphodium (Acremonium) coenophialum*–infected fescue had half the concentration of T_3 of control foals; however, no difference existed in T_4 and rT_3 concentrations compared with controls. Endophyte alkaloids are dopamine D_2-receptor agonists, and dopamine is a known inhibitor of TSH secretion. Whether decreased TH concentrations in foals born of mares grazing fescue results from the effects of the endophyte alkaloids on the fetal HPT axis or from the effects of these alkaloids in placental and fetal development is unclear.

18.3.3 Diagnostic Tests

Thyroid tests can be classified according to the information they provide in functional, etiologic, and anatomic tests. Thyroid tests are direct when one measures serum concentrations of THs or of thyroid-regulating hormone and indirect when one calculates TH concentrations by index or mathematic models or when one determines the relation of other metabolites to thyroid function.

18.3.3.1 DIRECT TESTS

Irvine[61] was one of the first investigators to develop and use quantitative thyroid function tests for horses. The T_4 secretion rate was calculated after injecting iodine-131 and plotting the fall of protein-bound iodine. The volume of distribution was calculated by extrapolation from the disappearance curve to zero time. The product of volume of distribution and the protein-bound iodine was used to calculate the T_4 pool. From the half-life of protein-bound ^{131}I, one obtained the fractional turnover of the pool and thus the daily loss of T_4. Assuming loss and replacement to be equal, the amount was designated the T_4 secretion rate. This method of measurement was not clinically practical.[31]

For years protein-bound iodine was the standard determination for estimating serum hormone concentrations.[2,37] All iodine, hormonal or not, was measured. This measurement is influenced greatly by substances containing iodine and by iodine in the diet.

With the development of new immunoassay technology, the approach to diagnosing thyroid gland dysfunction in human medicine has changed. Sensitive assays for TSH now are being accepted in the initial screening of thyroid function in patients with normal pituitary gland function. In these patients, increased TSH concentrations are expected in hypothyroidism and the opposite in hyperthyroidism. Free T_4 concentrations are determined when TSH concentrations are abnormal. This determination is known as the TSH-centered strategy for evaluation of thyroid gland function and is considered efficient and cost-effective.[83] Unlike human laboratories, in which tests for thyroid function are commercially available in preexisting

forms, in equine medicine veterinarians are far from assessing thyroid gland function in an organized manner. The traditional approach has been to determine TH concentrations, compare them to reference values, and make a diagnosis and specific recommendations, in many cases without considering the underlying cause of abnormal TH concentrations. 1345

Competitive immunoassays are the method of choice to measure total T_4 concentrations, and they measure 1346
free and protein bound T_4. To obtain an accurate measurement, T_4 must be dissociated from the transport proteins by using different blocking agents. These immunoassays can be isotopic and nonisotopic and are available to be used in automated systems. Serum is preferred to measure T_4 concentrations, although heparin or EDTA can be used. Thyroxine is stable in serum at 4° C for several weeks. One should avoid hemolyzed and turbid samples and samples from patients receiving thyroid supplements, a common practice in the equine industry.

To determine total T_3 concentrations, methods (isotopic and nonisotopic) to those similar for measuring T_4 concentrations are available. As with T_4, protein-bound T_3 must be released from the binding proteins; serum is preferred, and one must avoid hemolysis.

Free THs circulate in blood in equilibrium with protein-bound THs, and changes in thyroid-binding protein (thyroxine-binding globulin, thyroid-binding prealbumin, albumin) can affect the total TH concentration. At steady state, free TH concentrations remain constant despite changes in thyroid-binding protein. One also can estimate free TH concentrations using indirect methods. For example, the index method uses two separate tests to estimate free TH, one to determine total TH (T_3 or T_4) and another to determine thyroxine-binding globulin; and by mathematical models, one calculates free TH concentrations.

TABLE 18.3-1 Thyroid Hormone Concentrations in Adult Horses

TOTAL T_4	TOTAL T_3	fT_4	fT_3	TSH	REFERENCE
1.76 μg/dl	98.69 ng/dl	—	—	—	30
1.63 ± 0.51μg/dl (0.95–2.38 μg/dl)	77.1 ± 45.75 ng/dl (31–158 ng/dl)	—	—	—	42
1.46–3.38 μg/dl	—	—	—	—	147
1.79 ± 0.17 μg/dl	62 ± 4.3 ng/dl	—	—	—	62
1.57 ± 0.62 μg/dl (0.30–3.70 μg/dl)	—	—	—	—	41
1.56 ± 0.081 μg/dl	67.7 ± 10.2 ng/dl	5.9 ± 0.39 pg/ml	3.22 ± 0.18 pg/ml	—	43
2.13 ± 0.76 μg/dl (0.93–4.31 μg/dl)	47.38 ± 13.66 ng/dl (13.30–97.40 ng/dl)	—	—	—	48
19.87 ± 1.74 nmol/L	1.02 ± 0.16 nmol/L	11.55 ± 0.70 pmol/L	2.05 ± 0.33 pmol/L		53
6.2–25.1 ng/ml	0.21–0.80 ng/ml	—	0.07–0.47 ng/dl	—	148
12.9 ± 5.6 nmol/L	0.99 ± 0.51 nmol/L	12.2 ± 3.5 pmol/L	2.07 ± 1.14 pmol/L	0.40 ± 0.29 ng/ml	88
2.57 ± 0.71 μg/dl (1.5–3.5 μg/dl)	77.1 ± 45.75 ng/dl (31–158 ng/dl)	—	—	—	149
1.9 ± 0.4 μg/dl[*]	89.3 ± 26.2 ng/dl[*]	—	—	—	150
0.25–3.70 μg/dl	10.0–127.0 ng/dl	—	—	—	149

T4, Thyroxine; *T3,* triiodothyronine; *fT4,* free thyroxine; *fT3,* free triiodothyronine; *TSH,* thyroid-stimulating hormone.

†Miniature horses.

* Ponies.

Thyroxine-binding globulin is the most important T_4-binding protein, and measuring thyroxine-binding globulin concentrations is relevant for assessing TH concentrations. Immunoassays and two-site immunometric assays are available to determine human thyroxine-binding globulin concentrations but are not readily available for horses at this time.

Radioimmunoassay has been used to measure THs in horses since 1978[41] and is validated for horses.[84] Radioimmunoassay quantification of T_4 and T_3 is not affected by the presence of hemolysis of the blood sample.[85] Enzyme-linked immunosorbent assays (ELISA) techniques are available to measure TH concentrations.[2,86] The big advantage of this method is that no radioactive agents are required. New

immunometric assays with higher sensitivity than radioimmunoassay and ELISA are available to measure TH concentrations. The most precise way to quantify the actual amount of hormone available to target tissues is to determine the unbound or free fractions.[46]

Normal TH concentrations have a wide range of values and vary among laboratories and measurement techniques (Tables 18.3-1 and 18.3-2), complicating the interpretation of results, in particular when one uses different laboratories. For most consistent results the practitioner always should use the same laboratory and become familiar with its values. Ratios of circulating hormones may indicate dysfunction. In general, among species the ratio of circulating total T_4 to T_3 is 20:1. In horses the ratio was found to be 23:1. The ratio of fT_4 1346 1347
to fT_3 was found to be 1.83:1 in horses.[43] An increased ratio of T_3 to T_4 has been associated with 1347 1348
hypothyroidism in dogs[87] and in human beings. With iodine deficiency, the thyroid produces and secretes more T_3 as a way of conserving iodine, thus increasing the ratio of T_3 to T_4.[23] If the rT_3 value is available in addition to T_4 and T_3 values, certain patterns may suggest and support dysfunction. For instance, rT_3 may be increased in hyperthyroidism and in euthyroid sick syndrome (NTIS).[23] Because rT_3 is a breakdown product of T_4, its concentrations depend on the T_4 concentrations. Thus rT_3 may be increased along with T_4 in hyperthyroidism. In euthyroid sick syndrome the activity of type I deiodinase is decreased. This enzyme is responsible for T_3 neogenesis and rT_3 breakdown; therefore in that syndrome, T_3 is decreased and rT_3 is increased. Likewise, in hypothyroidism, where T_4 is decreased, rT_3 also may be decreased.[23]

TABLE 18.3-2 Thyroid Hormone Concentrations in Foals

AGE	TOTAL T_4	TOTAL T_3	fT_4	fT_3	REFERENCE
1–10 hours	28.86 μg/dl	991 ng/dl	12.12 pg/ml	2.99 pg/ml	32
4 days	11.2 μg/dl	935 ng/dl	—	—	
4 days	231.7 ± 61.8 nmol/L	7.8 ± 4.2 nmol/L	—	—	151
28 days	30.6 ± 17.4 nmol/L	3.1 ± 0.4 nmol/L	1.2 ± 0.4 ng/dl	3.4 ± 1.1 pg/ml	
1 day	4.4–25.1 μg/dl	26.0–732.7 ng/dl	—	—	94
1½-4 months	4.02–0.19 μg/dl (2.9–5.25 μg/dl)	192.86 ± 8.54 ng/dl (135–270 ng/dl)	—	—	30
5 minutes	29.3 μg/dl	—			
48 hours	20.5 μg/dl	—	—	—	122
Birth	233.0 ng/ml	7.9 ng/ml	—	—	
1 day	207 ng/ml	6.7 ng/ml	—	—	35
7 days	92 ng/ml	4.2 ng/ml			
14 days	49 ng/ml	2.4 ng/ml			
1 month	26 ng/ml	1.6 ng/ml			
6 months	35 ng/ml	0.9 ng/ml			

T4, Thyroxine; *T3,* triiodothyronine; *fT4,* free thyroxine; *fT3,* free triiodothyronine.

Sensitive immunometric assays to determine TSH concentrations have been developed. In these assays, one monoclonal antibody (to β-subunit) is fixed to a solid phase, and the bound hormone then is quantitated by a second detection antibody (i.e., directed to α-subunit). The detection antibody is labeled with ^{125}I (immunoradiometric assays) or with alkaline phosphatase or peroxidase (immunochemiluminometric assays). Immunometric assays to measure human TSH are currently available for use in automated systems and are more sensitive than radioimmunoassay or ELISA. The validity of human TSH assays to measure equine TSH is unknown; however, the results are expected to be acceptable based on a 90% homology between human and equine TSH amino acid sequences. Serum TSH concentrations from horses with hypothyroidism are not available.

Breuhaus[88] recently validated an equine-specific double-antibody TSH radioimmunoassay in an equine model of hypothyroidism. In that study the author induced a hypothyroid state in healthy horses by administering propylthiouracil for 6 weeks. Serum T_3 and fT_3 fell rapidly in the first week of propylthiouracil administration, whereas fT_4 and T_4 did not decrease until weeks 4 and 5, respectively. TSH remained steady during propylthiouracil administration and then increased after week 5. Furthermore, the TSH response to TRH stimulation (1 and 5 mg) was exaggerated on weeks 5 and 6 of propylthiouracil administration, and the TH response was blunted. In this study, using the propylthiouracil model of

hypothyrodism, the equine-specific TSH assay together with the measurement of TH concentrations was able to differentiate euthyroid from hypothyroid horses.

No direct tests are available to evaluate the effect of THs on the target tissues. The most direct measure of metabolic activity is basal metabolic rate. Routine determination of basal metabolic rate is not clinically practical.

18.3.3.2

INDIRECT TESTS

Serum cholesterol is elevated in human beings and dogs with hypothyroidism,[2] but this is not a specific response to thyroid dysfunction. Increased cholesterol concentrations have been found in some hypothyroid horses but not others, so this is not a reliable indicator in horses.[61,89] A normochromic, nonresponsive anemia of chronic disease, once again, is not specific but may be seen with hypothyroidism.[2] This finding of anemia responsive to thyroid supplementation has been documented in horses.[89–91] Low body temperature and bradycardia may indicate hypothyroidism.

18.3.3.3

TROPHIC RESPONSE TESTS

Trophic hormone response tests are useful to help differentiate primary from secondary thyroid dysfunction, and they remove the variability of endogenous and exogenous factors that influence other tests.

18.3.3.3.1

Thyroid-Stimulating Hormone

The TSH stimulation test protocol consists of injecting 5 IU of TSH intravenously and comparing pre- and postinjection TH concentrations.[92,93] In normal horses, T_4 peaks 3 to 4 hours after injection at a concentration 2.4 times the preinjection value. Triiodothyronine doubles within 30 minutes and peaks at 2 hours at a concentration 5 times baseline.[87] Normal responses are characterized by a rise in T_3 that antedates the T_4 rise and is also greater. When 5 IU TSH was given intramuscularly, T_4 peaked at 2 times baseline value 3 to 12 hours after injection, and T_3 peaked in 1 to 3 hours.[49] The suggested protocol for the intravenous TSH response test is to measure hormone concentrations at baseline and 4 hours after injection.[87] No difference in T_4 concentrations was found between 2.5 and 5 IU of TSH.[92] For the intramuscular protocol, one should measure hormone concentrations at baseline and 3 and 6 hours after injection.[49] The protocol for 1-day-old foals is to measure T_3 concentrations at baseline and 1 and 3 hours after intravenous injection of 5 IU of TSH. A normal response is a 50% increase by 3 hours. Thyroxine values were too variable.[94] An insufficient increase in hormone indicates a primary thyroid dysfunction. The ability to perform a TSH stimulation test may be limited by the cost or availability of TSH.

Phenylbutazone administration to horses decreases TH concentrations but does not affect the response of the TSH stimulation test.[49] Dexamethasone decreases the TH response to the TSH stimulation test. When the TSH stimulation test was performed in healthy horses treated for 5 consecutive days with dexamethasone (0.04 mg/kg), the response to the TSH stimulation (5 IU intramuscularly) test was found to be blunted by dexamethasone administration.[77] This study raised questions in the interpretation of the TSH stimulation test from previous studies,[49,87] as well as concerning the utility of the test in differentiating thyroidal and nonthyroidal causes of hypothyroidism in the horse.

18.3.3.3.2

Thyroid-Releasing Hormone

Thyroid-releasing hormone stimulation tests have been done in horses.[92,95–98] One administers TRH intravenously at 1 mg to horses and 0.5 mg to ponies. An inadequate hormone response to TRH would occur in primary or secondary hypothyroidism. In normal animals, T_4 peaks at 4 hours[92,97] and T_3 at 2 hours after intravenous TRH administration.[97] In another study, T_3 increased significantly at 1 to 2 hours after TRH injection and peaked at 2 to 4 hours. Thyroxine increased by 2 hours and peaked at 4 to 10 hours. Both hormones should increase two- to threefold in a normal response. The suggested protocol is an intravenous dose of 1 to 3 mg TRH with hormone measurements at baseline and 4 to 5 hours later. Side effects of TRH administration include salivation, urination, defecation, vomiting, pupillary constriction, tachycardia, and tachypnea.[96] An abnormal (low) response to TRH administration suggests dysfunction at the pituitary gland or the thyroid gland. Additional tests (TSH concentrations, TSH stimulation test) may be necessary to differentiate primary thyroid disease from pituitary disease. Low baseline TH concentrations with high TSH concentrations and an abnormal response (low) to the TSH stimulation test suggest thyroid gland dysfunction. Low baseline TH concentrations with a normal or low TSH concentration suggest hypothalamic or pituitary gland dysfunction. Low baseline TH and TSH concentrations with a normal TSH and TH response to TRH stimulation test may suggest hypothalamic dysfunction as the cause of hypothyroidism. The TRH stimulation test also has been used as a diagnostic aid to evaluate pituitary adenomas in horses.[95]

18.3.3.3.3

Thyroid Suppression Test

One can demonstrate the presence of autonomous or TSH-independent thyroid gland function by the thyroid suppression test. The exogenous administration of THs in quantities sufficient to suppress TSH secretion results in decreased TH synthesis and secretion. The lack of TH suppression indicates that thyroid gland function is independent of TSH. The administration of T_3 (T_3 suppression test) to healthy individuals is expected to cause a decrease in serum T_4 concentrations, whereas hyperthyroid individuals are expected not to have a significant decrease in serum T_4 concentrations. This test was useful in diagnosing hyperthyroidism in a horse with a thyroid adenoma[99]; 2.5 mg of T_3 were administered intramuscularly, and serial sampling revealed no decrease in T_4 concentrations.

18.3.3.4

IMAGING

Pertechnetate (technetium-99m) is a diagnostically useful compound that is taken up by the iodine-trapping mechanism.[2] Scintigraphic imaging has been used clinically to evaluate the thyroid glands of horses.[100] Abnormal uptake patterns have been seen with thyroid carcinoma in horses.[101–103] Ultrasonographic 1348
evaluation of the glands can differentiate solid structures from cystic ones.[23] Ultrasonography has been used 1349
to evaluate thyroid gland morphology in horses.[99,104]

18.3.3.5

BIOPSY

Aspirate or biopsy sampling can help differentiate cysts, neoplasia, hyperplastic goiter, colloidal goiter, and inflammation.

18.3.4
Hypothyroidism

Hypothyroidism is defined as a deficiency of THs, a deficient thyroid activity, or as a disruption in the HPT axis. Hypothyroidism occurs from diseases that affect thyroid function or from exogenous compounds that may affect the thyroid tissue or interfere with TH synthesis and less frequently by disorders that affect the hypothalamus or the pituitary gland. Hypothyroidism occurs because of the actions of THs on cell differentiation and metabolic rate regulation.[27] Liver, muscle, kidney, heart, salivary gland, and pancreatic tissue are sensitive targets of thyroid hormone action.[23] Hypothyroidism can manifest in many ways, and the diagnosis can be a challenge because few tests for thyroid function are equine-specific and many nonthyroidal factors can lead to misinterpretation of the results. Hypothyroidism, or thyroprivia, results when thyroid tissue is removed or thyroid function is suppressed. Hypothyroidism can be classified as primary, secondary, or tertiary. Primary gland dysfunction can be caused by iodine deficiency (endemic goiter) or excess (Wolff-Chaikoff effect), thyroiditis, neoplasia, biochemical defects, thyroid agenesis, or ingestion of goitrogenic compounds that can block hormone synthesis. Of these, iodine excess, iodine deficiency, and neoplasia have been reported as causes of hypothyroidism in horses. Based on the existing knowledge on thyroid gland function, individuals with primary hypothyroidism have decreased T_4 and T_3 concentrations and increased TSH concentrations; this seems to be true in horses with experimentally induced hypothyroidism.[88] Secondary (central) hypothyroidism results from pituitary (trophoprivic) or hypothalamic dysfunction. Authors report hypothyroid activity in horses with pituitary adenoma,[90,105] but all horses with pituitary adenoma do not have concomitant hypothyroidism.[95] Tertiary hypothyroidism denotes a defect in hormone use at the peripheral tissues. The condition has not been identified or reported in horses. In one report, a defect in T_4 to T_3 deiodination was suspected, and a hair coat started to grow after T_3 supplementation began.[106] In many of the reported cases of hypothyroidism in horses, a specific cause has not been identified.

The consequences of inadequate circulating THs are potentially devastating and life threatening to fetus and foal. The poor prognosis is associated with the essential role of THs in normal growth and development. Chances of recovery are unlikely once critical developmental stages are passed. Tests to evaluate thyroid gland function may be within normal limits at the time of examination, making it difficult to confirm a previous transient or in utero TH deficiency.[27] The equine placenta is permeable to iodine but is impermeable to T_3 and T_4, and excessive ingestion of iodine may result in hyperplastic goiter.

18.3.4.1
SIGNS

Because decreased iodine intake is considered the main cause of neonatal hypothyroidism and goiter, pituitary gland function in these foals may be normal or increased. Decreased TH concentrations stimulate the pituitary gland to secrete excessive amounts of TSH, which then stimulate the thyroid cells, resulting in thyroid enlargement. Most clinical signs thus far reported in hypothyroid foals are attributable to the crucial role of THs in the development and maturation of the nervous and musculoskeletal systems. Thyroid hormones have major effects in brain development during the pre- and postnatal periods, and TH deficiency results in decreased axonal growth, dendritic arborization, and myelinization in several areas of the central nervous system. Thyroid hormones are so important in neonatal brain development that early treatment of hypothyroid infants with T_4 prevents intellectual impairment and is a major reason for screening thyroid function in human neonates. Hypothyroid foals often are affected at birth, have difficulty standing, and have a weak or absent suckle reflex. Physeal dysgenesis[107]; weakness, death, or incoordination in newborns[32];

defects in ossification with tarsal bone collapse[108]; hypoplastic carpal bones, common digital extensor tendon rupture, forelimb contracture, and mandibular prognathism[109,110]; stillbirths, weakness, and long haircoat[111]; weak suckle reflex[112]; respiratory insufficiency[113]; and premature, weak foals that die shortly after birth[5] have been reported. Foals can be born apparently normal but may develop skeletal lesions weeks later.[114] Thyroid hormones are required for the biochemical maturation, hypertrophy, and capillary penetration of growing cartilage. Triiodothyronine may act indirectly to promote chondrogenesis by stimulating anterior pituitary synthesis and secretion of growth hormone. Thyroid hormones also increase fetal skeletal growth hormone receptor and insulin-like growth factor I gene expression,[115] and therefore THs influence the growth and skeletal development by local activity of the somatotrophic axis.

An age-related, carbohydrate diet–responsive, insulin-T_4-T_3 interaction has been identified that may play a role in cartilage maturation defects in weanlings.[116] Thyroxine also plays a role in maturation of the lungs 1349
and in surfactant production.[23] One report in the literature describes a foal with respiratory distress syndrome 1350
and hypothyroidism.[113] Doige and McLaughlin[117] described two foals that died in respiratory distress. Neonatal thermogenesis is also T_4 dependent[27]; thus cold intolerance and hypothermia could be a problem. In one study, when young, growing animals were thyroidectomized, physeal plate closure and incisor eruption were delayed. Very young foals suffered severe growth retardation and died.[118] In a second study in which day-old foals were thyroidectomized, lethargy, depression, and rough, dry hair coats were seen.[114]

18.3.4.2 CAUSE

The major cause of hypothyroidism in foals is believed to be nutritional. Congenital thyroid enlargement (goiter) (Figure 18.3-1) with decreased function is associated with inadequate iodine intake and excessive iodine intake by the mare. Thyroxine is transported transplacentally and via the milk.[119] No cases of hypothyroidism caused by an inborn error of metabolism have been described in foals.

Several reports in the literature describe goiter and hypothyroidism in foals associated with ingestion of excessive amounts of iodine in kelp-supplemented rations.[5,111,119–121] The dams of these foals may or may not be affected. Feeding 40 mg or more of iodine daily can produce this syndrome.[112]

18.3.4.3 DIAGNOSIS

Foals that exhibit any of the aforementioned signs, with or without enlarged glands, are suspect. One should examine the mare and determine her nutritional status. Because enlargement of the thyroid gland alone does not automatically imply hypo- or hyperthyroidism, one should perform function tests to confirm a diagnosis. One crucial point to remember is that high TH concentrations are normal in foals (see Table 18.3-2). Not only does a powerful central drive exist to stimulate the thyroid axis late in gestation,[27] but also thyroxine-binding globulin concentrations are increased in neonates[23] and hormone protein binding is more pronounced in neonates than in adults. Thyroid hormone concentrations measured in umbilical cord blood of newborn foals were found to be 14, 5, 7, and 3 times adult concentrations, respectively for total T_4, fT_4, total T_3, and fT_3. For most of fetal life, T_4 is deiodinated to rT_3, concentrations of T_3 are negligible, and concentrations of T_4 and rT_3 are high. At birth, rT_3 production decreases, and the deiodination pathway leads to T_3. The plasma rT_3 concentrations in foals fall rapidly as T_3 rises for 24 to 48 hours after birth, plateaus for 2 to 3

days, then declines to parallel the T_4 value.[27] A decline in T_4 concentrations occurs during the first 16 days of life. Free T_4 concentrations decline over the first 3 months of life.[32] In term foals, serum T_3 concentrations are elevated far above adult concentrations for the first 6 to 12 hours; by 24 hours they have declined significantly. The rT_3 concentrations in premature foals are lower than those of term foals, but a further decline is not observed up to 48 hours. This observation is consistent with a surge of 5′-deiodination resulting in T_3 production and rT_3 degradation about 6 to 12 hours after birth for term foals. Deiodination is impaired in preterm foals.[122] McCall, Potter, Kreider, et al.[40] found T_3 concentrations in foals 4 months of age comparable to adult concentrations; however, mean T_4 concentrations were higher at 2.9 µg/dl. When evaluating TH concentrations in foals suspected of having hypothyroidism, one must compare their TH concentrations to age-matched healthy foals. A foal may have clinical evidence of hypothyroidism, but THs may be within the normal range, suggesting that perhaps a TH deficiency occurred during development. Hypothyroidism in foals carries a poor prognosis, even after thyroid supplement therapy.

Figure 18.3-1 Goiter in a foal. The dam inadvertently was given a ration that contained too much iodine.

18.3.4.4 CONGENITAL HYPOTHYROIDISM AND DYSMATURITY SYNDROME IN FOALS

A syndrome characterized by thyroid gland hyperplasia and multiple congenital musculoskeletal deformities has been described in neonatal foals in western Canada.[40,109,117,123–126] In some farm outbreaks, this syndrome is reported to affect 30% to 100% of foals.[126] The syndrome is unique, with no sex or breed predilection.[124] Abnormal musculoskeletal findings include prognathia, osteochondrosis and inappropriate ossification of carpal and tarsal bones, angular limb deformities, and rupture of the common digital extensor tendons. These foals have signs of dysmaturity, including a silky and short hair coat, floppy ears, tendon 1350

laxity, and incomplete closure of the abdominal wall.[126] Thyroid gland hyperplasia also is associated with hypothyroidism.[125] The syndrome is referred as *thyroid hyperplasia and musculoskeletal deformities* or as *congenital hypothyroidism and dysmaturity*. The cause of this condition is unknown. A case-control study found that foals with congenital hypothyroidism and dysmaturity had a longer gestation time and that mares grazing irrigated pastures or fed green feed or mares that were not receiving mineral supplements or that left their home farm were statistically more likely to produce affected foals.[126] High dietary nitrate concentrations have been proposed as the possible cause of this syndrome. Nitrates can impair thyroid gland function[78] and also can cross the placenta in different species. Green feed can concentrate high levels of nitrates and nitrites. Alfalfa, ryegrass, timothy, and cereal crops such as wheat, oats, rye, and barley also accumulate nitrates. In addition to the high content of nitrates in these plants, they also have low levels of iodine. Nitrates also can be present in high concentrations in the water, in particularly in areas with high use of fertilizers, feedlots, and dairies.[126] 1351

18.3.4.5 HYPOTHYROIDISM IN ADULT HORSES

Hypothyroidism in adults is a rare condition and unlike that in foals is not life threatening. Low-grade anemia responsive to thyroid supplementation has been described in thyroidectomized horses[89,90] and in racehorses with myopathy.[91] Decreased body temperature, heart rate, respiratory rate, and cardiac output were reported in thyroidectomized horses.[89,127] Reproductive problems in mares are irregular and absent estrus cycles.[5,89] Thyroidectomized mares could conceive and carry a foal to term.[128] Stallions exhibit decreased libido.[89,106] Thyroidectomized stallions had decreased total sperm count but normal semen characteristics and testicular histology, and they could sire foals.[118,128] Bradycardia, obesity, and lethargy were seen in mares with thyroid function suppressed because of ingestion of excess iodine.[5] Lethargy, rear limb edema, coarse hair coat, and decreased appetite were seen in thyroidectomized mares.[128] One case describes failure to grow a normal hair coat in a stallion.[106] Alopecia attributed to iodinism and low circulating TH values were documented in a horse being treated with an expectorant containing iodide and topical povidone-iodine.[129] The hair coat grew and thyroid function returned to normal when the medications were discontinued. One report described agalactia associated with decreased thyroid function.[15] Thyrotropin-releasing hormone has been shown to stimulate pituitary secretion of prolactin in many species; however, TRH injections have minimal or no effect stimulating prolactin release in mares.[14–16] Poor performance in racehorses during the season when ambient temperatures are increasing may be related to seasonal thyroid function changes. At this time hormone concentrations may drop too low to support optimal muscle performance.[63]

Hypothyroidism has been implicated as a cause of anhidrosis. In one study, mean T_3 and T_4 values were found to be lower in anhidrotic horses than in unaffected horses.[130] Another report found that thyroid function was not abnormal in some horses selected from a group affected with anhidrosis.[131] Low TH concentrations may be present in horses with laminitis, but whether hypothyroidism is a cause or a contributing factor in laminitis has yet to be proved.[132] Many horses with laminitis and Cushing's disease also have decreased TH concentrations; however, hypothyroidism in these horses has not been documented.

18.3.4.6

TREATMENT OF HYPOTHYROIDISM

When the diagnosis is low circulating hormone concentrations or hyperplastic goiter, one must ascertain whether dietary intake of iodine is adequate. In addition, differentiating hypothyroidism from NTIS is important because animals with NTIS may not require treatment for thyroid hypofunction. Furthermore, TH therapy to euthyroid horses with NTIS may be harmful, causing inhibition of the HPT axis and suppressing endogenous TH synthesis and secretion.

The National Research Council recommends a minimum daily intake of iodine of 0.1 mg/kg of feed. Total daily intake of 1 to 2 mg iodine per animal also has been reported.[5] The soils of some geographic areas are low or marginal in iodine, for instance, the Great Lakes region.

Iodinated casein or thyroprotein, 5 g orally per day, reversed the effects of thyroidectomy in adults.[118] Iodinated casein, 5 to 10 g orally per day, reversed the thyroid-associated anemia and myopathy in racehorses.[91] Desiccated thyroid extract, 2 mg/kg body mass given orally each day, has been used successfully.[111] The responses to the iodinated casein and thyroid extract may not be consistent, for hormone concentrations in the products can vary.

Hormone supplementation with T_4 should be effective unless a deiodination defect exists. A starting dose of 20 μg of T_4 per kilogram body mass given orally daily is recommended.[38] If T_3 is to be supplemented, an oral dose of 1 mg/kg body mass once a day is recommended.[133] Whether the use of thyroid supplements is beneficial remains controversial and requires large scale studies.

One should monitor the animal for clinical response to therapy, which can take at least 2 weeks. One should monitor hormone concentrations periodically and reassess function and dosages, particularly if the desired response is not achieved. 1351

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18.3.5

Hyperthyroidism

Hyperthyroidism is a hypermetabolic disorder resulting from high concentrations of free T_4 and T_3. Few reports of equine hyperthyroidism document the condition. A syndrome of tremors, excitability, tachycardia, sweating, and weight loss in spite of a good appetite has been described in racehorses.[63,134] Plasma thyroid hormone concentrations were elevated, and the horses improved greatly in response to antithyroid treatment of 1 g potassium iodide orally per day.[61,63] The use of thyroidectomy as a means of behavior modification for high-strung, unmanageable horses has had variable success.[118] In these cases, whether a diagnosis of hyperthyroidism was confirmed by hormone concentrations or function tests was not stated. High concentrations of THs can occur in physiologic states not associated with abnormal clinical signs, such as pregnancy[45] and the well-documented high concentrations needed for normal fetal growth and development.[30,32] No reports describe hyperthyroidism associated with an autoimmune condition in horses. Hyperthyroidism associated with a thyroid adenocarcinoma recently was reported in a 21-year-old gelding.[104] The presenting complaint was an enlarging mass on the right cervical region, weight loss, and tachypnea. Serum T_3 and T_4 concentrations were elevated. The T_3-suppression test decreased serum T_4 concentrations in control horses but not in the affected horse.

Horses are at risk for accelerated TH production because of exposure to increased quantities of iodine, as in expectorants, counterirritants, contrast media, drugs (e.g., iodochlorhydroxyquin), and leg paints and shampoos containing iodine. This is called the Jod-Basedow phenomenon.[23] The phenomenon is recognized in human medicine, and certain persons (such as those with multinodular goiter) are predisposed to this effect. The phenomenon would be typified in a horse by transiently elevated THs that return to normal after the source of the excess iodine is discontinued. Human beings who respond this way to excessive iodine would not be expected to respond to the supplemental potassium iodide that causes suppression of TH production in others (Wolff-Chaikoff effect). In a racehorse that shows appropriate signs and elevated hormone concentrations, one must determine whether an iodine compound has been used or whether the horse may have received a TSH "jug." If severe distress of thyrotoxicosis is present, administration of glucocorticoids should alleviate the signs.[23]

18.3.6 Thyroid Tumors

Tumors of the thyroid in horses tend to occur more frequently in light-weight breeds than in Draft horses and in general more often in aged horses than in young ones.[135] Based on immunohistochemical techniques, three cell types are distinguishable in the thyroid glands of horses. The first type is undifferentiated and does not react to antibodies; the second type is parafollicular cells, which have calcitonin-positive secretory granules; and the third type is thyroglobulin-positive follicular epithelial cells. Moreover, cell aggregations have three classifications. The first is ultimobranchial remnant embedded in thyroid tissue without compression of adjacent cells. The second is nodular hyperplasia of the ultimobranchial remnant that compresses adjacent follicular cells. The third is adenoma of the ultimobranchial segment surrounded by fibrous capsule. Researchers found that in horses all cell aggregations had undifferentiated cells that retained embryonic characteristics such as those of ultimobranchial remnant.[135]

In a survey of thyroid glands of aged horses, gross tumors were found in 11 of 29 horses.[136] No tumors were found in horses younger than 18 years old, and most tumors were microfollicular adenomas.

18.3.6.1 ADENOMA

Adenoma is the most common neoplasia of the equine thyroid.[121,136–142] The phenomenon is age related, occurring mainly in horses older than 16 years; is benign; is usually unilateral; and is not associated with thyroid dysfunction. Occasionally the size of the adenoma may warrant surgical excision. Prognosis is good. Horses with thyroid adenoma or pituitary adenoma were found to have exaggerated insulin responses following oral glucose tolerance testing and failed to maintain cortisol suppression 24 hours after a dexamethasone suppression test. These findings were observed also in preclinical cases that were diagnosed at necropsy.[140] A thyroid adenoma associated with hyperthyroidism was diagnosed in a 23-year-old gelding.[99] In this case the diagnosis was aided by percutaneous biopsy and the use of a T_3-suppression test, in which T_4 concentration was not suppressed after the intramuscular administration of 2.5 mg of T_3. Hemithyroidectomy of the affected gland restored TH concentrations.

18.3.6.2

ADENOCARCINOMA

Malignant neoplasia of the thyroid occurs less frequently. In four reported cases of thyroid adenocarcinoma, one horse was euthyroid, two were hypothyroid, and one was hyperthyroid, based on serum T_3 and T_4 concentrations.[101–104] One case of thyroid carcinoma with systemic metastasis and concurrent pituitary adenoma is reported.[143] One report describes a horse with a diagnosis of carcinoma and adenoma in the same gland. The low T_3 concentration seen with this horse was felt to be caused by euthyroid sick syndrome because the TSH stimulation response was normal.[139] 1352

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18.3.6.3

MEDULLARY CARCINOMA

Medullary carcinoma (C cell or parafollicular cell tumors) have been reported in horses.[3,144,145] In these cases the youngest horse was 8 years old and the tumors were unilateral. In two horses a complaint of constant gulping was alleviated by surgical removal of the enlarged glands, and the horses returned to athletic function.[3] Two horses had no associated clinical signs.[145,146] A C cell adenoma was reported in a 13-year-old horse with no clinical evidence of endocrine disease.[146] In this case the diagnosis of C cell adenoma was confirmed by immunohistochemistry. In one horse a C cell adenoma associated with multiple endocrine neoplasia was a necropsy finding.[144] Thyroid function tests were not done. C cell hyperplasia has been described in horses as young as 3 years.[138] Electron microscopy or immunohistochemical techniques may be necessary to distinguish C cell tumors from other thyroid neoplasms.

18.3.6.4

MULTIPLE ENDOCRINE NEOPLASIA

Multiple endocrine neoplasia is a human syndrome characterized by multiple neoplasia of glands with a neuroendocrine origin. One case of a 22-year-old Thoroughbred mare with a C cell adenoma, a pheochromocytoma, and a multicentric bilateral nodular hyperplasia of the adrenal medulla was reported recently.[144] A retrospective evaluation of endocrine tumors in horses that underwent necropsy suggested that hyperplasia and neoplasia of the thyroid and adrenal glands, as occur in human beings with multiple endocrine neoplasia syndrome, also occur in the horse.[143,144]

18.3.6.5

TREATMENT

In circumstances that warrant surgical excision, prognosis is best when the condition is unilateral without a concurrent systemic abnormality. Potential complications of the surgery include infection, hemorrhage, laryngeal hemiplegia on the side of the surgery, and scar tissue at the surgical site. The parathyroid glands of the horse generally are not connected to the thyroid glands, and hypocalcemia is not a complication. When hypo- or hypercalcemic states were induced in bilaterally thyroidectomized horses, normocalcemia was not achieved until T_4 was supplemented, suggesting that the absence of calcitonin alone was not responsible for the abnormal calcium homeostasis.[29]

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18.4

18.4—The Adrenal Glands

Ramiro E. Toribio

The adrenal glands in the horse lie retroperitoneally, embedded in the fat on the medial cranial pole of each kidney. They are red-brown, 7 to 8 cm long, 3 to 3.5 cm wide, and 1.2 to 1.5 cm thick and weigh 15 to 20 g each. Blood supply to the adrenal gland is through the adrenal artery, which branches from the aorta or the renal artery.

Innervation involves sympathetic fibers from the splanchnic nerve. In the horse, accessory adrenal cortical tissue may be found in the capsule of adrenal glands, in the periadrenal or perirenal adipose tissue, in the mesorchium, and in the vicinity of the equine testis.

The adrenal gland comprises two different organs that differ in their embryologic origin, type of secretion, and function: the medulla and the adrenal cortex. The medulla is related functionally to the sympathetic nervous system and secretes epinephrine and norepinephrine in response to sympathetic stimulation. The adrenal cortex secretes an entirely different group of hormones: corticosteroids, which are synthesized from cholesterol. The adrenal cortex consists of three zones: the outermost zona glomerulosa, which secretes mineralocorticoids (aldosterone) in response to the renin-angiotensin system and to changes in extracellular fluid osmolality; the zona fasciculata that secretes glucocorticoids (cortisol) in response to adrenocorticotropic hormone (ACTH, corticotrophin) stimulation; and the zona reticularis that secretes androgens.

The adrenal cortex is involved in a variety of body functions, including maintenance of fluid and electrolyte balance, immunity, defense, and energy metabolism. Removal or destruction of the adrenal glands leads to death unless exogenous adrenocortical hormones are administered. Aldosterone increases renal sodium reabsorption and increases renal excretion of potassium. Hyperkalemia and increased activity of the renin-angiotensin system are the most important factors to increase aldosterone secretion. Hypernatremia decreases aldosterone secretion. Cortisol increases gluconeogenesis by stimulating the enzymes required to convert amino acids into glucose in the liver. Cortisol also causes mobilization of amino acids from extrahepatic tissues to enter the gluconeogenesis. In addition, cortisol decreases the rate of glucose use. The increased rate of gluconeogenesis and the decreased rate of glucose use cause blood glucose concentrations to rise. The increase in the glucose concentration from increased adrenal glucocorticoid secretion is occasionally great enough that this condition in human beings is called adrenal diabetes.[1] Cortisol promotes fatty acid mobilization, apparently from decreased transport of glucose into the adipocytes. Cortisol is also important in stress and by increasing fat and amino acid mobilization can make these substances immediately available for energy. In addition, during trauma, endogenous cortisol concentrations are antiinflammatory. Increased concentrations of cortisol result in eosinopenia and lymphopenia.

18.4.1 Adrenal Insufficiency

Adrenal insufficiency—also known as adrenal exhaustion, hypoadrenocorticism, turnout or steroid letdown syndrome—is a poorly documented condition in horses. Horses with adrenal insufficiency often have decreased cortisol concentrations and do not respond to the ACTH stimulation test. Hypoadrenocorticism may occur in critically ill horses (colic, enterocolitis, endotoxemia, sepsis, disseminated intravascular coagulation) because the adrenal gland is a shock organ, and severe adrenal damage from sepsis, hemorrhage, venous thrombosis, and cortical necrosis may lead to adrenal atrophy and dysfunction. Most documented cases of adrenal insufficiency involve discontinuation of chronic administration of glucocorticoids or anabolic steroids[2,3]; however, adrenal insufficiency does not seem to be a common condition in horses that have not been treated with steroids. Wilson, Kingery, and Snow found in Thoroughbreds in training that the adrenal gland response to exogenous ACTH was not altered by training and that baseline ACTH and cortisol concentrations decreased as the fitness of the horses increased.[4] Furthermore, low serum cortisol concentrations were not found in stressed racehorses with a history of poor performance,[5] and high plasma cortisol and ACTH concentrations were found in horses after maximal exercise.[6–8] Dybdal, Gribble, Madigan, et al.[9] measured plasma cortisol concentrations in endurance horses in a 160-km ride and found that midride cortisol concentrations were significantly higher than preride cortisol concentrations. They also found that postride cortisol concentrations were lower than midride concentrations. The ACTH stimulation test was not performed in these horses to determine their

adrenal gland function. Lassourd, Gayrard, Laroute, et al.[10] found that exercise resulted in a sixfold increase in adrenal gland cortisol secretion rate and in a two- to threefold increase in plasma cortisol concentrations.

Horses suspected of having adrenal insufficiency have a history of depression, anorexia, exercise intolerance, weight loss, poor hair coat, and lameness, and therefore one must obtain a complete history, including performance, previous diseases, drug administration, and stressing conditions. Endogenous and exogenous glucocorticoids suppress the hypothalamus-pituitary-adrenal axis, resulting in atrophy of the zona fasciculata of 1357
the adrenal gland from decreased concentrations of ACTH. The zona glomerulosa is affected minimally, 1358
although electrolyte abnormalities may be present in some cases of adrenal insufficiency.

Clinical signs include depression, anorexia, exercise intolerance, weight loss, mild abdominal discomfort, poor hair coat, lameness, and seizures.[2,3,11,12] Serum biochemical analysis may be normal or hyponatremia, hypochloremia, hyperkalemia, and hypoglycemia may be present.[2,3,11]

Although adrenal insufficiency is an uncommon clinical diagnosis in equine practice, one should suspect it in any horse with a history of anorexia, lethargy, poor body condition, poor exercise performance, glucocorticoid administration, electrolyte imbalances (hyponatremia, hypochloremia, hyperkalemia), and hypoglycemia. Because cortisol has daily fluctuations, a single measurement of cortisol concentrations does not provide enough information to make a diagnosis of hypoadrenocorticism. Therefore one must take multiple measurements of cortisol concentrations and more importantly perform an ACTH stimulation test.[11,12] Horses with adrenal insufficiency have decreased cortisol concentrations and do not respond or minimally respond to the ACTH stimulation test.[11,13] Measuring ACTH concentrations may be important to determine other causes of hypoadrenocorticism. Exogenous glucocorticoid administration is expected to result in decreased ACTH concentrations (secondary hypoadrenocorticism), whereas adrenal insufficiency from adrenal gland dysfunction (primary hypoadrenocorticism) results in increased ACTH concentrations from decreased endogenous glucocorticoid concentrations (lack of negative feedback). In horses with increased plasma ACTH concentrations, one must rule out pars intermedia dysfunction (see Chapter 18.2).

In the ACTH stimulation test, one administers 1 IU/kg of natural ACTH gel intramuscularly[13]; takes a pre-ACTH administration sample in a heparinized tube to measure plasma cortisol concentrations and administers ACTH between 8 and 10 am; and takes post-ACTH samples for cortisol concentrations 2 and 4 hours after ACTH administration. Horses with a functional adrenal gland should have a two- to threefold increase in plasma cortisol concentrations compared with baseline concentrations.[3,13] In the cosyntrophin stimulation test, one administers 100 IU (1 mg) of synthetic ACTH intravenously between 8 am and noon.[14] One takes a pre-ACTH sample for plasma cortisol concentrations, administers ACTH, and takes a post-ACTH sample 2 hours later. As in the ACTH stimulation test, plasma cortisol concentrations should be at least twice baseline. Doses of $ACTH_{1\text{-}24}$ as little as 25 IU intravenously may increase cortisol concentrations up to twice baseline.[15]

Necropsy findings of horses with hypoadrenocorticism include adrenocortical hemorrhages and necrosis, as well as adrenal atrophy and fibrosis.

Treatment of hypoadrenocorticism involves rest and glucocorticoid supplementation. The duration and dosage of exogenous steroid treatment that induces adrenocortical atrophy in healthy horses is unknown. Total dexamethasone doses as low as 4 mg may suppress the hypothalamus-pituitary-adrenal axis for 18 to 24 hours.[3] Doses of 0.044 and 0.088 mg/kg of dexamethasone every 5 days for six treatments did not change the response to ACTH stimulation, even though dexamethasone administration reduced cortisol concentrations for up to 4 days. Both doses of dexamethasone resulted in maximal cortisol concentrations suppression; however, this

protocol did not result in adrenal atrophy.[16] Toutain, Brandon, de Pomyers, et al. found that dexamethasone (50 μg/kg intravenously or intramuscularly) suppressed adrenocortical function for up to 4 days; prednisolone sodium succinate (0.6 mg/kg intravenously or intramuscularly), for less than 24 hours; and prednisolone acetate suspension (0.6 mg/kg intramuscularly), for up to 21 days.[17] A single dose of dexamethasone (0.044 mg/kg intramuscularly) or triamcinolone (0.044 mg/kg intramuscularly) suppressed the hypothalamus-pituitary-adrenal axis for 7 and 14 days, respectively.[18] Based on this information, prednisolone sodium succinate (up to 300 mg daily) is the first choice to treat hypoadrenocorticism, low doses of dexamethasone are the second choice, and one must avoid triamcinolone. A case of hypoadrenocorticism following prolonged anabolic steroid administration was treated successfully with 300 mg of prednisone orally every 48 hours for 9 months.[2] With this therapeutic regimen, glucocorticoids were supplied and adrenal gland function was restored. Prednisone (40 mg p.o. b.i.d. or 50 mg intravenously) also was used to treat a foal diagnosed with adrenal insufficiency.[11] The efficacy of prednisone as a therapeutic agent in horses is questionable because prednisone in poorly absorbed from the intestinal tract, and the active metabolite, prednisolone, rarely is produced.[19] In contrast, orally administered prednisolone has excellent bioavailability and may be more useful as a therapeutic agent in horses. As with any long-term glucocorticoid therapy, slow withdrawal is recommended.

Some of the clinical features of equine hypoadrenocorticism (hyponatremia and hyperkalemia without evidence of renal disease) resemble Addison's disease in human beings and small animals; however, Addison's disease has not been documented in the horse.

18.4.2 Pheochromocytoma

Pheochromocytomas are tumors that arise from the chromaffin cells of the adrenal medulla, which are part of the sympathochromaffin system (together with the sympathetic nervous system). During fetal life, the 1358
chromaffin cells are associated with the sympathetic ganglia, and after birth most of them degenerate, and the 1359
few remaining cells constitute the adrenal medulla. In human beings, approximately 90% of pheochromocytomas arise from the adrenal medulla and often are diagnosed associated with a condition known as multiple endocrine neoplasia.[20] Extraadrenal pheochromocytomas have been found in human beings, but not in horses. Pheochomocytomas may be functional or nonfunctional. In the horse these are tumors of low incidence of malignancy and predominantly are unilateral.[21,22] Functional pheochromocytomas secrete catecholamines at a rate sufficient to cause clinical signs.[22] In horses, functional pheochromocytomas are diagnosed with more frequency than nonfunctional pheochromocytomas, probably as a result of the clinical signs,[23] although most of the pheochromocytomas in horses are believed to be nonfunctional and therefore go undiagnosed.[24] Epinephrine and norepinephrine have been identified as the predominant catecholamines produced by equine pheochromocytomas.[25]

Functional pheochromocytomas in horses have no breed or gender predilection[22] and have been described only in horses older than 12 years.[23,26] Because clinical signs exhibit acute onset and rapid progression and result from intense adrenergic stimulation, the clinical signs resemble those of more common conditions such as colic, rhabdomyolysis, acute laminitis, impending enterocolitis, and hyperkalemic periodic paralysis. The most common clinical signs include anxiety, tachycardia, tachypnea, profuse sweating, muscle tremors, and mydriasis (with intact pupillary light reflex).[22] Horses also may show abdominal pain from large hematomas or gastric and intestinal distention caused by ileus,[23,26] hyperthermia, dry and pale mucous membranes, increased capillary refill time, bladder paralysis, and ataxia. Ileus may result from increased intestinal adrenergic stimulation. Noninfectious abortion also has been documented.

Hematologic abnormalities associated with functional pheochromocytomas include hemoconcentration, stress leukogram (mature neutrophilia with lymphopenia),[26] and leukopenia with neutropenia.[23] Hemoconcentration may result from epinephrine-induced splenic contraction rather than from dehydration.[23,24,26] Epinephrine and epinephrine-induced steroid release may be responsible for the stress leukogram; however, in severe cases, tumor rupture with gastrointestinal disease and neutropenia with or without a leftward shift may be present.[23,24]

The serum biochemical profile of horses with a functional pheochromocytoma is nonspecific. Azotemia, metabolic acidosis, hyperkalemia, and hyperglycemia are among the most consistently abnormal laboratory findings.[22,26] Renal and muscular vasoconstriction induced by catecholamines is believed to result in reduced renal blood flow (causing azotemia) and reduced muscle blood flow (causing lactic acidosis and exit of potassium from cells). The mechanisms of hyperglycemia involve direct and indirect actions of the catecholamines, as well as stimulation of glucose production and limitation of glucose use; these effects are mediated through α- and β-adrenergic receptors.[20] A combination of hyponatremia, hyperkalemia, hypocalcemia, and hyperphosphatemia suggests acute renal failure; however, these findings have been reported in horses with pheochromocytoma without evidence of renal lesions at necropsy.[23,24] Hyperkalemia, hypocalcemia, and hyperphosphatemia in part could result from prolonged muscle activity and ischemia, although serum concentrations of creatine kinase were normal or only mildly increased.[22–24,26] Hyperkalemia is unlikely to result from increased catecholamine concentrations because catecholamines induce hypokalemia rather than hyperkalemia by inducing shift of potassium from the extracellular to the intracellular compartment (β-receptor–mediated) and by increasing the activity of the renin-angiotensin system.[27] Glucosuria is also a frequent finding, likely the result of the hyperglycemic action of epinephrine. Pheochromocytomas also may secrete other hormones of neuroendocrine origin (calcitonin, parathyroid hormone, ACTH, corticotropin-releasing hormone, somatostatin, vasoactive intestinal peptide, leu-enkephalin).[23,28–30] Increased concentrations of some of these hormones are expected to be present in horses with functional pheochromocytomas; however, they remain to be determined. Increased concentrations of calcitonin also may be responsible for hypocalcemia in some of these horses.

Functional pheochromocytomas are infrequent in equine practice; however, one may suspect a functional pheochromocytoma in old horses with paroxysmal attacks of anxiety, tachycardia, tachypnea, increased sweating, and muscle tremors, along with azotemia, metabolic acidosis, hyperkalemia, and hyperglycemia.[22,23] Once one rules out other common conditions (colic, impending enterocolitis, acute laminitis, rhabdomyolysis) associated with these clinical signs, one can pursue a specific diagnosis of pheochromocytoma. The clinician and the owner should be aware that this condition carries a poor prognosis, and euthanasia may be a reasonable decision. Measurement of high serum concentrations of catecholamines or their metabolites in urine is valuable in establishing a diagnosis; however, catecholamines are labile substances and not many laboratories are equipped to measure them.[22,23,26,31] Alternatively, ultrasonographic evaluation and measurement of blood pressure can support the diagnosis.

If one reaches a diagnosis, one may attempt adrenalectomy; however, surgery may be difficult because of the 1359
anatomic proximity of the adrenal glands and major blood vessels, tumor size, arrhythmias before surgery, the 1360
risk of inducing fatal arrhythmias (interaction between catecholamines and anesthetic agents),[24] and the complications of prolonged muscle ischemia and recumbency.[22,24,26] Hypotensive α-adrenergic antagonists such as phentolamine, phenoxybenzamine hydrochloride, and prazosin hydrochloride are used in human beings with functional pheochromocytomas before surgery.β-Adrenergic blockers (i.e., propanolol hydrochloride) in general are not recommended before surgery unless an arrhythmia is present. Another alternative is to use the

tyrosine hydroxylase inhibitor α-methyl-L-tyrosine. None of these compounds have been used in the horse to treat functional pheochromocytomas.

Functional pheochromocytomas in horses do not appear to metastasize, are unilateral, tend to bleed, and the adrenal capsule initially may contain the hemorrhage, although at necropsy the tumor frequently is found to be ruptured. Myocardial infarction and degeneration may be present, probably the result of increased concentrations of catecholamines.[22–24]

Nonfunctional pheochromocytomas in general result in clinical signs consistent with abdominal pain.[22,24,26]

Malignant pheochromocytomas have been reported in young and old horses.[32,33] A malignant pheochromocytoma was reported in a 6-month-old filly with a history of hindlimb lameness and spinal cord disease.[33] Both adrenal glands had multiple and well-circumscribed yellow masses, and metastasis were found in the liver, lungs, vertebral canal, left scapula, and azygous vein. A malignant pheochromocytoma also was diagnosed in a 22-year-old mare that died of massive intrauterine hemorrhage.[32] This mare also had a thyroid C cell medullary adenoma and bilateral nodular hyperplasia of the adrenal medulla, findings that were consistent with multiple endocrine neoplasia. This was the first documented case of multiple endocrine neoplasia in the horse, although a previous report was also consistent with multiple endocrine neoplasia.[34]

Other tumors of neuroectodermal origin such as paragangliomas have been reported in horses.[35,36]

18.4.3 Hyperadrenocorticism

Hyperadrenocorticism resulting from pituitary pars intermedia dysfunction (equine Cushing's disease) is discussed elsewhere in this text. Iatrogenic and adrenal hyperadrenocorticism, although far less common than pituitary-dependent hyperadrenocorticism, also has been described in horses.[37–39]

Iatrogenic hyperadrenocorticism was induced in a horse treated for a pruritic skin condition after injection of 12 mg of triamcinolone acetonide followed by two injections of 200 mg of the same compound within 6 weeks.[37] Clinical signs included depression, polyuria and polydipsia, weight loss, and laminitis. Blood and urinalysis abnormalities (neutrophilia, lymphopenia, hyperglycemia, glucosuria) were similar to those of pituitary-dependent hyperadrenocorticism. However, this horse also had increased serum concentrations of γ-glutamyltransferase, aspartate transaminase, and bile acids, suggesting hepatopathy. Steroid-induced hepatopathy has been reported only in iatrogenic hyperadrenocorticism. Diagnosis of iatrogenic hyperadrenocorticism is based on clinical history, abnormally low baseline cortisol concentration, and abnormally low cortisol response to an ACTH-stimulation test. Treatment for this condition consists in discontinuing administration of exogenous steroids. Steroid-induced hepatopathy was reversible in this case.

One case of pituitary-independent (adrenal-dependent) hyperadrenocorticism has been documented in the literature.[39] In this case a 12-year-old gelding had clinical signs consistent with pituitary-dependent hyperadrenocorticism. Baseline cortisol and ACTH concentrations were within the normal range, and the horse had a normal cortisol response to the ACTH-stimulation test. The horse was treated unsuccessfully with bromocriptine, and over the next 11 months the clinical signs worsened. On necropsy the horse had unilateral enlargement of one adrenal gland and the pituitary gland was small. Histologically, significant cortical atrophy was present in the opposite adrenal gland, the pituitary gland and pancreas were normal, and an adrenocortical adenoma was present in the affected gland. The small pituitary gland in this horse probably resulted from a negative feedback from increased glucocorticoid concentrations, and decreased secretion of ACTH from the

pituitary gland may have resulted in adrenocortical atrophy on the opposite adrenal gland. Adrenal-dependent hyperadrenocorticism also may be diagnosed by a negative dexamethasone suppression test and a delayed peak concentration of cortisol (at 12 hours instead of 8) following ACTH gel administration. [38] No treatment for this condition has been tried.

18.4.4 Adrenocortical Neoplasia

Adrenocortical tumors are rare in horses,[15,39–41] and the few cases reported in the literature were nonfunctional tumors. One case of a 12-year-old Dutch Warmblood gelding with a functional adrenocortical adenoma and clinical signs consistent with Cushing's disease (hyperadrenocorticism) was reported recently.[39]

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18.5

18.5—Endocrine Pancreas

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The islets of Langerhans are endocrine functional units of the pancreas. Histologically, four cell types can be identified: the α- or A cells that secrete glucagon, the β- or B cells that secrete insulin, the δ- or D cells that secrete somatostatin, and the PP-cells that secrete pancreatic polypeptide. The β-cells represent approximately 80% of the islet, and the α-cells the remainder. β-Cells are stimulated by glucose and glucagon and are inhibited by somatostatin. Blood glucose and amino acids concentrations are important regulators of glucagon and insulin secretion; however, circulating islet hormones are the dominant means of pancreatic endocrine secretory control. Pancreatic endocrine function also is regulated by sympathetic and parasympathetic fibers that have nonsynaptic release of norepinephrine, epinephrine, and acetylcholine. The sympathetic system is important in inhibiting insulin and stimulating glucagon secretion, whereas the parasympathetic system is important in stimulating insulin secretion during food intake.[1,2]

Insulin is the primary hormone controlling the metabolism and storage of body fuels. Therefore insulin actions involve three major fuels (carbohydrates, proteins, and fats) and three major tissues (liver, skeletal muscle, and adipose tissue). In the liver, insulin decreases glycogenolysis, gluconeogenesis, and ketogenesis and stimulates gluconeogenesis and fatty acid synthesis. In adipose tissue, insulin decreases lipolysis and stimulates fatty acid uptake, synthesis, and esterification. In skeletal muscle, insulin decreases proteolysis and amino acid output and increases amino acid uptake, protein synthesis, and glycogen synthesis.[3]

Insulin resistance is defined as a decreased response to endogenous insulin (hyperinsulinemia associated with normal or elevated glucose concentrations) or exogenous insulin. Insulin resistance results from changes in insulin receptors, postreceptor effects, or both. Because a number of insulin receptors must be occupied to achieve biologic action, if most receptors are nonfunctional, higher insulin concentrations may be required to activate functional or spare receptors. If higher insulin concentrations are required to achieve a biologic effect, then *decreased insulin sensitivity* exists. If a maximal effect is not achieved, then *decreased insulin responsiveness* exists.

Insulin resistance is reported in horses and ponies with pars intermedia dysfunction, granulosa cell tumors, and hyperlipemia. Obese ponies are less sensitive to endogenous and exogenous insulin, and because of this, insensitivity hyperinsulinemia develops. Defective insulin receptors have not been reported in the horse, and postreceptor kinetic studies are lacking. Few studies have been done on glucose transporters in the horse,[4] and at least in human beings, decreased glucose transporters (in particular GLUT-4) are reported in cases of insulin resistance. An increase in muscular activity increases the GLUT-4 protein expression in the skeletal muscle fibers, and good evidence indicates that exercise is beneficial in human patients with insulin resistance because it increases insulin sensitivity and GLUT-4 protein expression and translocation.[5] An increased number of GLUT-4 transporters have been demonstrated in human athletes with increased insulin sensitivity.[6] Moderate-intensity exercise training in horses increased skeletal muscle GLUT-4 protein content.[4]

Glucagon and insulin have opposing effects on the hepatic glucose metabolism. Glucagon increases glucose concentrations by stimulating hepatic glycogenolysis and gluconeogenesis. Glucagon is important in amino acid uptake, degradation, and conversion to glucose. Glucose inhibits glucagon secretion and stimulates insulin secretion.

Catecholamines inhibit glucose-stimulated insulin secretion. In addition, catecholamines raise glucose concentrations by stimulating glycogenolysis and gluconeogenesis, inhibiting insulin-mediated glucose uptake, increasing lipolysis, and stimulating glucagon secretion.

Growth hormone counteracts insulin action on lipid and glucose metabolism.[7] Growth hormone decreases glucose use and storage and promotes protein synthesis and lipolysis. Growth hormone is important in setting the basal glucose concentrations, also known as the glucostat effect.[3] Low growth hormone concentrations have been associated with insulin resistance in human beings; however, little is known on the role of growth hormone on the pathogenesis of insulin resistance in the horse. Growth hormone deficiency in human beings is associated with the metabolic syndrome X, a condition associated with insulin resistance, central obesity, hyperlipidemia, and hypertension. Some of these clinical findings are similar to those of ponies with Cushing's disease and hyperlipemia and with the recently described peripheral cushingoid syndrome in horses (see Chapter 18.2). Patients with metabolic syndrome X have an abnormal peripheral glucocorticoid metabolism, which is associated with increased cortisol concentrations in adipose tissue.[8]

Glucocorticoids stimulate proteolysis, increase glycogen formation, and stimulate gluconeogenesis. Glucocorticoids also increase glycemia by decreasing the skeletal muscle and adipocyte response to insulin-stimulated glucose uptake. The insulin-antagonistic effects of glucocorticoids in general are accompanied by hyperglycemia and hyperinsulinemia.[3] Increased glucocorticoid concentrations may be associated with insulin 1362
resistance; however, information is lacking on the role of glucocorticoids on insulin resistance in the horse. 1363

18.5.1 Diabetes Mellitus

Diabetes mellitus (DM) is defined as persistent hyperglycemia and glucosuria from hypoinsulinemia or insulin resistance. Diabetes mellitus is characterized by changes in the fuel metabolism (carbohydrates, fats, and proteins) and is associated with disturbances in the secretion and sensitivity to hormones such as insulin, glucagon, catecholamines, growth hormone, and glucocorticoids.[3]

In human beings with Cushing's disease and in horses with pituitary pars intermedia dysfunction (PID, equine Cushing's disease), hyperglycemia, glucosuria, hyperinsulinemia, and insulin resistance are common findings.

Diabetes mellitus is a rare condition in horses and most frequently is associated with insulin resistance following PID.[9–13] *Primary DM* is a term used to describe DM resulting from pancreatic β-cell dysfunction, with decreased insulin concentrations (insulin-dependent DM, type I DM). This distinction is important because most cases of equine DM develop as a consequence of Cushing's disease and abnormal pars intermedia pituitary function. Increased concentrations of adrenocorticotropic hormone and cortisol are considered responsible for the development of secondary DM in horses with Cushing's disease, and the role of growth hormone is unknown.

Horses suspected of having DM often have with a history of depression, polyuria, polydipsia, polyphagia, progressive weight loss, and a rough haircoat. These same clinical signs are present in horses with PID.

Hypoinsulinemia (primary DM) is an uncommon cause of DM in horses and results from chronic pancreatitis, often induced by parasite migration (*Strongylus equinus* larvae).[14–17] Because invasion of the pancreas by *S. equinus* larvae occurs around 8 to 10 weeks after infection, one can observe insulin-dependent DM in younger horses.[15] Primary DM resulting from chronic pancreatitis was reported in a 7-year-old pony that had weight loss, depression, polyuria, polydipsia, polyphagia, hyperglycemia, glucosuria, and ketonuria.[16] Treatment with regular insulin failed to bring the glucose concentration to the normal range; however, protamine zinc insulin successfully decreased glucose concentrations and improved clinical signs.

Insulin resistance and the resulting DM may be induced by different hormonal imbalances. Diabetes mellitus was suspected in a mare with bilateral granulosa cell tumors.[18] The exact cause of DM was not determined, and no lesions were found in the pituitary gland and pancreas; however, the mare had abnormal glucose and insulin tolerance tests, suggesting insulin resistance probably resulting from endocrine factors released by the ovarian tumors. Insulin resistance has been demonstrated in another mare with DM, hyperinsulinemia, normal plasma concentration of growth hormone, and no lesions in the pituitary gland or adrenal glands.[19]

Because persistent hyperglycemia and glucosuria defines DM, to reach a correct diagnosis, one must avoid factors in the horse that can result in temporary hyperglycemia such as carbohydrate diets, stress, exercise, glucocorticoid treatment, and sedation with xylazine and detomidine.[20] Ketonemia, ketonuria, and ketone odor have been reported in diabetic ponies and in a horse with insulin resistance.[9,13,14,16,19] Laboratory abnormalities suggestive of cholestasis may be present in insulin-dependent DM when an inflamed pancreas compresses the common bile duct.[15] Persistent hyperglycemia may be present in horses with pheochromocytomas.[21]

Once one has established a clinical diagnosis of DM, one must rule out PID as the cause of hyperglycemia and insulin resistance. Many test protocols are available for the diagnosis of PID; however, the dexamethasone suppression test is considered the test of choice (see Chapter 18.2).

The *glucose tolerance test* is indicated to characterize the type of DM further. The test can be oral or intravenous, and depending on the situation, one may be preferred over the other. The intravenous administration of glucose has less confounding factors (gastrointestinal motility and absorption, fasting) than the oral test.[22] One administers a 50% solution of glucose, 0.5 g/kg, intravenously and determines serum concentrations of glucose and insulin. In a healthy horse, insulin is released immediately and serum glucose concentration returns to baseline value within 3 hours. In the case of insulin-dependent DM, insulin concentration does not rise and the glucose concentration remains elevated. In the case non–insulin-dependent DM (insulin resistance, type II DM), insulin concentrations rise, but the return of glucose concentration to normal is delayed more than 3 hours.

One also can perform the *insulin tolerance test*.[9,13,16,18,23] One administers soluble (regular) insulin at 1 to 8 IU/kg intravenously and determines the plasma concentration of glucose every 15 minutes for 3 hours. Alternatively, one administers 0.05 IU/kg of crystalline insulin intravenously and takes blood samples every 15 minutes for 3 hours. In healthy horses, one can expect a 30% to 50% decrease in glucose concentrations at 15 minutes, 60% at 30 minutes, and normal glucose concentrations at 2 hours. Failure of insulin to lower the concentration of glucose to normal values suggests insulin-resistant DM. When performing the insulin tolerance test, one must have glucose available for rapid intravenous administration because insulin administration may result in hypoglycemic shock.[16,24] 1363

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Measurement of serum insulin concentrations may aid in the diagnosis of DM.[12,18] An elevated concentration suggests insulin resistance, and a reduced concentration suggests insulin-dependent DM.

The treatment of DM depends on the primary cause. Obese ponies with insulin resistance require a decrease in caloric intake and increase in exercise. Treatment of insulin resistance and hyperglycemia following PID is discussed in the section on equine Cushing's disease. If one reaches a diagnosis of primary DM, treatment with insulin is indicated. During treatment with insulin, one must monitor blood glucose concentrations to adjust insulin dosage. In cases of neoplasia (granulosa cell tumor, pheochromocytoma) resulting in hyperglycemia, surgical removal is indicated if possible.

Treatment with insulin (Lente) was attempted in a valuable stallion with pituitary adenoma. Relief of symptoms was achieved initially for 6 weeks, but subsequent worsening of hyperglycemia, polyuria, and polydipsia, along with swelling at the injection sites and development of antibodies against porcine and bovine insulin suggested refractoriness to treatment by exacerbation of the hyperadrenocorticism (insulin resistance) or insulin interference by antibodies.[12] Treatment with regular insulin (8 IU/kg intravenously) failed to bring the glucose concentration to the normal range in a pony with insulin-dependent DM. However, treatment with protamine zinc insulin (8 IU/kg intramuscularly) successfully decreased glucose concentrations. For the next 6 weeks the pony was treated with a daily dose of protamine zinc insulin of 1 IU/kg (0.5 IU/kg intramuscularly b.i.d.), and glucose remained stable (67 to 140 mg/dl). Treatment with this protocol resulted in improvement of body condition and remission of clinical signs. When treatment was discontinued, the clinical signs returned. Insulin administration to this pony often resulted in hypoglycemic shock that responded to intravenous administration of dextrose.[16] A possible complication of insulin administration is insulin-induced hyperglycemia (Somogyi phenomenon), a rebound or paradoxical hyperglycemic result of catecholamine, cortisol, and growth hormone release from acute hypoglycemia.[3,25] The Somogyi phenomenon has been reported in dogs and human beings, but not in horses.

New antihyperglycemic compounds such as biguanides (metformin), sulfonylureas, and thiazolidinediones are indicated to treat insulin-resistant DM but have not been evaluated in the horse.

Exercise may be indicated in affected horses based on information that exercise increases insulin sensitivity and glucose transport across the muscle fiber cell membrane in human beings[5] and that exercise increases skeletal muscle GLUT-4 protein content in horses.[4]

18.5.2 Hyperinsulinemia and Hypoglycemia

Hyperinsulinemia may result in hypoglycemic shock. Hyperinsulinemia with accompanying hypoglycemia may result from therapeutic[16] or fraudulent[24,26] injections of insulin or from increased secretion of insulin by pancreatic tumors.[27]

Depending on the degree of hypoglycemia, the affected horse may show trembling, ataxia, hyperexcitability, tachycardia, tachypnea, mydriasis, nystagmus, profuse sweating, and unawareness of surroundings followed by recumbency, violent seizures, coma, and death. One may suspect injection of insulin in insured horses that suddenly show these clinical signs.[24,26] Hyperinsulinism following an adenoma of pancreatic islet cells was documented in a 12-year-old Shetland pony broodmare.[27] The clinical signs were episodic and consistent with seizures and often were associated with feeding. The pony was hypoglycemic during seizure episodes.

The *glucagon tolerance test* has been used in dogs to diagnose hyperinsulinemia but has not been validated in the horse. Glucagon administration increases glucose and insulin concentrations. The glucagon tolerance test was performed in a pony suspected of having hyperinsulinism from a pancreatic adenoma. Glucagon administration (1500 μg intravenously) to the subject and to a control pony resulted in a rapid increase in glucose concentrations; however, the ponies showed no differences. Insulin concentrations increased in both ponies; however, the subject pony had an exaggerated and sustained insulin response.[27] Major side effects of glucagon administration are cardiovascular effects.

In addition to increased insulin concentrations, hypoglycemia also may result from an abnormal hypothalamus-pituitary-adrenal axis (pituitary destruction, adrenal gland insufficiency). Measurement of insulin and adrenocorticotropic hormone concentrations and the adrenocorticotropic hormone stimulation test to evaluate adrenal gland function may be useful to determine the cause of the hypoglycemia.

During the second half of their pregnancy, mares may develop hyperinsulinemia and have an enhanced β-cell response to exogenous glucose infusion.[28]

One can differentiate between endogenous (pancreatic neoplasia) and exogenous (injected) insulin using high-pressure liquid chromatography.[26]

18.5.3 Neoplasia

Exocrine pancreas tumors (adenocarcinomas) are more frequent than endocrine (islet cell) tumors. Pancreatic
adenocarcinomas reported in the literature resulted in anemia, hyperammonemia, protein-losing enteropathy, 1364
and biliary obstruction.[29–31] No pancreatic endocrine dysfunction has been documented in horses with 1365
pancreatic adenocarcinoma. Likewise, no endocrine dysfunction has been documented in horses with inflammatory bowel disease or lymphosarcoma involving the pancreas.

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18.6
18.6—Magnesium Disorders

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18.6.1
Magnesium

Magnesium is an essential intracellular cation that is required for cellular energy-dependent reactions involving adenosine triphosphate (ATP), including nucleic acid and protein synthesis, ion pump function, glycolysis, and oxidative phosphorylation. Magnesium has an important role in regulating the calcium channel function and therefore neurotransmitter release, neuronal excitation, skeletal muscle contraction, vasomotor tone, and cardiac excitability. Because of the importance of Mg in several physiologic processes, homeostatic mechanisms normally maintain intracellular and extracellular concentrations within narrow limits. Severe Mg deficiency 1365
results in neuromuscular disturbances, but such overt clinical signs rarely are documented in horses. In contrast, 1366
subclinical hypomagnesemia is common in critically ill human beings and animals. Subclinical hypomagnesemia increases the severity of the systemic inflammatory response syndrome, worsens the systemic response to endotoxin, and can lead to ileus, cardiac arrhythmias, refractory hypokalemia, and hypocalcemia.

18.6.1.1
CHEMISTRY

The atomic weight of magnesium is 24.3. Therefore 1 mEq (0.5 mmol) of Mg is equal to 12.156 mg. Magnesium concentration in body fluids may be reported as milliequivalents per liter, milligrams per

deciliter, or millimoles per liter. Values reported as milliequivalents per liter can be converted to milligrams per deciliter by multiplying by 1.2156 and to millimoles per liter by dividing by 0.5. Magnesium concentration in feed material may be reported in grams per kilogram of feed, which can be converted to parts per million or milligrams per kilogram by multiplying by 1000 or which can be expressed as a percentage by dividing by 10. Magnesium for oral use is commonly available as MgO (Magox), which contains 60.25% elemental Mg. One also can feed horses $MgCO_3$ and $MgSO_4$. For intravenous injection, $MgSO_4$ is commercially available as $MgSO_4 \cdot 7H_2O$ in a 50% solution (Abbott Laboratories, North Chicago, Illinois). Although the $MgSO_4$ compound is 20.2% elemental Mg, the $MgSO_4 \cdot 7H_2O$ solution contains only 9.9% elemental Mg. Each milliliter of 50% $MgSO_4 \cdot 7H_2O$ solution contains 99 mg (8 mEq or 4 mmol) of elemental Mg. The 50% solution (500 mg/ml or 4 mEq/ml) is hypertonic, with an osmolarity of 4000 mOsm/L and should be diluted to at least a 10% solution before intravenous administration.

18.6.1.2

DISTRIBUTION OF MAGNESIUM WITHIN THE BODY

Magnesium is the fourth most abundant cation in the mammalian body and the second most common intracellular cation after potassium. Fifty percent to 60% of total body Mg is in bone, of which only one third is readily exchangeable.[1–3] Most Mg in bone is combined in a hydroxyapatite lattice and can be released only during active bone resorption. The remaining 40% to 50% of Mg present in the body is located in soft tissues (skeletal muscle, heart, and liver), with the intracellular concentration proportional to the metabolic activity of the cell. Red blood cells contain approximately 3 times the concentration of Mg in serum. Less than 1% of the total body Mg is contained in the extracellular fluid; therefore serum Mg concentration may not reflect total body Mg stores adequately. Serum Mg is present as protein-bound, chelated, or ionized forms. In human beings, 25% of serum total magnesium (tMg) is bound to albumin and 8% to globulins; 55% is ionized, and the remainder is chelated to divalent anions such as phosphate and sulfate. The protein-bound and chelated Mg is unavailable for biochemical processes and only ionized Mg (Mg^{2+}) has biologic activity. Therefore measuring serum ionized rather than total Mg concentrations is preferable. In the horse 65% to 80% of serum Mg is the ionized form,[4] which is much higher than in human beings. Serum tMg concentration depends on protein concentration, whereas Mg^{2+} concentration depends on acid-base status. Feeding a diet with a low dietary cation-anion balance, which is acidifying, increases the percentage of Mg in the ionized form (unpublished observation).

Within the cell, most Mg is bound to proteins and negatively charged molecules, with 80% to 90% of Mg complexed to ATP. Intracellular ionized Mg makes up only 1% to 5% of the total cellular Mg. The intracellular ionized Mg concentration is similar to the extracellular ionized Mg concentration, providing little concentration gradient, which is in contrast to calcium, for which a 10,000-fold extracellular to intracellular concentration gradient exists. Intracellular ionized magnesium concentration is maintained relatively constant even when the Mg concentration in the extracellular fluid varies to high or low concentrations[5] and is due to limited permeability of the plasma membrane to Mg.

18.6.1.3

PHYSIOLOGIC ROLE OF MAGNESIUM

Magnesium serves as an essential cofactor for more than 300 enzymatic reactions involving ATP, such as replication, transcription and translation of genetic information, and the cellular energy metabolism reactions of glycolysis and oxidative phosphorylation.[2,6] Magnesium is also necessary for membrane stabilization, nerve conduction, ion transportation, and regulation of calcium channel activity.[6] Magnesium is required for

normal function of the sodium-potassium activated adenosinetriphosphatase (Na^+,K^+-ATPase) pump, which maintains the Na^+/K^+ gradient across all membranes and regulates intracellular potassium balance. The Ca^{2+}-ATPase and proton pumps also require Mg as a cofactor. Consequently, Mg plays an important role in excitable tissues. Defective function of ATPase pumps and ion channels may result in interference with the electrochemical gradient, alteration in resting membrane potential, and disturbances in repolarization, resulting in neuromuscular and cardiovascular abnormalities.[6–9] The role of Mg in regulating movement of calcium into the myocyte is pivotal for cardiac contractile strength, peripheral vascular tone,[9] and visceral peristalsis.

18.6.1.4 MAGNESIUM ABSORPTION

Metabolic and hormonal effects on gastrointestinal absorption and renal excretion regulate body stores of 1366
Mg. In human beings, Mg is absorbed uniformly from the small intestine. In horses, 25% of ingested Mg is 1367
absorbed in the proximal half of the small intestine and 35% from the distal small intestine and only 5% from the cecum and large and small colons. The mechanisms of Mg absorption and regulation are not well understood, but serum concentrations can be elevated in response to dietary intake and digestibility. The average absorption of Mg by horses fed a variety of feedstuffs was 49.5% (30% to 60%),[10] which is higher than the range of absorption reported in ruminants (10% to 40%).[11] Meyer found that in clover or meadow hay, Mg was 31% digestible, whereas in rations containing hay and grain Mg was 38% digestible.[12] The type of diet does not appear to affect the site of Mg absorption.[10] Forms of MgO, $MgSO_4$, and $MgCO_3$ for oral use have equivalent digestibilities, with absorption rates higher than from organic sources, being approximately 70% in growing foals.[13]

In contrast to Ca, for which increasing the intake of Ca decreases the digestibility of Ca,[14] an increase in Mg intake increases the digestibility of Mg. When MgO concentration in feed was increased from 1600 to 3100 ppm, the absorption in the horse increased from 53% to 62%, with no further increase in absorption with excessive supplementation at 8600 ppm. Apparently the response of Ca absorption to Mg intake is biphasic, with increased Ca absorption with Mg deficiency or excess.[15] High levels of dietary phosphorus decrease absorption of magnesium.[10]

18.6.1.5 MECHANISMS OF RENAL MAGNESIUM EXCRETION AND REABSORPTION

Free or ionized Mg is filtered by the glomerulus, whereas protein-bound Mg passes directly through the renal efferent arteriole without passing into the glomerular filtrate. In human beings, serum Mg concentration is regulated by tubular reabsorption, and tubular secretion is minimal. Renal excretion of Mg varies directly with dietary changes and thus the serum concentration of Mg presented to the kidney. During Mg depletion, the kidney avidly conserves Mg and virtually no Mg is excreted into the urine.[16] Conversely, when excess Mg is ingested, it is excreted rapidly into the urine because of diminished renal tubular reabsorption. Microtubule nephron puncture studies in several mammalian species indicate that the proximal tubule reabsorbs 5% to 15%, and the thick ascending limb of the loop of Henle absorbs 70% to 80% of the Mg filtered by the glomerulus.[17,18] The distal convoluted tubule only absorbs approximately 10% of the filtered Mg, but this is 70% to 80% of that delivered from the loop of Henle. Because absorption is minimal beyond the distal tubule, this segment is responsible for determining the final urinary Mg excretion.

Reabsorption of Mg in the proximal convoluted tubule is via the paracellular route. The process is passive and is driven by positive luminal voltage and the high concentration gradient of Mg in the glomerular filtrate. In the cortical thick ascending loop of Henle, transport of Mg is also passive, with a permeability of 60%, and is mediated by paracellin-1.[19] The driving force provided by the $Na^+/K^+/^-$ cotransporter creates a transepithelial voltage gradient that maintains a positive luminal voltage that drives divalent cations such as Ca and Mg down their respective concentration gradients.[20]

18.6.1.5.1 Hormonal Regulation of Magnesium

Despite the precise regulation of serum Mg concentration by the kidney, Mg does not have a complex homeostatic endocrine regulating mechanisms.[21] This lack of regulation is in contrast to calcium, which is tightly regulated by parathyroid hormone (PTH), calcitonin, and calcitriol (1,25-dihydroxyvitamin D) (see Chapter 18.1). Parathyroid hormone, vitamin D, calcitonin, arginine vasopressin, glucagons, and calcium concentrations influence Mg absorption and excretion to some degree.

Parathyroid hormone acts on the renal tubules to increase Mg reabsorption.[22] Micropuncture studies have shown that PTH changes the cortical thick ascending limb of the loop of Henle potential difference, which increases the transepithelial voltage gradient and paracellular Mg reabsorption.[16–18,23–25] Other peptide hormones such as calcitonin, arginine vasopressin, and glucagon enhance Mg absorption in the distal convoluted tubule similar to PTH, but the physiologic relevance of these actions is unknown.[25] Magnesium uptake in the renal tubules is concentration and voltage dependent.[23] In the kidney the calcium receptor regulates Ca and Mg resorption independent of PTH. When Ca or Mg interact with the calcium receptor, they inhibit the $Na^+/K^+/2Cl^-$ transporter, which reduces the transepithelial voltage gradient[20] and directly decreases the reabsorption of Ca and Mg.

In other species, vitamin D increases intestinal Mg absorption, but the effect of calcitriol and calbindins on renal handling of Mg is unclear. Vitamin D_3 administration in other species causes increased intestinal absorption of Ca and Mg, resulting in hypercalcemia and hypermagnesemia, which decreases Mg reabsorption in the loop of Henle and distal tubule because of stimulation of the extracellular Ca^{2+}/Mg^{2+}-sensing receptor, increasing urinary excretion of Mg and Ca.[23] In human beings with sepsis, vitamin D synthesis is impaired because of a deficiency of renal 1α-hydroxylase because of the action of calcitriol.[26] The importance of vitamin D in Ca and Mg metabolism in the horse is poorly understood.[27] Horses have low plasma concentrations of vitamin D metabolites (calcitriol, calcidiol) and no detectable 1α-hydroxylase activity in renal cortex homogenates.[28] Vitamin D has little effect on Ca and phosphorus 1367
homeostasis in the horse (see Chapter 18.1), and whether vitamin D has any influence on Mg regulation is 1368
unknown.

18.6.1.5.2 Pathophysiologic Consequences of Hypomagnesemia and Inflammation

Recent information implicates Mg in cell messaging and cytokine production. Hypomagnesemic rats exhibit elevated circulating cytokine concentrations (interleukin-1, interleukin-6, tumor necrosis factor) indicative of a generalized inflammatory state.[29,30] Hypomagnesemic rats are acutely sensitive to the effects of experimentally administered endotoxin, and this vulnerability is correlated with higher plasma tumor necrosis factor concentrations.[30] In a rodent model, progressive Mg deficiency lead to increasing

mortality rates from the effects of endotoxin administration, whereas Mg supplementation reduced the endotoxin-induced mortality.[31] Hypomagnesemia also predisposed animals to free radical–associated injury,[32,33] leading to the formation of cardiomyopathic lesions and altered vascular tone.[34,35] The author and colleagues have found that experimental endotoxin administration in the horse results in an acute decrease in Mg^{2+} concentration (personal communication with Joanne Hardy and Ramiro Toribio, 2002). Not only does endotoxemia appear to induce acute hypomagnesemia, but also Mg administration may have a protective effect in patients with hypomagnesemia and endotoxemia. Human beings with endotoxemia and concurrent hypomagnesemia have a worse outcome compared with normomagnesemic patients.[31] Considering that approximately 40% of horses with colic are endotoxic and that free radical injury is an important mechanism in intestinal ischemia-reperfusion injury,[36] the role of Mg and its therapeutic importance in equine disease warrants investigation.

18.6.2 Hypomagnesemia

18.6.2.1 INCIDENCE OF HYPOMAGNESEMIA IN HUMAN PATIENTS

Hypo- and hypermagnesemia are reported to be the most common and most underdiagnosed electrolyte disorders in adult and pediatric human medicine.[37–39] The prevalence of Mg deficiency has been documented in 61% and 65% of surgically and medically critically ill human patients, respectively.[40,41] Whether one should use assessment of tMg or Mg^{2+} to define serum hypomagnesemia is controversial. In 115 critically ill human patients, 51.3% had serum tMg below reference range, but 71% of these had normal serum Mg^{2+}. None of these patients had mononuclear blood cell or erythrocyte Mg concentrations below the reference limit.[42] In contrast, 60% (40 of 67) of critically ill pediatric patients had low Mg^{2+}, and of these, 60% (24 out of 40) had normal tMg concentrations.[43] Other studies also revealed Mg^{2+} to be a more sensitive indicator of hypomagnesemia than tMg.[44] Measurement of Mg^{2+} generally is recommended over tMg concentration as an assessment of the physiologically active component in the serum.[7,43,45–48]

18.6.2.2 INCIDENCE OF HYPOMAGNESEMIA IN VETERINARY PATIENTS

Until recently, Mg therapy was considered unconventional in veterinary medicine,[49] with serum Mg concentrations being assessed infrequently. In the Ohio State University canine and feline intensive care unit, 28% to 39% of patients were hypomagnesemic.[50] Recently a high prevalence of hypo- and hypermagnesemia was found in feline patients in intensive care, with period prevalence of 28% (16 of 57) and 18% (10 of 57), respectively.[51] A retrospective study found that 48.7% of hospitalized horses had Mg values below the reference range. Hypomagnesemia was associated with gastrointestinal disease, infectious respiratory disease, and multiorgan system disease.[52] In an earlier study, 54% of equine surgical colic patients had low serum Mg^{2+} concentrations, and these horses had a significantly greater prevalence of postoperative ileus than normomagnesemic equine surgical colic patients.[44] Low Mg^{2+} was documented recently in 78% of horses with enterocolitis at the Ohio State University.[27]

18.6.2.3 OUTCOME IN HYPOMAGNESEMIC PATIENTS

Hypomagnesemia is a common observation in the critically ill patient, but whether the condition contributes to mortality or whether it is merely an association in patients with severe disease is unknown.[53] One study of 115 critically ill human patients found a lack of association with clinical outcome, suggesting hypomagnesemia to be an epiphenomenon.[42] In critically ill human pediatric patients, hypomagnesemia and hypocalcemia were predictors of high mortality, with 62% of nonsurvivors having abnormal Mg concentrations.[39] Human beings with endotoxemia and concurrent hypomagnesemia were predisposed to a poorer outcome.[31] In human and canine intensive care unit populations, hypomagnesemic patients had a longer length of hospitalization.[40,54]

In a hospitalized equine population in which 48.7% (401 of 823) were hypomagnesemic, no association with mortality was found, but the length of hospitalization was longer for horses with hypomagnesemia.[52] In a study of equine surgical patients with colic, horses that were euthanatized at the time of surgery (7 of 35) had significantly lower preoperative serum concentrations of Mg^{2+} compared with horses that survived, but serum Mg concentration did not predict hospitalization time or survival.[44] Cats with abnormalities of serum tMg had a longer length of hospitalization compared with normomagnesemic cats (5 verses 4 days: $P = 0.03$) 1368
and a lower survival rate than normomagnesemic cats (54% verses 77%: $P = 0.05$).[51] 1369

18.6.2.4 ASSOCIATION OF HYPOMAGNESEMIA WITH HYPOKALEMIA

Hypomagnesemia frequently is associated with hypokalemia and kaliuresis in human beings and dogs.[37, 40, 54–56] Magnesium deficiency has been associated with loss of cellular potassium stores, and as is the case in hypocalcemic patients, restoring normokalemia until the concurrent Mg deficiency is corrected may be difficult.[57,58] Associations between low serum Mg and K concentrations have been reported in critically ill dogs and human beings.[59] Hypomagnesemia occurred in 36% to 61% of hospitalized human patients with hypokalemia,[37,41,55,60,61] and 31% of dogs with hypomagnesemia concurrently had hypokalemia.[54]

Hypomagnesemia affects the ability of Mg to act as a coenzyme for the Na^+,K^+-ATPase pump. Hypomagnesemia leads to decreased intracellular K and increased intracellular Na concentrations that lower the resting membrane potential, predisposing cells to spontaneous depolarization and impairment of transmission of electric impulses.[62] Hypomagnesemia can result in the blockade of voltage-gated K channels,[63] which interferes with electric repolarization and the propagation of the action potential. Hypomagnesemia also can lead to increased Purkinje fiber excitability, which predisposes to arrhythmia generation.[64]

18.6.2.5 ASSOCIATION OF HYPOMAGNESEMIA WITH HYPOCALCEMIA

Hypocalcemia is a common finding in human patients with hypomagnesemia. Although the mechanism of action is not understood completely, serum Mg may influence serum Ca concentrations.[65,66] Hypocalcemic patients with concurrent hypomagnesemia are often refractory to Ca therapy unless the low serum Mg concentrations are identified and corrected.[67–70] Most human beings with hypocalcemia and concurrent hypomagnesemia have inappropriately low serum PTH concentrations based on their degree of

hypocalcemia, suggesting impairment of PTH synthesis or secretion from the parathyroid gland.[65,69,71–73] Administration of Mg to these patients results in elevation of their serum PTH concentration and normalization of serum Ca concentrations. This response contrasts to normal human beings, in whom administration of Mg results in a decrease in PTH concentrations.[74] Low Mg concentrations increase peripheral organ (renal and skeletal) resistance to PTH,[74–77] with resulting decreases in serum concentration of $1,25(OH)_2D_3$. In a dietary-induced Mg deficiency model in human volunteers, in addition to the induced decrease in serum Mg concentrations were concurrent reductions in serum Ca and $1,25(OH)_2D_3$ concentrations and an inappropriate fall or lack of elevation of PTH in 20 of 25 subjects who had low serum Ca and Mg concentrations. The significant decrease in $1,25(OH)_2D_3$ concentration was suggested to be caused by hypomagnesemic-induced decreases in PTH secretion and renal resistance to PTH.[68] Mild hypocalcemia and hypomagnesemia stimulate PTH release, but severe Mg depletion and acute hypermagnesemia decrease PTH release.[75,78–82] Consequently, parallel determination of Ca and PTH concentrations is important in the investigation of Mg homeostasis.

Magnesium is considered the physiologic calcium blocker of nature because it reduces the release of calcium from and into the sarcoplasmic reticulum and protects the cell against calcium overload under conditions of ischemia.[83] The calcium channel–blocking effect of Mg appears to be decreased in the hypomagnesemic state with a subsequent increase in intracellular Ca concentration leading to enhanced cellular sensitivity to cardiotoxic drugs or ischemic events. In human beings, these physiologic processes support the associations made between hypomagnesemia and various cardiac arrhythmias and organic myocardial diseases.[34,41,63,84–91]

Sepsis-induced hypocalcemia and hypomagnesemia may be associated with intracellular ionic shift, hemodilution, or sequestration. In human beings, inflammation causes elevations in calcitonin and procalcitonin concentrations and may help to explain the hypocalcemia frequently observed in cases of sepsis and trauma.[92,93] Magnesium may function as a Ca antagonist, and low Mg may enhance intracellular entry of Ca in sepsis and endotoxemia.[31] Acute hypomagnesemia following experimental endotoxin administration has been observed in horses (personal communication with Ramiro Toribio and Joanne Hardy, 2002). Whether Mg administration to acutely hypomagnesemic patients is beneficial in reducing mortality or length of hospitalization is undetermined, but to maintain therapeutic serum concentrations within reference range during times of severe illness when homeostatic mechanisms are overwhelmed seems reasonable.

18.6.2.6 AMINOGLYCOSIDE-INDUCED RENAL MAGNESIUM WASTING

Gentamicin administered at standard clinical doses to healthy human subjects results in immediate and transient renal Ca and Mg wasting.[94] Although the mechanism is not defined fully, the phenomenon does not appear to be caused by aminoglycoside tubular injury as previously theorized.[95,96] Magnesium is absorbed primarily in the thick ascending limb of the loop of Henle, but the distal convoluted tubule is the major site 1369
of hormonal regulation of Mg absorption and therefore the final determinant of urinary Mg excretion. 1370
Gentamicin therapy results in Mg loss by its effect on the distal convoluted tubule.[95] Aminoglycosides inhibit PTH-mediated cyclic adenosine monophosphate formation and reduce PTH-stimulated renal tubular cell entry of Mg. The hypothesis is that the positively charged aminoglycosides act via a polyvalent cation–sensing receptor on the extracellular surface of the distal convoluted tubule cells, thereby inhibiting hormone-stimulated renal tubular absorption of Mg.[97] This proposed mechanism helps explain the clinically observed Mg wasting. Gentamicin is a common antimicrobial used to treat gram-negative infections in the

horse, but the effects of gentamicin on renal Mg absorption have not been investigated in the domestic species.

18.6.2.7

MAGNESIUM REQUIREMENTS IN THE HORSE

Obligatory urinary and fecal Mg loss in the horse was estimated at 2.8 mg/kg body mass per day and 1.8 mg/kg body mass per day, respectively, by the use of regression analysis of 72 metabolic balance studies. The horse therefore must absorb a total of 4.6 mg of Mg per kilogram of body mass to replace daily obligatory losses. With an average of 49.5% absorption, the dietary requirement to replace obligatory losses was estimated at 9.3 mg/kg body mass per day. However, the same study determined the regression analysis of intake versus retention to predict Mg balance to occur with ingestion of 13.1 mg Mg per kilogram of body mass per day.[10] The maintenance Mg requirement of 13 mg/kg body mass per day can be provided by a diet containing approximately 0.16% Mg (1600 ppm, 1600 mg/kg, or 1.6 g/kg of feed), which is considered the low-level maintenance requirements for adult horses.[15] Growing, lactating, and exercising animals have a higher requirement of dietary Mg. Mare's milk contains approximately 90 mg/kg of Mg in early lactation and 45 mg/kg in late lactation. Substantial amounts of Mg also can be lost in sweat. During early lactation and for horses undergoing moderate to intense exercise, Mg intake should be increased between 1.5 to 2 times the maintenance level.

Roughage in the form of alfalfa hay had an increased digestibility of Mg (51%) compared with a pelleted complete feed (31%), with a similar Mg content of 1700 ppm, when fed to ponies. In ponies fed alfalfa hay, postprandial plasma concentrations of Mg rose rapidly in the first hour after feeding, peaking after 2 hours at 0.84 mmol/L and returning to 0.75 mmol/L after 12 hours, whereas ponies fed a concentrate pelleted diet had two plasma peaks of 0.67 mmol/L after 2 and 6 hours and then returned to a baseline of 0.6 mmol/L after 12 hours. Therefore the concentration of Mg in the plasma was higher in the group fed roughage and was consistent with the increased Mg absorption from roughage-based feeds. The alfalfa diet resulted in a greater renal excretion of Ca and Mg within 12 hours of feeding (56% and 57% of intake, respectively) than the concentrate diet (19% and 33% of intake, respectively), which reflects increased Mg availability and hence greater excess than with concentrate feeding.[98]

18.6.2.8

EXPERIMENTAL DIETARY MANIPULATION OF MAGNESIUM IN THE HORSE

Hypomagnesemia was induced in mature ponies by feeding Mg at 5 to 6 mg/kg body mass per day (using a 370-ppm diet), whereas 20 mg/kg/day met Mg requirements.[99] A deficiency state is more readily inducible in growing animals because of their higher dietary requirement of Mg. Foals fed a diet extremely deficient in Mg (7 to 8 ppm or 0.0007%) developed severe mineralization of the aorta with severe clinical signs of hypomagnesemia becoming apparent after 90 days in 2 of 11 foals.[100]

Urinary excretion of electrolytes is useful for assessing dietary supply of minerals. The urinary Mg concentration decreased from a baseline of 30 mg/dl to 4 mg/dl after 6 days on a Mg-deficient diet (370 ppm) and increased to more than 300 mg/dl on a high-Mg diet supplemented with 36 g of MgO per day.[99] Increasing the Mg content of a diet from 3100 to 8600 ppm increased Mg digestibility, retention, and excretion in urine and feces and increased serum concentrations from 2.21 mg/dl to 3.39 mg/dl.[15] In foals fed a diet severely deficient in Mg (7 to 8 ppm), serum Mg concentration decreased rapidly from a baseline of 0.78 mmol/L to 0.53 mmol/L after 7 days and then decreased steadily to 0.26 mmol/L after 150 days. The slower rate of reduction in serum Mg concentrations was presumed to be because of mobilization of Mg from

bone. Bone magnesium content decreased in response to Mg depletion, but surprisingly no effect on tissue (brain, liver, kidney, spleen, lung, and cardiac or skeletal muscle) concentrations of Mg, Ca, or P was found after 71 to 180 days.[101]

18.6.2.9 CLINICAL SIGNS AND CONSEQUENCES OF MAGNESIUM DEFICIENCY

Magnesium deficiency may cause muscle weakness, tremors, seizures, cardiac arrhythmias, hypokalemia, and hypocalcemia and may be associated with increased mortality. Hypomagnesemia results from reduced Mg intake (poor nutrition, low Mg content of the diet, or intravenous fluids deficient in Mg), reduced absorption (chronic diarrhea, malabsorption, or bypass/resection of bowel), redistribution (exchange transfusion, third space sequestration), and increased excretion (medication [gentamicin or furosemide], 1370
alcoholism, diabetes mellitus, renal tubular disorders, hypercalcemia [from medication and other causes of 1371
hypercalcemia], hyperthyroidism, aldosteronism, stress, or heavy lactation).

Severe hypomagnesemia can lead to ventricular arrhythmias, supraventricular tachycardia, or atrial fibrillation. Characteristic findings on electrocardiogram include prolongation of the P-R interval, widening of the QRS complex, ST segment depression, and peaked T waves.[102]

18.6.2.10 CLINICAL SIGNS AND CONSEQUENCES OF MAGNESIUM DEFICIENCY IN THE HORSE

Grass tetany, hypomagnesemia associated with grazing lush green winter pastures, is a source of substantial economic loss in lactating beef cattle. However, clinical signs of hypomagnesemia are rarely reported in horses grazing similar pastures. Clinical manifestations of hypomagnesemia include muscle weakness, muscle fasciculations, ventricular arrhythmias, seizures, ataxia, and coma. Hypocalcemic tetany complicated by hypomagnesemia was reported in Welsh mountain ponies.[103,104] Similar signs were induced experimentally after 90 days in 2 of 11 foals fed a diet extremely deficient in Mg (7 to 8 ppm or 0.0007%). The signs of hypomagnesemic tetany were precipitated by loud noises, with foals initially exhibiting nervousness, muscular tremors, and ataxia, followed by collapse, profuse sweating, hyperpnea, and convulsions. One foal died during its third seizure on day 150 of the deficiency trial. Total serum magnesium concentrations decreased from 1.9 ± 0.1 mg/dl to 0.7 ± 0.4 mg/dl. Severe mineralization of the elastic fibers of the aorta and pulmonary artery occurred in all 11 foals fed the Mg-deficient ration for between 30 and 180 days.[100]

Concurrent hypocalcemic and hypomagnesemic tetany was reported in two Thoroughbred broodmares that had been transported for breeding. The mares were nursing foals that were 4 and 7 weeks old. The serum total Ca (tCa) was 4.0 mg/dl and 5.4 mg/dl, whereas the tMg was 1.0 mg/dl and 1.9 mg/dl, respectively. The mares responded to intravenously administered calcium borogluconate and magnesium chloride.[105]

Hypomagnesemia and hypocalcemia are common perioperatively in horses requiring exploratory celiotomy for colic, particularly in horses with strangulating intestinal lesions and ileus. In 35 equine surgical colic cases, preoperative serum tMg and Mg^{2+} concentrations were less than the reference range in 17% (6 of 35) and 54% (19 of 35), respectively. Serum concentrations of tCa and Ca^{2+} were below reference range in 57% (20 of 35) and 86% (30 of 35) of horses before surgery. Serum concentrations of tMg and tCa were less sensitive than Mg^{2+} and Ca^{2+} in detecting horses with hypomagnesemia and hypocalcemia. Significantly lower serum concentrations of Mg^{2+} occurred in horses that developed postoperative ileus. Serum

concentrations of Mg and Ca (total and ionized) correlated significantly with the P-R, QRS, QT, and QT corrected for heart rate (QTc) intervals. Horses that were euthanized at the time of surgery ($n = 7$) had significantly lower preoperative serum concentrations of Mg^{2+} compared with horses that survived, but neither serum Mg nor Ca concentrations were predictors of hospitalization time or survival. Horses with strangulating lesions were more likely to be hypomagnesemic and hypocalcemic and also had significantly shorter PR, QRS and QTc intervals compared with horses with nonstrangulating lesions. Horses with strangulating lesions were more likely to be hypomagnesemic and hypocalcemic and have electrocardiogram changes than horses with nonstrangulating lesions.[44] Multiple factors probably contribute to the observed electrocardiogram disturbances, but the routine detection and correction of the electrolyte abnormalities (including Mg^{2+} and Ca^{2+}) are recommended.

Serum Mg^{2+} is significantly lower in horses with enterocolitis and hypocalcemia compared with healthy horses. Most horses (48 of 64; 75%) with enterocolitis had decreased serum tCa, Ca^{2+}, and Mg^{2+} concentrations and increased phosphorus concentrations. Differential diagnoses for the mechanism of the enterocolitis-induced hypomagnesemia include sequestration of Mg within cells, in the peritoneal fluid, or in the lumen of gastrointestinal tract (increased gastrointestinal secretion or impaired absorption from inflamed bowel) or decreased renal reabsorption of Mg resulting in excessive urinary losses. Fractional clearances of Mg were not calculated in this group of horses. Fractional clearances of Ca were appropriately low in hypocalcemic enterocolitis patients compared with healthy horses, and thus urinary loss of Ca could not explain the observed hypocalcemia. In this group of horses, serum PTH varied. Of the horses with hypocalcemic/hypomagnesemic enterocolitis, 20% (10 of 51) had high PTH, which may be explained by hypomagnesemia-induced end organ resistance to PTH. Some horses (15 of 51; 29%) had inappropriately low serum PTH concentrations in the face of hypocalcemia. One explanation involves hypomagnesemia-induced intracellular accumulation of Ca and reduced cyclic adenosine monophosphate production by the parathyroid gland, resulting in low PTH synthesis and release.[27] The exact mechanisms of the interdependence of Ca and Mg on PTH secretion are unknown.

18.6.2.11 MAGNESIUM AND BRAIN INJURY

Based on evidence from human medicine, Mg infusions recently have gained popularity in the empirical treatment of hypoxic ischemic encephalopathy in neonatal foals.[106] Although such therapy appears safe, results of clinical trials in foals have not yet been published. 1371

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Perinatal brain damage may result from asphyxia after reduction of uterine or umbilical blood flow.[107] The resultant cerebral hypoxia decreases oxidative phosphorylation and energy production, resulting in failure to maintain ionic gradients across cell membranes. With the loss of membrane potential, calcium influxes into neuronal cells down its large extra- and intracellular gradient through voltage-gated ion channels. Hypoxic conditions also lead to the release of glutamate sequestered within neurons. The excitatory neurotransmitter glutamate is itself neurotoxic but also allows further influx of calcium into neurons by glutamate-regulated ion channels. Calcium overload results in neuronal cell death by damage resulting from activation of calcium-dependent proteases, endonucleases, and lipases.

Apoptosis (programmed cell death) is thought to occur because of the neurotoxicity of accumulated glutamate and aspartate and the associated increase in intracellular calcium concentrations. Traumatic brain injury induces the activation of the *N*-methyl-d-aspartate (NMDA) subtype of the glutamate receptor[108] and has been implicated in the pathophysiology of hypoxic ischemic encephalopathy.[109] Magnesium is important

in the voltage-dependent blockade of NMDA channels. In the normal resting state, without glutamate-receptor interaction, Mg blocks the NMDA calcium channel, preventing calcium entry into the cell and decreasing neurotransmitter release. Magnesium also blocks the entry of calcium through the voltage-gated calcium channels in the presynaptic membrane.[110] Normal blood-brain vessel vasodilation also depends on Mg. Magnesium can block calcium channels in vascular smooth muscle, thereby decreasing vascular smooth muscle contraction, resulting in vasodilation.[111]

In brain injury, increased glutamate receptor interaction results in phospholipase C activation. A subsequent release of phosphate groups occurs after the cleavage of phospholipid glycerophosphate that binds free Mg. These magnesium-phosphate complexes are removed from the site of injury, resulting in the tissue loss of Mg. Lower Mg concentrations increase vascular smooth muscle tone, potentiating vasospasm with reduction of oxygen and substrate delivery to tissues. Focal traumatic brain and spinal cord injury in rats can reduce free brain Mg concentrations by as much as 60%, with the reduction proportional to the extent of the injury.[110] Therefore brain injury reduces brain Mg concentration and results in the loss of the protective role of Mg and potentiation of further brain injury.

As the excitotoxic cascade occurs over a period of several days, a window for therapeutic intervention exists to prevent secondary brain injury.[112] The reduction in the voltage-dependant Mg^{2+} blockage of NMDA current in mechanically injured neurons can be restored by increasing extracellular Mg concentration. Magnesium sulfate has been shown greatly to improve the immediate recovery of rats from hypoxia[113] and to improve the motor outcome in rats treated after severe traumatic axonal brain injury.[114] Magnesium sulfate also has been shown to protect the fetal brain during severe maternal hypoxia.[115] The available experimental literature and reasoning suggests that in most cases Mg therapy may be advantageous in protecting against and in treating hypoxic ischemic encephalopathy in foals, but further evidence still is required before the benefits, if any, can be proved.

18.6.2.12 DIAGNOSTIC TESTING

The clinical laboratory evaluation of Mg status is limited primarily to measurement of serum Mg concentration, 24-hour urinary excretion, and percent retention following parenteral Mg loading. However, results for these tests do not necessarily correlate with intracellular ionized concentrations. No universally accepted, validated, and readily available test exists to determine intracellular and total body magnesium status.

The diagnosis of Mg deficiency can be a challenge. Only 1% of body stores are located in serum as protein-bound, chelated, or ionized forms. The lack of representative value of serum Mg concentrations, the influence of other constituents on serum Mg concentration, and the slow equilibrium of Mg between tissue pools limits the value of serum Mg determination as an index of total body Mg content.[1,116] Determination of tMg content in blood monocytes or erythrocytes also has been shown to be unreliable for estimation of total body status.[1,116–118] The protein-bound and chelated Mg are unavailable for biochemical processes, and only Mg^{2+} has biologic activity. Because the Mg^{2+} concentration represents the functional pool of serum Mg, determination of Mg^{2+} may provide a better assessment of physiologic Mg status as opposed to measuring tMg.[7,43,45–48]

Even though Mg^{2+} concentrations may not reflect total body stores, an association exists between changes in Mg^{2+} concentrations and acute disease processes.[1,27,44,54] The recent advent of a commercially available ion-selective electrode for determining Mg^{2+} concentrations has allowed quantification of Mg^{2+}, and this test is performed routinely in hospitalized critical equine patients in the author's hospital. Although not specifically proven in the horse, in human beings, one cannot predict Mg^{2+} concentration from concentrations of tMg, albumin, Ca^{2+}, or pH.[43] Hypoalbuminemia results in a low measured serum tMg concentration (pseudohypomagnesemia) and does not require Mg supplementation if the serum Mg^{2+} concentration is normal. One must recognize this association, but formulae to correct Mg concentration based 1372
on adjustment for protein concentrations are not accurate and should not be used (Dennis Chew, personal 1373
communication, 2002). If possible, one should measure Mg^{2+} concentration whenever one finds the total Mg concentration to be abnormal. Similar to calcium, Mg binds to anionic (negatively charged) protein binding sites, with the binding affinity dependent on pH. During acidosis, the increased hydrogen ion concentration displaces Ca^{2+} and Mg^{2+} from their protein binding sites, increasing the percent of these cations in their ionized form, resulting in increased serum Ca^{2+} and Mg^{2+} concentrations. In animals with respiratory or metabolic alkalosis (often observed after prolonged strenuous endurance exercise), Ca^{2+} and Mg^{2+} concentrations may be low because of increased protein binding. Because Mg^{2+} is the physiologically active component, ionized hypomagnesemia supplementation is recommended, especially if one observes clinical signs of synchronous diaphragmatic flutter (thumps), ileus, or (rarely) muscle fasciculations, ataxia, or tetany. Although not likely to be of consequence in an animal with adequate renal function, resolution of the alkalosis may result in elevations of the serum Mg^{2+} concentration. In contrast, animals with metabolic acidosis following sepsis, systemic inflammatory response syndrome, and severe gastrointestinal disease rarely have serum ionized hypermagnesemia, rather their serum Mg^{2+} concentration tends to be low from altered Mg homeostasis, cellular or third space redistribution, gastrointestinal loss of Mg, or diuresis following aggressive fluid therapy with intravenous fluids unsupplemented with Mg.

One may use renal excretion of Mg to evaluate Mg balance. When Mg intake is deficient, urinary Mg excretion falls to negligible levels, whereas serum Mg remains within the normal range.[16,119] Renal Mg excretion is measured in urine collected over 24 hours and expressed as total amount excreted (milligrams per kilogram per day). Renal clearance, also measured by 24-hour urine collection, estimates the amount of blood cleared of Mg daily and uses concurrent serum and urine Mg concentrations for calculations. One can determine renal electrolyte clearance, without quantitative urine collection, by expressing the renal electrolyte clearance relative to the endogenous creatinine clearance, which clinically is referred to as fractional clearance. Fractional clearance relates the amount of substance excreted to the amount filtered by the glomerulus. Reference values for the fractional clearance of Na, K, Cl, and P have been reported in horses.[120–123] The use of spot urine and serum collection as estimates of 24-hour clearance has been validated in horses for Na, K, Cl and P.[122] Spot sample fractional clearance of Mg has been determined in normo- and hypomagnesemic human beings and was found to be useful in the diagnosis of hypomagnesemia.[124,125] Currently, little information has been published on renal handling of Mg in horses. The reference range for the fractional clearance of Mg in normal horses fed grass hay was found to be 29% ± 8%. Fractional clearance of less than 6% would indicate inadequate dietary intake.[4]

Tissue Mg content has been used as an estimate of total body stores. In one study in horses, muscle was found to contain 1.29 ± 0.09 mg/g dry matter of Mg, accounting for 32.3% of the body Mg, whereas the skeleton accounted for 62.5%.[126] Skeletal muscle is therefore important in the assessment of Mg status. The

skeleton buffers the majority of acute Mg loss, and in periods of chronic Mg deficiency, skeletal Mg depletion may be significant.[127]

Investigation of the role of hypomagnesemia in human patients with cardiac disease found that cardiac muscle biopsy Mg concentrations correlated well with Mg status.[128] In human patients undergoing surgery for acute myocardial infarction, a good correlation between intracellular Mg concentration within cardiac cells and that in sublingual epithelial cells was observed. These patients had significantly lower sublingual intracellular Mg concentrations than healthy control subjects and acutely ill patients without myocardial ischemia. Intravenous administration of $MgSO_4$ raised sublingual intracellular Mg concentrations within 24 hours. Intracellular Mg was measured using energy-dispersive x-ray microanalysis using a specially configured scanning electron microscope to irradiate cells with a focused electron beam. Sublingual cells were chosen because they are noncornified and aerobic, have a turnover time of less than 3 days, are readily accessible, and therefore provide a noninvasive measurement of intracellular Mg stores.[129]

Estimation of total body Mg status has been evaluated recently in human beings using an intravenous Mg retention test, which now provides the gold standard for identification of Mg deficiency in human beings.[130,131] One calculates the percentage of Mg retained after an intravenous load after measuring the amount of Mg excreted in urine during a 24-hour period. Magnesium sulfate solution ($MgSO_4 \cdot 7H_2O$) is used commonly and contains 10% elemental Mg. For example, one can provide a dose of 10 mg/kg of elemental Mg using 100 mg/kg of $MgSO_4$ solution. Magnesium sulfate is commercially available as a 50% solution, which should be diluted to 10% before administration. Percent retention (%Ret) is calculated as follows[132]:

$$\%\text{Ret} = \frac{1-(\text{Mg excretion in 24 hours})}{\text{Mg infused}} \times 100$$

In human beings, retention of 40% to 50% of the administered Mg load indicates hypomagnesemia, whereas retention of less than 20% indicates that Mg deficiency is less likely.[133] In one clinical study, 1373
normomagnesemic human beings retained a mean of 14%, whereas patients suspected of having Mg 1374
deficiency retained up to 85%.[134] Development of a Mg retention test for diagnosis of hypomagnesemic states in horses has been investigated using a reduced Mg diet. The Mg retention test is labor intensive. Spot sample fractional clearance was determined to reflect 6- and 24-hour Mg excretions adequately and was more sensitive in detecting reduced Mg intake than the Mg loading test. In states of extreme and prolonged dietary Mg deficiency, the Mg loading test may provide additional information when urinary excretions have dropped to negligible amounts, but such situations are not considered likely to occur clinically. Reference ranges for urinary Mg excretion, clearance, and fractional clearance in normal horses fed grass hay containing 0.24% Mg, were 9.5 ± 4.8 mg/kg/day, 0.43 ± 0.18 ml/kg/min, and 29% ± 8%, respectively. In horses fed a reduced Mg diet of 0.06% Mg for 4 weeks, the urinary Mg excretion, clearance, and fractional clearance were 2.0 ± 1.3 mg/kg/day, 0.09 ± 0.05 ml/kg/min, and 6% ± 3%, respectively. The conditions investigated in this study were not severe enough to induce serum hypomagnesemia but indicate that one can use urinary indexes to reflect Mg intake and determine the necessity of dietary supplementation.[4]

18.6.2.13 TREATMENT FOR HYPOMAGNESEMIA

When supplementing Mg, one carefully must determine whether the dose reported is for elemental Mg or for the compound $MgSO_4$. For example, a dose of 100 mg/kg of $MgSO_4$ solution provides 9.7 mg/kg of elemental Mg. Confusion and subsequent overdose may be fatal.

Recommended dose rates for $MgSO_4$ in adult horses are 25 to 150 mg/kg/day (0.05 to 0.3 ml/kg of a 50% solution) diluted to a 5% solution in normal saline, dextrose, or a polyionic isotonic solution and given by slow intravenous infusion. A constant rate infusion of 150 mg/kg/day intravenously of $MgSO_4$ solution (0.3 ml/kg/day of the 50% solution) would provide the daily requirements of the horse.[135]

Of the commonly available replacement crystalloid fluids for intravenous administration, only Plasmalyte-A (Baxter Healthcare, Deerfield, Ill.) and Normosol-R (Abbott Laboratories, North Chicago, Ill.) contain any Mg. These fluids contain 3 mEq/L (3.6 mg/dl) of elemental Mg. If a horse received 60 ml/kg/day of the replacement fluid, it would receive 2.16 mg/kg/day of elemental Mg, which is equivalent to the amount of Mg from 20 mg/kg of $MgSO_4$. A horse receiving 120 ml/kg/day would receive the same amount of Mg as if it had been given 40mg/kg of $MgSO_4$. Therefore if long-term fluid therapy is required to support an inappetent animal, the horse still may require additional Mg. Based on the available knowledge, one should make an effort to determine and correct serum Mg^{2+} concentrations as is performed routinely for other electrolytes.

Magnesium sulfate also is used to treat refractory ventricular arrhythmias including those caused by idiosyncratic quinidine reactions (especially torsades de pointes) in hypomagnesemic and normomagnesemic horses.

Emergency therapy for ventricular arrhythmias under anesthesia or in response to quinidine-induced ventricular arrhythmias is a dose of 2 to 6 mg/kg/min of $MgSO_4$ intravenously (1.8 to 5.4 ml of 50% $MgSO_4$ per 450-kg horse per minute) to effect. Some authors recommend a maximum dose of 25g (56 mg/kg) of $MgSO_4$,[102,136] but the author's studies in normal horses indicate that at least 100 mg/kg of $MgSO_4$ can be administered safely over 30 minutes, with mild sedation occasionally being noted. Therefore one could exceed the 25 g total if necessary at a reduced infusion rate.

Studies in a canine model of cardiopulmonary arrest found that 100 mg/kg of $MgSO_4$ over 5 to 15 minutes raised the ventricular fibrillation threshold and prevented cerebral hypoperfusion following resuscitation without any adverse effects.[137,138]

Magnesium sulfate also has been recommended as a muscle relaxant as an adjunct treatment for tetanus.

For the treatment of hypoxic ischemic encephalopathy in neonatal foals, Wilkins[106] suggests a constant rate infusion of $MgSO_4$ at an initial dose of 50 mg/kg/hr intravenously for 1 hour, followed by 25 mg/kg/hr constant rate infusion for 24 hours. This dose provides 600 mg/kg/day of $MgSO_4$ and is therefore much higher than that required for maintenance. Therapy has been continued for up to 3 days without visible detrimental effects other than possible trembling.

Magnesium sulfate can be infused with a high therapeutic safety index, with the safety depending on the dose and the infusion rate, but is contraindicated with undiagnosed disturbances in cardiac conduction, renal failure, or elevated serum Mg concentration. In human beings with moderate hypomagnesemia (serum tMg <1.2 mg/dl), the recommendation is that 6 g of $MgSO_4$ solution (86 mg/kg; dose divided for average 70-kg person) be infused over 3 hours in 500 ml of isotonic saline, followed by 5 g over the next 6 hours, and then 5 g every 12 hours by continuous infusion for the next 5 days. In cases of hypomagnesemia associated with life-threatening arrhythmia, one can infuse 2 g of $MgSO_4$ (29 mg/kg) over 2 minutes in 500 ml of isotonic

saline, followed by the second two steps of the protocol for moderate hypomagnesemia.[139] Similar doses have been extrapolated for dogs.[140]

The typical equine diet contains sufficient Mg for maintenance, with supplementation rarely required. If necessary, one can provide Mg orally MgO, $MgCO_3$, or $MgSO_4$, which have equivalent digestibilities of 1374
approximately 70%. One could provide the maintenance requirement of 13 mg/kg/day of elemental Mg by 31 1375
mg/kg/day of MgO, 64 mg/kg/day of $MgCO_3$, or 93 mg/kg/day of $MgSO_4$. This may be important when one formulates oral replacement fluids for inappetent horses.

Magnesium sulfate (Epsom salts) commonly is used as an osmotic cathartic to treat large colon impactions. One can administer a dose of 0.5 to 1.0 g/kg of $MgSO_4$ in 6 to 8 L of water by stomach tube when the horse is metabolically stable. One can administer a second dose 24 to 36 hours later in severe cases only if serum Mg concentrations have returned to normal. Diarrhea may result with clearance of the impaction after the aggressive oral and intravenous fluid therapy often required to resolve large colon impactions. Hypermagnesemic neuromuscular paralysis has been reported after administration of 1.5 to 2 g/kg of $MgSO_4$. Freeman, Ferrante, and Palmer did not observe clinical signs of hypermagnesemia or diarrhea on adminstration of 1.0 g/kg of $MgSO_4$ orally to normal horses.[141]

18.6.3 Hypermagnesemia

Hypermagnesemia is rare in all species and most commonly results from iatrogenic Mg overdose or excessive supplementation to a patient in renal failure. Serum hypermagesemia also occurs after severe rhabdomyolysis or in a patient suffering from systemic inflammatory response syndrome in which cellular breakdown releases Mg into the extracellular fluid. Hyperkalemia and hyperphosphatemia also may occur in cases of severe cellular damage.

The cardiac effects of excessive Mg doses were examined in a canine model in which infusion of 0.12 mEq/kg/min of elemental Mg (15 mg/kg/min of $MgSO_4$ solution) was given safely for 16 minutes. After 16 minutes of infusion a total of 233 mg/kg of $MgSO_4$ solution had elevated the serum tMg concentration to 12.2 mEq/L (14.8 mg/dl) without changing hemodynamic parameters. With continued infusion the heart rate accelerated. Extreme doses of intravenous $MgSO_4$ can be toxic. Dogs given a cumulative infusion dosage of 2.0 mEq/kg of elemental Mg (243 mg/kg of $MgSO_4$ solution) became hypotensive, and dangerous arrhythmias were provoked at a total dosage of 3.9 mEq/kg of elemental Mg (474 mg/kg of $MgSO_4$ solution). A cumulative dose of between 5.9 mEq/kg and 10.9 mEq/kg of Mg, when infused at 0.12 mEq/kg/min, caused ventricular fibrillation or cardiac arrest.[142]

Hypermagnesemia was reported in two horses given excessive Epsom salts ($MgSO_4$) in addition to dioctyl sodium sulfosuccinate for the treatment of large colon impaction. The 450-kg and 500-kg horses were given 750 g and 1000 g of $MgSO_4$, respectively. Four to 6 hours after the $MgSO_4$ overdose the horses showed signs of agitation, sweating, and muscle tremors followed by recumbency and flaccid paralysis. Tachycardia and tachypnea developed, peripheral pulses were undetectable, and capillary refill time was prolonged at 4 seconds. Serum tMg concentrations rose to 5 times the reported reference range. The horses were treated with 250 ml of a 23% solution of calcium gluconate (diluted in 1 L of 0.9% NaCl) administered slowly intravenously. One horse was able to stand 10 minutes after the completion of the infusion. Intravenous fluids were given to induce diuresis. A second calcium infusion was required when muscle tremors reoccurred 1 hour later in this horse. The second horse remained weak for several hours, being only able to stand for short periods. These two horses

were given $MgSO_4$ at 1.5 to 2 times the recommended maximum dose, but this dose of Epsom salts alone normally would not likely be able to induce such severe clinical signs. The authors suggested that the concurrently administered dioctyl sodium sulfosuccinate may have increased intestinal permeability and increased the Mg absorption, with exacerbation of the signs of hypermagnesemia caused by the concurrent low serum calcium concentration. The most severely affected horse also showed signs of endotoxemia, which may have contributed to the observed hypotension and delayed recovery. One should give Epsom salts only to treat large colon impactions after correction of dehydration and metabolic imbalances. One should avoid simultaneous administration of excessive doses of Epsom salts with dioctyl sodium sulfosuccinate.[143]

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19 CHAPTER 19 DISORDERS OF FOALS

Pamela A. Wilkins

19.1 Neonatal and Perinatal Diseases

Before the 1980s, intensive management of the compromised neonate was unusual and little was known regarding many of the problems of this special patient population. Although some specific conditions had been described by astute clinician-researchers, most notably the "dummy" foal syndrome[1] and respiratory distress syndrome caused by primary surfactant deficiency,[2] little information regarding the diagnosis and management of conditions of the foal during the neonatal period was available, although at least one active group was investigating fetal and neonatal physiology of the horse in Great Britain.[1–14] When treatment of compromised foals was undertaken, the approach most commonly resembled treating them as small adults with little understanding of the different physiology of the equine neonate. The advent of improved management of reproductive efficiency of mares led naturally to increased interest in preservation of the conceptus to parturition and the foal thereafter. Interested clinicians, taking their lessons from the field of human perinatology/neonatology and sometimes working hand-in-hand with their counterparts in the human field, pioneered investigations into these small patients and created the fields of equine perinatology and equine neonatal intensive care.[1–19] Because of the foresight and energy of these early investigators, the field of veterinary perinatology/neonatology exploded in the 1980s, leading to the creation of equine neonatal intensive care units throughout the United Sates and the world. From these units information about the normal and abnormal physiology of foals, the medical conditions affecting them, and methods for treatment and management of these problems has been developed through observational, retrospective, and prospective studies. This veritable explosion of information over the last 20 years has improved greatly the ability of all practitioners to provide appropriate care for these patients, whether in the field or at an equine neonatal intensive care unit. The ability not only to save the lives of these patients but also to treat them in such a manner as to allow them to fulfill their purposes, whether as pleasure animals or racing athletes, has improved almost exponentially from those early days.[20–23] This chapter aims to provide the clinician with some of the most current information regarding the management of these patients, recognizing that much still remains unknown and that advances will continue to be made in this dynamic field. The reader is cautioned that much of this chapter is flavored by the experiences of the author and that variation in approach and treatment of specific problems exists between neonatal intensive care units (NICUs) and between clinicians in the same NICU and that each year results in change. In some cases, information that is presented has been gleaned from human NICU studies, essentially using the critically ill infant as the experimental model.

Many of the problems of the newborn foal have their genesis in utero. Identification of high-risk pregnancies is an important component of prenatal care of the foal, and some of the most commonly encountered problems of the dam resulting in abnormal foals include previous or concurrent disease, poor reproductive history, poor perineal or pelvic conformation, poor general health, poor nutritional condition, prolonged transport, history of previous abnormal foals, placental abnormalities, and twins.[24]

Some of the more common causes of abortion can result in the birth of severely compromised foals of variable gestation lengths (Box 19-1). These include infectious causes such as equine herpesvirus (EHV) types 1 (most commonly) and 4 (rarely), equine infectious anemia, equine arteritis virus, bacterial and fungal placentitis, leptospirosis, equine ehrlichiosis, and gram-negative septicemia/endotoxemia.[25–27] Noninfectious causes of

abortion include twinning and noninfectious placental abnormalities such as extensive endometrial fibrosis, body pregnancy, and abnormal length (long or short) of the umbilical cord.[24,28]

19.1.1 BOX 19-1 CONDITIONS ASSOCIATED WITH HIGH-RISK PREGNANCY

19.1.1.1 Maternal Conditions

Colic/endotoxemia

Abdominal hernia

Pelvic anatomic abnormalities

Malnutrition

Any debilitating disease

Uterine torsion

Uterine abnormalities

Mare reproductive loss syndrome

Hyperlipemia

Hypogalactia

History of previous abnormal foals

19.1.1.2 Conceptional Conditions

Placentitis

Twins

Hydrops

Prolonged gestation

Fetal abnormalities

Dystocia

Fescue toxicosis

Umbilical abnormalities

Congenital deformity

To the equine neonatologist opportunities for intervention may appear limited, and in the case of many of the aforementioned causes of fetal loss, this is true. However, one can do much in an attempt to preserve the

pregnancy and in effect treat the fetus. When one is faced with a threatened pregnancy, one has various ways of evaluating the fetus and its environment and may use many potential therapies.

19.2 Prepartum Evaluation Of The Fetus And Placenta

Once one identifies a pregnancy as high risk, one should evaluate the fetus for viability. Evaluation should include as thorough an evaluation as possible of the reproductive tract, placenta, and fetal fluids. Prepartum disorders in the mare usually are readily recognizable, but disorders of the fetus and placenta can be more subtle and difficult to determine. The first step is to take a thorough history of the mare. Of particular interest is any history of previous abnormal foals, but the history taking should include questions regarding transportation; establishment of an accurate breeding date (sometimes more difficult than one would suspect); any pertinent medical history including any diagnostic testing performed for this pregnancy such as culture, endometrial biopsy, and cytologic results; and any rectal and ultrasound examination results. Additionally, one should obtain information regarding possible ingestion of endophyte-infected fescue or exposure to potential infectious causes of abortion.[29,30] A complete vaccination and deworming history is requisite, as is a complete history of any medications and supplements administered during pregnancy.

After obtaining a history, one examines the mare per rectum. This examination should include palpation of the cervix, uterus, fetus, and all palpable abdominal contents. One should note any abnormalities. The cervix should be tight throughout gestation; the late gestation uterus will be large and distended with fluid and usually pulled craniad in the abdomen. Palpation of the fetus frequently results in some fetal movement; however, one should interpret lack of movement with caution, for some normal fetuses do not respond. Ultrasonographic evaluation of the uterus and conceptus per rectum can provide valuable information, particularly regarding placental thickness if placentitis is a concern. One may evaluate fetal fluids and estimate fetal size from the size of the eye later in gestation.[31] In the author's hospital the practitioners choose not to perform vaginal examinations or speculum examinations because of an association between these examinations and the subsequent development of placentitis. Unless placentitis is recognized with ultrasonograhic evaluation per rectum and culture is desirable, these types of examinations are generally not necessary.

Following examination per rectum, one performs transabdominal ultrasonographic evaluation of the uterus and conceptus.[28] One can generate a biophysical profile of the fetus from this examination in the late-term fetus and readily determine viability.[32,33] One also readily can determine the presence or absence of twins in the late pregnant mare in this manner. One performs the sonogram through the acoustic window from the udder to the xiphoid ventrally and laterally to the skinfolds of the flank. Imaging of the fetus usually requires a low-frequency (3.5-MHz) probe, whereas examination of the placenta and endometrium requires a higher-frequency (7.5-MHz) 1382
probe. A complete description of this examination is beyond the scope of this chapter, but the reader will find 1383
several complete descriptions of the technique and normal values for specific gestation lengths within the relevant veterinary literature.[33] The utility of this examination lies in its repeatability and low risk to the dam and fetus. Sequential examinations over time allow the clinician to follow the pregnancy and to identify changes as they occur.

A companion to transabdominal ultrasongraphy is evaluation of the fetal electrocardiogram (ECG). One can measure fetal ECGs continuously using telemetry or can obtain them using more conventional techniques several times throughout the day.[24,28,34] One places electrodes on the skin of the mare in locations aimed at maximizing the magnitude of the fetal ECG. Because the fetus frequently changes position, multiple sites may be needed in any 24-hour period. To begin, one places an electrode dorsally in the area of the sacral prominence with two electrodes placed bilaterally in a transverse plane in the region of the flank. The fetal ECG maximal amplitude is

low, usually 0.05 to 0.1 mV, and can be lost in artifact or background noise, so one commonly must move electrodes to new positions to maximize the appearance of the fetal ECG. The normal fetal heart rate during the last months of gestation ranges from 65 to 115 beats/min, a fairly wide distribution. The range of heart rate of an individual fetus can be narrow, however. Bradycardia in the fetus is an adaptation to in utero stress, most commonly thought to be hypoxia. By slowing the heart rate, the fetus prolongs exposure of fetal blood to maternal blood, increasing the time for equilibration of dissolved gas across the placenta and improving the oxygen content of the fetal blood. The fetus also has altered the distribution of its cardiac output in response to hypoxia, centralizing blood distribution.[35,36] Tachycardia in the fetus can be associated with fetal movement, and brief periods of tachycardia should occur in the fetus in any 24-hour period. Persistent tachycardia is a sign of fetal distress and represents more severe fetal compromise than bradycardia. The author has recognized dysrhythmias in the challenged fetus, most commonly as atrial fibrillation but also apparent runs of ventricular tachycardia.

The ability to monitor the fetus in a high-risk pregnancy inevitably has led to questions of whether, how, and when to intervene. Most equine neonatologists would agree that removal of the fetus from the uterus before its attainment of readiness for birth is not desirable. One of the difficulties in determining fetal preparedness for birth is that prediction of parturition is difficult in these mares. Many of the parameters used in normal mares are unreliable in the high-risk pregnant mare. One must have an accurate history of any previous gestation length in terms of days for the specific mare in question to allow a more accurate estimate of her usual gestational length. Evaluation of the usual mammary gland parameters, including size, the presence of “wax,” and alteration of electrolyte concentrations, is not generally predictive in the high-risk mare, for in the author's experience many of these mares have changes predictive of parturition for weeks before actual parturition.[37,38] This circumstance may be related to the observation that many high-risk pregnant mares, particularly those with placentitis, are presented for a primary complaint of early onset lactation. Although pulmonary system maturity in human beings can be assessed with some degree of accuracy using measurement of lecithin/sphingomyelin ratios, this measurement—along with sphingomyelin, cortisol, and creatinine concentrations in the amnionic fluid—has proved to be of no benefit in the horse.[39–41] Amniocentesis carries a high risk of abortion in the horse, even with ultrasound guidance, and is not a clinically useful technique at this time.[41] Currently, no clear-cut guidelines are available as to when to intervene, but the presence of persistent fetal tachycardia or prolonged absence of fetal movements, including breathing movements, as determined by transabdominal ultrasound evaluation, should initiate discussion regarding the appropriateness of induction of parturition or elective cesarean section. The goal of induction or cesarean section is to remove a pregnancy that is threatening the survival of the dam with no thought to fetal survival or to remove the fetus from a threatening environment to improve its likelihood for survival. Preterm induction is ill advised if fetal survival is desirable because of the limited ability to treat severely immature neonates. Timing of intervention in these circumstances remains an art, not a science.

The approach to management of the high-risk pregnancy is dictated to some degree by the exact cause for concern, but for many mares therapy is similar. Many high-risk mares have placentitis, primarily caused by ascending bacterial or fungal infections originating in the region of the cervix. These infections can cause in utero sepsis or compromise the fetus by local elucidation of inflammatory mediators or altered placental function.[42,43] Premature udder development and vaginal discharge are common clinical signs. Treatment consists of administration of broad-spectrum antimicrobial agents and nonsteroidal antiinflammatory drugs (Table 19-1). In the author's clinic, trimethoprim-sulfonamide drugs have been the antimicrobial of choice based on unpublished studies performed at the facility demonstrating increased concentration of these agents in the fetal fluids compared with penicillin and gentamicin. However, if culture and sensitivity results are available, one should institute directed therapy. Nonsteroidal antiinflammatory agents such as flunixin meglumine are useful to combat alterations in prostaglandin balance that may be associated with infection and inflammation. Although the efficacy 1383
of these agents is best when administered before the development of clinical signs, to date no detrimental effects 1384

have been reported in the fetus or dam when chronically used at low doses in well-hydrated patients. Tocolytic agents and agents that promote uterine quiescence have been used and include altrenogest, isoxuprine, and clenbuterol.[44–48] Altrenogest usually is administered, although its need in late gestation has been challenged. The efficacy of isoxuprine as a tocolytic in the horse is unproven, and bioavailability of orally administered isoxuprine appears to be highly variable.[48] The long-term use of clenbuterol is inadvisable because of receptor population changes associated with chronic use and its unknown effects on the fetus at this time. Clenbuterol may be indicated during management of dystocia in preparation for assisted delivery or cesarean section.[46] The intravenous form of clenbuterol is not currently available in the United States.

TABLE 19-1 Drugs Used to Treat High-Risk Pregnancy

DRUG	DOSE/FREQUENCY/ROUTE	REASON
Trimethoprim-sulfonamide	25 mg/kg b.i.d. p.o.	Antimicrobial
Flunixin meglumine	0.25 mg/kg t.i.d. p.o. or IV[*]	Antiinflammatory
Altrenogest	0.44 mg/kg s.i.d. p.o.	Tocolytic
Isoxuprine	0.4–0.6 mg/kg b.i.d. IM or p.o.	Tocolytic
Clenbuterol	0.8 μg/kg as needed p.o.[†]	Tocolytic
Vitamin E	6000–10,000 IU s.i.d. p.o.	Antioxidant

* *IV,* Intravenously; *IM,* intramuscularly.

† Intravenous form currently not available in the United States.

One can use three additional strategies in managing high-risk pregnancy patients. In mares with evidence of placental dysfunction, with or without signs of fetal distress, the author provides intranasal oxygen supplementation in the hope of improving oxygen delivery to the fetus. Intranasal oxygen insufflation of 10 to 15 L/min to the mare significantly increases PaO_2 and percent oxygen saturation of hemoglobin.[49] Because of the placental vessel arrangement of the horse, improvement of these two arterial blood gas parameters should result in improved oxygen delivery to the fetus. Blood gas transport is largely independent of diffusion distance in the equine placenta, particularly in late gestation, and depends more on blood flow. Information from other species cannot be extrapolated to the equine placenta because of its diffuse epitheliochorial nature and the arrangement of the maternal and fetal blood vessels within the microcotyledons.[50,51] Umbilical venous pO_2 is 50 to 54 mm Hg in the horse fetus, compared with 30 to 34 mm Hg in the sheep, whereas the maternal uterine vein to umbilical vein pO_2 difference is near 0. Also unlike the sheep, the umbilical venous pO_2 values decrease 5 to 10 mm Hg in response to maternal hypoxemia and increase in response to maternal hyperoxia.[52–54]

Vitamin E (tocopherol) is administered orally to some high-risk mares as an antioxidant. Administration of large doses of vitamin E before traumatic brain injury improves neurologic outcome in experimental models and has been examined as possible prophylaxis for human neonatal encephalopathy.[55–57] Extrapolation of that information to the compromised equine fetus suggests that increased antioxidant concentrations in the fetus may mitigate some of the consequences of uterine and birth hypoxia, but no evidence is available to date demonstrating that protection occurs or that vitamin E accumulates in the fetus in response to supplementation of the mare. Finally, many high-risk mares are anorectic or held off feed because of their medical condition. These mares are at particularly great risk for fetal loss because of their lack of feed intake, which alters prostaglandin metabolism.[58]

Therefore one should administer 2.5% to 5% dextrose in 0.45% saline or water (5% dextrose) intravenously at maintenance fluid rates to these patients.

Perhaps the most important aspect of managing high-risk pregnancy mares is frequent observation and development of a plan. One should observe mares at least hourly for evidence of early-stage labor and should put them under constant video surveillance if possible. Depending on the primary problem, the team managing the mare should develop a plan for handling the parturition once labor begins and for fetal resuscitation following delivery. Any equipment that might be needed should be readily available stallside, and a call sheet, listing contact numbers for all involved, should be posted on or near the stall. The plan should include a decision as to how to handle a complicated dystocia, should it occur, with permission for general anesthesia and cesarean section obtained before the event so that time is not wasted. An important question to be posed to the owner at the outset is which is most important to the owner, the mare or the foal, for the answer may dictate the direction of the decision tree once labor begins.[59]

19.3 Evaluation Of The Newborn Foal

Early recognition of abnormalities is of utmost importance for successful management of critically ill foals. To recognize the abnormal, one must know the normal. Immediately following birth, foals effect several important physiologic and behavioral changes. Chief among these changes is the adaptation of the cardiovascular and respiratory systems to extrauterine life. The normal transition of the respiratory tract involves opening closed 1384
alveoli and absorption of fluid from the airway, accomplished by a combination of breathing efforts, expiration 1385
against a closed glottis (grunting), and a change in sodium flux across the respiratory membrane from net secretion to net absorption.[60–64] The transition from fetal to neonatal circulatory patterns requires resolution of the pulmonary hypertension present in the fetus, normally shunting blood flow through the lower resistance ductus arteriosus in the fetal state, to direct cardiac output to the pulmonary vasculature for participation in gas exchange. This change is achieved by the opening of alveoli, decreasing airway resistance and providing radial support for pulmonary vessels, functional closure of the ductus arteriosus, and increasing the oxygen tension in the lung, reversing pulmonary vasoconstriction mediated by hypoxia.[65,66] Pulmonary tree vasodilators (prostacyclin, nitric oxide [NO]) and vasoconstrictors (endothelin-1, leukotrienes) play apparently well-coordinated, but as yet not fully elucidated, roles. In the normal newborn this change is smooth and rapid. These critical events are undermined by factors such as inadequate lung development, surfactant deficiency (primary or secondary), viral or bacterial infection, placental abnormalities, in utero hypoxia, and meconium aspiration.

Spontaneous breathing should begin in the neonate within 1 minute of birth, many foals attempt to breathe as their thorax clears the pelvic canal. During the first hour of life, the respiratory rate of a healthy foal can be as high as 80 breaths per minute but should decrease to 30 to 40 breaths per minute within a few hours. Similarly, the heart rate of a healthy newborn foal has a regular rhythm and should be at least 60 beats/min at the first minute.[67,68] One usually can auscultate a continuous murmur over the left side of the heart, although its loudness may vary with position. This murmur is thought to be associated with some shunting through the ductus arteriosus. One may auscultate variable systolic murmurs, thought to be flow murmurs, during the first week of life.[69] One should investigate more thoroughly murmurs that persist beyond the first week of life in an otherwise healthy foal, along with any murmur associated with persistent hypoxia. Auscultation of the thorax shortly after birth reveals a cacophony of sounds as airways open and fluid is cleared. End-expiratory crackles are consistently audible in the dependent lung during and following lateral recumbency. For a normal newborn foal to appear slightly cyanotic during this initial adaptation period is not unusual, but this should resolve within minutes of birth. The equine fetus, as do all fetuses, exists in a moderately hypoxic environment, but the equine fetus has a greater partial pressure of oxygen, around 50 mm Hg.[70] Because the fetus is well adapted to low oxygen tensions, cyanosis is

rarely present in newborn foals once adaption occurs, even those with low oxygen tensions. Although in many species the fetal blood oxygen affinity is greater than the maternal blood, in the equine fetus the oxygen affinity of its hemoglobin is only about 2 mm Hg greater than the maternal blood because of decreased levels of 2,3-diphosphoglycerate compared with other species.[71] The result is enhanced oxygen unloading in the equine fetus compared with others. 2,3-Diphosphoglycerate concentration increases after birth in the foal and reaches mature levels by 3 to 5 days of age. The major blood adaptation of the equine fetus to chronic hypoxia is an increase in packed cell volume of up to 20%, increasing the oxygen content of the blood as compensation for decreased oxygen delivery at the placenta.[72] A larger than expected packed cell volume in any newborn foal should alert the clinician for possible sequelae from chronic hypoxia. The presence of significant cyanosis that persists should prompt the clinician to evaluate the foal thoroughly for cardiac anomalies resulting in significant right-to-left shunting or separated circulations, such as transposition of the great vessels.

The chest wall of the foal is compliant, facilitating passage through the pelvic canal during parturition. This compliance requires that the foal actively participate in inspiration and expiration with several potential consequences. First, restriction of the thorax or the abdomen can result in impaired ventilation, which can occur easily when one restrains a foal and may result in spuriously abnormal arterial blood gas values (see the discussion on arterial blood gas evaluation, Respiratory Diseases Associated with Hypoxemia in the Neonate). Second, foals with primary pulmonary parenchymal disease resulting in poorly compliant lungs develop paradoxical chest wall motion, with the thorax moving inward during inspiration.[73–76] The work of breathing can increase greatly, resulting in respiratory failure because of respiratory muscle fatigue. A foal that appears suddenly to improve a previously abnormal respiratory rate and pattern may in fact be in greater respiratory difficulty because of fatigue. One can observe a reduction in respiratory rate or abnormal breathing pattern in premature/dysmature foals or foals subjected to peripartum hypoxia/asphxia. Although the genesis of these patterns is not understood fully, Cheyne-Stokes (lengthy periods of apnea interrupted by short breaths that wax and wane in depth), cluster (short periods of apnea interspersed with long periods of breathing), and Biot's breathing (periods of apnea and breathing with no discernible pattern) may occur in these cases. Foals attempting to maintain an adequate lung volume expire against a partially closed glottis, called Valsalva's maneuver, producing an audible grunt.

Foals are normally nonresponsive while in the birth canal but should respond to stimulation immediately after
birth.[67] The lack of responsiveness while in the birth canal has lead to presumption of fetal death during dystocia. 1385
Because of this, one should attempt other tests before determining that a foal is dead intrapartum. One possibly 1386
may detect pulses in the tongue, neck, or any presented limbs or palpate the thorax for a heartbeat. In the author's facility, nasotracheal intubation of the foal combined with measurement of CO_2 tensions in the exhaled gas aids practitioners in cases where they can reach the nose. Nasotracheal intubation of foals under these circumstances actually can be performed readily with minimal practice. Having long endotracheal tubes available of several different diameters (7 to 12 mm outer diameter) with an inflatable cuff is important. One can pass the tube blindly using a finger in one nostril for guidance and can check the position frequently by palpation of the throatlatch region. One inflates the cuff and begins manual ventilation with 100% oxygen or room air using an Ambu-bag or equivalent. One can obtain continuous measurement of CO_2 tension using a capnograph or single-use disposable end-tidal CO_2 monitor attached to the Ambu-bag or the nasotracheal tube. In a dead foal the end-tidal CO_2 measurement will be negligible after the first 10 to 20 breaths. One must ensure tube placement and seal integrity and allow for multiple breaths. Some CO_2 will “wash out” with the first few breaths and can result in false hope initially. End-tidal CO_2 varies in living intrapartum foals, depending on cardiac output and ventilation frequency, but should be consistently greater than 20 mm Hg and is usually closer to 30 mm Hg. Once one establishes manual ventilation of a living foal, one must continue ventilation until the foal is delivered satisfactorily. The

author has resuscitated and maintained many foals successfully in this manner throughout induction of general anesthesia in the mare and cesarean section delivery of the foal. The nasotracheal tube also provides a convenient site for administration of intratracheal medications such as epinephrine used for extrauterine intrapartum resuscitation of the foal. The reader is cautioned that intratracheal epinephrine increases end-tidal CO_2 measurements transiently, even in a dead foal, because of local actions on tissues. One should allow a washout period after intratracheal administration of epinephrine.

The righting reflex is present as the foal exits the birth canal, as is the withdrawal reflex. Cranial nerve responses are intact at birth, but the menace response may take as long as 2 weeks to develop fully. One should not consider lack of a menace reflex diagnostic of visual deficits in the newborn foal. Within an hour of birth the normal foal will demonstrate auditory orientation with unilateral pinna control. The normal pupillary angle is ventromedial in the newborn foal; this angle gradually becomes dorsomedial over the first month of life. Foals should begin attempting to stand shortly after birth and should be able to achieve this on their own within 2 hours of birth.[67] The normal newborn foal has a suck reflex shortly after birth and should be searching for an udder even before it stands. The expectation is that a normal foal will be sucking from the dam unaided by 3 hours post partum; many foals are overachievers and will be sucking well before this time. The normal foal may defecate shortly after standing but may not attempt defecation until after it first successfully sucks from the dam. Urination varies more, with filly foals usually urinating before colt foals, but both usually do not urinate for several hours following birth, up to 12 hours for some colts.[67] For colt foals to fail to drop their penises when urinating over the first few days of life is not unusual.

The gait of the newborn foal is hypermetric and the stance is base wide. Extreme hypermetria of the forelimbs, usually bilateral but occasionally unilateral, has been observed in some foals and is associated with perinatal hypoxic/ischemic insults, but this gait abnormality usually resolves without specific therapy within a few days. Spinal reflexes tend to be exaggerated, whereas the crossed extensor reflex may not be fully present until 3 weeks of age.[77] Foals also exhibit an exaggerated response to external stimuli (noise, sudden visual changes, touch) for the first few weeks of life. Foals are not bonded strongly to their mother for the first few weeks of life and will follow any large moving object, including other horses and human beings. Orphan foals bond with surrogate mothers until they are several months of age; their primary motivation appears to be appetite. Conversely, mares strongly bond with their foals shortly after parturition; the process begins once the chorioallantois ruptures and is driven more by olfaction and taste than by vision or hearing. Interference with this process, by medical intervention or excessive owner manipulation of the foal, can disrupt normal bonding and result in foal rejection by the dam.[78]

19.4 Evaluation Of The Weak Or Depressed Foal

19.4.1 NEONATAL RESUSCITATION

Most newborn foals make the transition to extrauterine life easily. However, for those in difficulty, recognition of the condition immediately and institution of appropriate resuscitation is of utmost importance. A modified Apgar scoring system has been developed as a guide for initiating resuscitation and assessing probable level of fetal compromise (Table 19-2).[79] One also must at least perform a cursory physical examination before initiating resuscitation, for issues of humaneness are associated with with serious problems such as severe limb contracture, microophthalmia, and hydrocephalus, among others.

The initial assessment begins during presentation of the fetus. Although the following applies primarily to 1386
attending the birth of a foal from a high-risk pregnancy, one can perform quiet and rapid evaluation during any 1387
attended birth. The goal in a normal birth with a normal foal is to disturb the bonding process minimally. This goal also applies to high-risk parturitions, but some disruption of normal bonding is inevitable. The lead clinician should control tightly the number of persons attending, and the degree of activity surrounding, the birth.

TABLE 19-2 Apgar Score in the Foal

	ASSIGNED VALUE		
PARAMETER	0	1	2
Heart/pulse rate	Undetectable	<60 beats/min irregular	>60 beats/min regular
Respiratory rate/pattern	Undetectable	Slow, irregular	40–60 beats/min regular
Muscle tone	Limp/absent	Lateral, some tone	Sternal
Nasal mucosal stimulation	Unresponsive	Grimace, mild rejection	Cough or sneeze

From Martens RJ: Pediatrics. In Mansmann RA, McAllister ES, Pratt PW, editors: *Equine medicine and surgery,* ed 3, vol 1, Santa Barbara, Calif, 1982, American Veterinary Publications.

One should determine the score at 1 and 5 minutes after birth. Scores of 7 to 8 generally indicate a normal foal; 4 to 6, mild to moderate asphyxia; and 0 to 3, severe asphyxia. A score of 4 to 6 should prompt stimulation, intranasal oxygen, or mechanical ventilation. For a score of 0 to 3, one should begin cardiopulmonary resuscitation.

One should evaluate the strength and rate of any palpable peripheral pulse and should evaluate the apical pulse as soon as the chest clears the birth canal. Bradycardia (pulse <40 beats/min) is expected during forceful contractions, and the pulse rate should increase rapidly once the chest clears the birth canal. Persistent bradycardia is an indication for rapid intervention.

The fetus is normally hypoxemic compared with the newborn foal, and this hypoxemia is largely responsible for the maintenance of fetal circulation by generation of pulmonary hypertension. The fetus responds to conditions producing more severe in utero hypoxia by strengthening the fetal circulatory pattern, and the neonate responds to hypoxia by reverting to the fetal circulatory pattern.[80] During a normal parturition, mild asphyxia occurs and results in fetal responses that pave the way for a successful transition to extrauterine life. If more than mild transient asphyxia occurs, the fetus is stimulated to breathe in utero; this is known as *primary asphyxia*.[81] If the initial breathing effort resulting from the primary asphyxia does not correct the asphyxia, a second gasping period occurs in several minutes, known as the *secondary asphyxia* response. If no improvement in asphyxia occurs during this period, the foal enters *secondary apnea,* a state that is irreversible except with resuscitation.

Therefore the first priority of neonatal resuscitation is establishing an airway and breathing pattern. One should assume that foals not spontaneously breathing are in secondary apnea and should clear the airway of membranes as soon as the nose is presented. If meconium staining is present, one should suction the airway before delivery of the foal is completed and before the foal breathes spontaneously. One should continue to the

trachea if aspiration of the nasopharynx is productive. Overzealous suctioning worsens bradycardia as it worsens hypoxia. One should stop suctioning once the foal begins breathing spontaneously, as hypoxia will worsen with continued suction. If the foal does not breathe or move spontaneously within seconds of birth, one should begin tactile stimulation. If tactile stimulation fails to result in spontaneous breathing, one immediately should intubate the foal and manually ventilate the foal using an Ambu-bag or equivalent. One can use mouth-to-nose ventilation if nasotracheal tubes and an Ambu-bag are not available. The goal of this therapy is to reverse fetal circulation, and hyperventilation with 100% oxygen is the best choice for this purpose. However, recent evidence suggests that no clinical disadvantages are apparent in using room air for ventilation of asphyxiated human neonates rather than 100% oxygen.[82,83] Human infants resuscitated with room air recovered more quickly than those resuscitated with 100% oxygen in one study as assessed by Apgar scores, time to the first cry, and the sustained pattern of breathing.[84] In addition, neonates resuscitated with 100% oxygen exhibited biochemical findings reflecting prolonged oxidative stress, present even after 4 weeks of postnatal life, which did not appear in the group resuscitated with room air. Thus the current accepted recommendations for using 100% oxygen in the resuscitation of asphyxiated neonates needs further discussion and investigation.[85,86] Almost 90% of foals requiring resuscitation respond to hyperventilation alone and require no additional therapy. One can initiate nasotracheal intubation while the foal is in the birth canal if the foal will not be delivered rapidly, such as with a difficult dystocia. This technique is “blind” and requires some practice but 1387
may be beneficial and lifesaving. Once spontaneous breathing is present, one should provide humidified oxygen 1388
via nasal insufflation at 8 to 10 L/min.

One should initiate cardiovascular support in the form of chest compression if the foal remains bradycardic despite ventilation and a nonperfusing rhythm is present. One should make sure the foal is on a hard surface in right lateral recumbency with the topline against a wall or other support. Approximately 5% of foals are born with fractured ribs and an assessment for the presence of rib fractures is in order before initiating chest compressions.[87] Palpation of the ribs identifies many of these fractures, which usually are multiple and consecutive on one side of the thorax and located in a relatively straight line along the part of the rib with the greatest curvature dorsal to the costochondral junction. Unfortunately, ribs 3 to 5 frequently are involved, and their location over the heart can make chest compression a potentially fatal exercise. Auscultation over the ribs during breathing results in a recognizable click, identifying rib fractures that may have escaped detection by palpation.

One should initiate drug therapy if a nonperfusing rhythm persists for more than 30 to 60 seconds in the face of chest compression. Epinephrine is the first drug of choice (Table 19-3). Practitioners pose various arguments regarding the best dose and the best frequency of administration for resuscitation. However, most of the data are acquired from human cardiac arrest studies and are not strictly applicable to the equine neonate because the genesis of the cardiovascular failure is different.[88,89] Vasopressin is gaining attention as a cardiovascular resuscitation drug, and although the author has used this drug in resuscitation and as a pressor, experience is limited at this time.[90] The author does not use atropine in bradycardic newborn foals because the bradycardia usually is caused by hypoxia, and if the hypoxia is not corrected, atropine can increase myocardial oxygen debt.[89] The author also does not use doxapram because it does not reverse secondary apnea, the most common apnea in newborns.

TABLE 19-3 Resuscitation Drugs Used for Cardiopulmonary Resuscitation of Foals*

DRUG	HOW SUPPLIED	DOSE PER kg	ml/kg	ml/30 kg	ml/40 kg	ml/50 kg
Epinephrine (low dose)	1 mg/ml (1:1000)	0.01–0.02 mg	0.01–0.02	0.3–0.6 3–5 minutes	0.4–0.8	0.5–1
Epinephrine (high dose)	1 mg/ml (1:1000)	0.1–0.2 mg	0.1–0.2	3–6 3–5 minutes	4–8	5–10
Lidocaine	2% (20 mg/ml)	1.5 mg	0.075	2.25 Every 5 minutes for a maximum of 3 mg/kg	3	3.75
Bretylium	50 mg/ml	5–10 mg (30–35 mg/kg maximum dose)	0.1–0.2	3–6 10 minutes	4–8	5–10
Atropine	0.54 mg/ml	0.02 mg	0.037	1.1 Maximum of 2 times	1.5	1.8
CaCl	10% solution	0.5–1.0 mEq	0.2	6	8	10
$NaHCO_3$	1 mEq/ml	0.5–1 mEq	0.5–1	15–30	20–40	25–50
$MgSO_4$	50% (500 mg/ml)	14–28 mg	0.028–0.056	0.8–1.7	1.1–2.2	1.4–2.8

* Epinephrine is the most commonly used of the drugs.

Because birthing areas are generally cold, one should dry the foal and place it on dry bedding once resuscitation is complete. The fetus has some homeothermic mechanisms, but its size in relation to its mother and its position within her body means that it is in effect a poikilotherm. The body temperature of the foal generally reflects that of its environment, namely its mother, although the human fetal temperature directly measured at cesarean section, induction of labor, or during labor is approximately 0.5° C higher than the mothers.[91,92] Adaptation from poikilothermy to homeothermy normally takes place rapidly following birth. The fetus is capable of nonshivering thermogenesis, primarily through the oxidation of brown fat reserves, but this type of thermogenesis is inhibited in utero, probably by placental prostaglandin E_2 and adenosine.[93,94] Immediately after birth the foal must adapt to independent thermoregulation. Local physical factors, including ambient temperature and humidity, act to induce cold stress, and the newborn must produce heat by metabolic activity. In response to the catecholamine surge associated with birth, uncoupling of oxidative phosphorylation occurs

within mitochondria, releasing energy as heat. This nonshivering thermogenesis is impaired in newborns undergoing hypoxia or asphyxiation and in those that are ill at birth. Infants born to mothers sedated with benzodiazepines are affected similarly, a consideration in the choice of sedative and preanesthetic medications 1388
in mares suffering dystocia or undergoing cesarean section.[95–97] Heat losses by convection, radiation, and 1389
evaporation are high in most areas where foals are delivered, resuscitated, and managed, and one must take care to minimize cold stress in the newborn and the critically ill foal. Supplementary heat, in the form of radiant heat lamps or warm air circulating blankets, may be required.

One should use fluid therapy conservatively during postpartum resuscitation, for the neonate is not volume depleted unless excessive bleeding has occurred. Some compromised newborn foals are actually hypervolemic. Fluid therapy of the neonate is discussed in more detail later in this chapter. Because the renal function of the equine neonate is substantially different from the adult, one cannot simply scale down fluid therapy from adult therapy.[98–100] If intravenous fluids are required for resuscitation and blood loss is identified, administration of 20 ml/kg of a non–glucose-containing polyionic isotonic fluid over 20 minutes (about 1 L for a 50-kg foal) once intravenous access is established can be effective. The author stresses non–glucose-containing polyionic intravenous fluids because hyperglycemia, but not hypoglycemia, immediately after fetal or neonatal asphyxia interfered with the recovery of brain cell membrane function and energy metabolism in neonatal piglets in one recent study.[101] These findings suggest that post–hypoxic-ischemic hyperglycemia is not beneficial and might even be harmful in neonatal hypoxic-ischemic encephalopathy. Indications for this shock bolus therapy include poor mentation, poorly palpable peripheral pulses, and the development of cold distal extremities, compatible with hemorrhagic shock. One should reassess the patient after the initial bolus and administer additional boluses as necessary. Ideally, one should follow up on blood pressures and ECG readings and initiate appropriate pressor therapy if needed. Again, these procedures are discussed in detail later in the chapter.

One can administer glucose-containing fluids after resuscitation at a rate of 4 to 8 mg/kg/min (about 250 ml/hr of 5% dextrose or 125 ml/hr of 10% dextrose) to the average 50-kg foal, particularly in the obviously compromised foal. This therapy is indicated to help resolve metabolic acidosis, to support cardiac output because myocardial glycogen stores likely have been depleted, and to prevent postasphyxial hypoglycemia. Under normal conditions, the fetal-to-maternal blood glucose concentration gradient is 50% to 60% in the horse, and glucose is the predominant source of energy during fetal development.[102,103] Glucose transport across the placenta is facilitated by carrier receptors (glucose transporter [GLUT] receptors), and a direct relationship exists between maternal and fetal blood glucose concentration when maternal glucose is in the normal range.[102] The GLUT receptors in the placenta are stereospecific, saturable, and energy independent.[104] Although the enzyme kinetics for GLUT isoform 1 suggest that they are not saturable under conditions of euglycemia, equine maternal hyperglycemia results in increased fetal glucose concentration to a plateau point, likely caused by GLUT saturation.

At term, the net umbilical uptake of glucose is 4 to 7 mg/kg/min, with most of the glucose being used by the brain and skeletal muscle.[105–107] The fetus only develops gluconeogenesis under conditions of severe maternal starvation. A certain percentage of the delivered glucose is used to develop large glycogen stores in the fetal liver and cardiac muscle in preparation for birth, and at birth the foal liver produces glucose at a rate of 4 to 8 mg/kg/min by using these stores. Fetal glycogen stores also are built using the substrates lactate, pyruvate, and alanine; fetal uptake of lactate across the placenta is about half that of glucose.[102,108] The transition to gluconeogenesis, stimulated by increased circulating catecholamine concentration from birth and by stimulation of glucagon release at the time the umbilical cord breaks takes 2 to 4 hours in the normal foal, and glycogenolysis supplies needed glucose until feeding and glucose production are accomplished.[109] In the

challenged foal, glycogen stores may have been depleted and gluconeogenesis delayed, so provision of glucose at rates similar to what the liver would normally produce during this period is requisite.

19.4.2 PERSISTENT PULMONARY HYPERTENSION

Persistent pulmonary hypertension (PPH) also is known as reversion to fetal circulation or persistent fetal circulation, and its genesis lies in the failure of the fetus to make the respiratory and cardiac transition to extrauterine life successfully or reversion of the newborn to fetal circulatory patterns in response to hypoxia or acidosis. Differentiating this problem from other causes of hypoxemia in the newborn requires some investigation, and multiple serial arterial blood gas analyses are necessary to confirm suspicion of this problem (see the section on arterial blood gas analysis, Respiratory Diseases Associated with Hypoxemia in the Neonate). However, one should suspect the condition in any neonate with hypercapnic hypoxemia that persists and worsens; these foals are in hypoxemic respiratory failure. The fetal circulatory pattern, with pulmonary hypertension and right-to-left shunting of blood through the patent foramen ovale and ductus arteriosus, is maintained in these cases.

Pulmonary vascular resistance falls at delivery to about 10% of fetal values, while pulmonary blood flow increases accordingly.[110] Early in the postnatal period these two changes balance each other, and mean pulmonary and systolic pressures remain increased for several hours. Systolic pulmonary pressures can remain 1389
equivalent to systemic pressure for up to 6 hours of age in human infants, although diastolic pulmonary 1390
pressures are well below systemic diastolic pressures by 1 hour.[111] Mean pulmonary artery pressures fall gradually over the first 48 hours.[112] The direct effects of lung expansion and increasing alveolar oxygen tension probably provide the initial stimulus for pulmonary arteriolar dilation and partly result from direct physical effects, but vasoactive substances are released in response to physical forces associated with ventilation, for example prostacyclin.[110] Other vasoactive mediators thought to play a role in regulating pulmonary arteriolar tone include NO, prostaglandins D_2 and E_2, bradykinin, histamine, endothelin-1, angiotensin II, and atrial natriuretic peptide. The increase in alveolar and arterial oxygen tensions at birth is required for completion of resolution of pulmonary hypertension. Much of this increase is thought to be mediated by NO, evidence for this being the parallel increase during gestation of the pulmonary vasodilation response to hyperoxia and the increase in NO synthesis.[113] However, inhibition of NO synthesis does not eliminate the initial decrease in pulmonary artery resistance occurring because of opening of the airways.[114]

When these mechanisms fail, one can recognize PPH. Right-to-left shunting within the lungs and through patent fetal conduits occurs and can result from many factors, including asphyxia and meconium aspiration, but in many cases the precipitating trigger is unknown. Inappropriately decreased levels of vasodilators (NO) and inappropriately increased levels of vasoconstrictors (endothelin-1) currently are being examined as potential mechanisms. Chronic in utero hypoxia and acidosis may result in hypertrophy of the pulmonary arteriolar smooth muscle.[115] In these cases, reversal of PPH can be difficult and cannot be achieved rapidly.

Treatment of PPH is twofold: abolishment of hypoxia and correction of the acidosis, for both abnormalities only bolster the fetal circulatory pattern. Initial therapy is provision of oxygen intranasally at 8 to 10 L/min. Some foals respond to this therapy and establish neonatal circulatory patterns within a few hours. Failure to improve or worsening of hypoxemic respiratory failure following intranasal oxygen administration should prompt intubation and mechanical ventilation with 100% oxygen. This serves two purposes, one diagnostic and one therapeutic. Ventilation with 100% oxygen may resolve PPH and, if intrapulmonary shunt and altered ventilation-perfusion relationships are causing the hypoxic respiratory failure, arterial oxygen tension (PaO_2)

should exceed 100 mm Hg under these conditions. Failure to improve PaO_2 suggests PPH or large right-to-left extrapulmonary shunt caused by congenital cardiac anomaly. The vasodilators prostacyclin and telazoline (an α-blocking vasodilator) cause pulmonary vasodilation in human infants with PPH, but the effects on oxygenation vary and the side-effects (tachycardia, severe systemic hypotension) are unacceptable.[116] Recognition of NO as a potent dilator of pulmonary vessels has created a significant step forward in the treatment of these patients, for inhaled NO dilates vessels in ventilated portions of the lung while having minimal effects on the systemic circulation.[117] Based on evidence presently available, use of inhaled NO in an initial concentration of about 20 ppm in the ventilatory gas seems reasonable for term and near-term foals with hypoxic respiratory failure and PPH that fails to respond to mechanical ventilation using 100% oxygen alone.[117,118] The author has used this approach in the clinic, administering a range of 5 to 40 ppm NO with success.

19.4.3 PERINATAL ASPHYXIA SYNDROME

Hypoxic ischemic encephalopathy (HIE), currently referred to as neonatal encephalopathy in the human literature, is one systemic manifestation of a broader syndrome of perinatal asphyxia syndrome (PAS), and management of foals with signs consistent with a diagnosis of HIE requires the clinician to examine other body systems fully and to provide therapy directed at treating other involved systems.[119] Although PAS primarily manifests as HIE, the gastrointestinal tract and kidneys frequently are affected by peripartum hypoxia/ischemia/asphyxia, and one should expect complications associated with these systems. Hypoxic ischemic encephalopathy also may affect the cardiovascular and respiratory systems, and one also may encounter endocrine disorders in these patients.

Hypoxic ischemic encephalopathy has been recognized as one of the most common diseases of the equine neonate for generations.[1,10,12] In the past HIE has been known as dummy foal syndrome and as neonatal maladjustment syndrome. The designation HIE, although not perfect, attempts to describe the syndrome in terms of the suspected underlying pathophysiology.

A wide spectrum of clinical signs is associated with HIE and can range from mild depression with loss of the suck reflex to grand mal seizure activity. Typically, affected foals are normal at birth but show signs of central nervous system abnormalities within a few hours after birth. Some foals are obviously abnormal at birth, and some do not show signs until 24 hours of age. Hypoxic ischemic encephalopathy commonly is associated with adverse peripartum events, including dystocia and premature placental separation, but a fair number of foals have no known peripartum period of hypoxia, suggesting that these foals result from unrecognized in utero hypoxia (Box 19-2). Severe maternal illness also may result in foals born with PAS. In human beings, 1390
ascending placental infection now is suspected of being a major contributor to neonatal encephalopathy in 1391
infants, and the incidence of neonatal encephalopathy increases with the presence of maternal fever, suggesting a role for maternal inflammatory mediators.[120]

19.4.3.1 BOX 19-2 CAUSES OF HYPOXIA IN THE FETUS AND NEONATE

19.4.3.1.1 Maternal Causes

Reduced maternal oxygen delivery

Maternal anemia

- Maternal pulmonary disease with hypoxemia
- Maternal cardiovascular disease

Reduced uterine blood flow

- Maternal hypotension (endotoxemia/colic)
- Maternal hypertension (laminitis/painful conditions)
- Abnormal uterine contractions
- Anything that increases uterine vascular resistance

19.4.3.1.2
Placental Causes

Premature placental separation

Placental insufficiency; for example, twins

Placental dysfunction

- Fescue toxicity
- Postmaturity
- Placentitis
- Placenta edema

Reduced umbilical blood flow

- General anesthesia of the dam
- Congenital cardiovascular disease
- Inappropriate fetal blood distribution
- Fetal hypovolemia

- Excessive length of umbilical cord

19.4.3.1.3 ### Intrapartum Causes

Dystocia

Premature placental separation

Uterine inertia

Oxytocin induction of labor

Cesarean section

- General anesthesia
- Poor uterine blood flow because of maternal positioning
- Decreased maternal cardiac output
- Reduced umbilical blood flow
- Effects of anesthetic drugs on fetus

Anything that prolongs stage 2 labor

19.4.3.1.4 ### Neonatal Period Causes

Prematurity

Recumbency

- Musculoskeletal disease
- Sepsis
- Prematurity
- Mild hypoxic ischemic encephalopathy

Pulmonary disease

  - Meconium aspiration
  - Milk aspiration
  - Persistent pulmonary hypertension
  - Septic pneumonia
  - Acute respiratory distress syndrome or acute lung injury
- Severe disturbance in breathing pattern
- Septic shock
- Anemia
  - Neonatal isoerythrolysis
  - Excessive umbilical bleeding
  - Fractured ribs (hemothorax) or long bone fracture
- Congenital cardiovascular disease

Adapted from Palmer JE: Perinatal hypoxic-ischemic disease. Proceedings of the International Veterinary Emergency Critical Care Symposium, San Antonio, Tex., 1998. p 717-718.

The underlying pathophysiologic details of HIE in the foal are unknown, and to date accurate experimental models of HIE and PAS in the foal have not been described. However, a great deal of attention has been paid to peripartum hypoxia/asphyxia by human counterparts because the effects of adverse peripartum events in the human neonate have far ranging implications for the affected human neonate and for society. Therefore equine neonatologists have long looked to human studies and models of the human disease for understanding of the syndrome in the equine neonate.

Perinatal brain damage in the mature fetus usually results from severe uterine asphyxia caused by an acute reduction of uterine or umbilical circulation. The fetus responds to this challenge by activation of the sympathetic adrenergic nervous system, causing a redistribution of cardiac output that favors the central organs: brain, heart, and adrenal glands.[121,122] If the hypoxic insult continues, the fetus reaches a point beyond which it cannot maintain this centralization of circulation, cardiac output falls, and cerebral circulation diminishes.[122] The loss of oxygen results in a substantial decrease in oxidative phosphorylation in the brain with concomitant decreased energy production. The Na^+/K^+ pump at the cell membrane cannot maintain the ionic gradients, and the membrane potential is lost in the brain cells. In the absence of the membrane potential, calcium flows down

its large extracellular/intracellular concentration gradient through voltage-dependent ion channels into the cell. 1391
This calcium overload of the neuron leads to cell damage by activation of calcium-dependent proteases, lipases, 1392
and endonucleases. Protein biosynthesis is halted. Calcium also enters the cells by glutamate-regulated ion channels as glutamate, an excitatory neurotransmitter, is released from presynaptic vesicles following anoxic cellular depolarization. Once the anoxic event is over, protein synthesis remains inhibited in specific areas of the brain and returns to normal in less vulnerable areas of the brain. Loss of protein synthesis appears to be an early indicator of cell death caused by the primary hypoxic/anoxic event.[123]

A second wave of neuronal cell death occurs during the reperfusion phase and is thought to be similar to classically described postischemic reperfusion injury in that damage is caused by production of and release of oxygen radicals, synthesis of NO, and inflammatory reactions.[124] Additionally, an imbalance between excitatory and inhibitory neurotransmitters occurs.[123] Part of the secondary cell death that occurs is thought to be caused by apoptosis, a type of programmed cell death termed *cellular suicide*. Secondary cell death also is thought be caused by the neurotoxicity of glutamate and aspartate resulting again from increased intracellular calcium levels.[125,126] In human infants the distribution of lesions with hypoxic-ischemic brain damage following prenatal, perinatal, or postnatal asphyxia falls into distinct patterns depending on the type of hypoxia-ischemia rather than on postconceptual age at which the asphyxial event occurs.[126] Periventricular leukomalacia was associated with chronic hypoxia-ischemia, whereas the basal ganglia and thalamus were affected primarily in patients experiencing acute profound asphyxia, providing direct evidence that the nature of the event determines the severity and distribution of neurologic damage in human beings. These remarkably selective patterns of injury in children, with differential variability in the damage caused to regions anatomically located within millimeters of each other, resulted in the hypothesis that location within neurotransmitter-specific circuitry loops is important. This hypothesis has important implications in the design of neuroprotective strategies and therapies for neonates experiencing hypoxic-ischemic-asphyxial events. Now the evidence is overwhelming that the excitotoxic cascade that evolves during HIE extends over several days from the time of insult and is modifiable.[125,126]

In brain injury, traumatic or hypoxic, the mechanisms underlying delayed tissue injury still are understood poorly. Many believe that neurochemical changes, including excessive neurotransmitter release, are pivotal in the pathophysiology of secondary neuronal death. Excitatory amino acid neurotransmitters and magnesium are known to play at least a minimal role in secondary cell death following brain injury; a fair body of literature regarding these factors has been generated over the last 10 years. The activation of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors is implicated in the pathophysiology of traumatic brain injury and is suspected to play a role in HIE.[125–127] Mechanically injured neurons demonstrate a reduction of voltage-dependent Mg^{2+} blockade of NMDA current that can be restored partially by increasing extracellular Mg^{2+} concentration or by pretreatment with calphostin C, a protein kinase C inhibitor.[128] This finding suggested that administration of Mg^{2+} to patients with brain injury could lead to improved outcome. Subsequently, magnesium sulfate solution was shown to improve dramatically the immediate recovery of rats from hypoxia.[129] However, although pretreatment with magnesium sulfate protected against hypoxic ischemic brain injury, postasphyxial treatment worsened brain damage in 7-day-old rats, suggesting an age-related response in the rat.[130] Delayed magnesium treatment of mature rats following severe traumatic axonal brain injury improved motor outcome when administered up to 24 hours after injury, with early treatments providing the most benefit.[131]

Maternal seizure in rats is associated with fetal histopathologic changes that are abolished by administration of magnesium sulfate to the mother, and magnesium sulfate has been demonstrated to protect the fetal brain from

severe maternal hypoxia.[132] Clinical trials investigating the efficacy of magnesium treatment following hypoxia in infants are under way, with few reports currently in the medical literature. Magnesium sulfate was used to treat nine infants after perinatal asphyxia in one study (no control group), and all children were neurologically normal at 1 year of age. Seizures did not occur in any of these children, nor were any adverse side effects noted.[133] Magnesium sulfate administration failed to delay the global impairment in energy metabolism after hypoxia ischemia, characteristic of severe brain damage, in newborn piglets; at 48 hours after hypoxia ischemia, no difference could be found in the severity of injury in piglets treated with magnesium compared with piglets treated with placebo, suggesting magnesium may not be protective with severe acute injury.[134]

In developing countries, birth hypoxia frequently is associated with HIE, and although this finding is attributed most frequently to inadequate obstetric care, poor nutrition also may play a role. Red blood cell magnesium levels were measured in more than 500 women in labor at a teaching hospital in South Africa.[135] Fifty five of the women delivered infants with HIE and had significantly lower levels of magnesium than controls; the infants with HIE also had significantly lower magnesium levels than controls. The large majority (54 of 55) of the women giving birth to HIE infants were from poor social circumstances, suggesting nutrition might play a 1392
role in some cases of HIE, with maternal magnesium levels affecting outcome in the infants. The authors 1393
suggested an early pregnancy intervention study may help determine the role of magnesium in the pathogenesis of HIE in human infants born to at-risk mothers.

Therapy for the various manifestations of hypoxia-ischemia involves control of seizures, general cerebral support, correction of metabolic abnormalities, maintenance of normal arterial blood gas values, maintenance of tissue perfusion, maintenance of renal function, treatment of gastrointestinal dysfunction, prevention and recognition and early treatment of secondary infections, and general supportive care. Control of seizures is important because cerebral oxygen consumption increases fivefold during seizures. One can use diazepam for emergency control of seizures (Table 19-4). If diazepam does not stop seizures readily or one recognizes more than two seizures, then one should replace diazepam with phenobarbital given to effect. The half-life of phenobarbital can be long in the foal (100 hours), and one should keep this in mind when monitoring neurologic function in these cases after phenobarbital administration (J.E. Palmer, personal communication, 1998).[136] Early-stage, preseizure administration of phenobarbital has been advocated by some investigators for prevention of neonatal encephalopathy. However, one recent study in asphyxiated human infants demonstrated that early phenobarbital treatment was associated with a threefold increase in the incidence of subsequent seizures and consequently a trend toward increased mortality. Seizures per se were associated with almost a twentyfold increase in mortality. Their findings suggest that early phenobarbital administration may produce adverse rather than beneficial effects following asphyxia. Because this was an observational study; the results need to be confirmed by appropriate randomized trials in similar clinical settings.[137] If phenobarbital fails to control seizures, one may attempt phenytoin therapy. In cases of HIE, one should avoid ketamine and xylazine because of their association with increased intracranial pressure. One must protect the foal from injury during a seizure and also ensure the patency of the airway to prevent the onset of negative pressure pulmonary edema[138] or aspiration pneumonia.

TABLE 19-4 Drugs Used to Control or Prevent Seizures in Foals

DRUG	DOSE	ROUTE	FREQUENCY	COMMENT
Diazepam	5–10 mg per foal	IV*	As needed	Short-term seizure control
Phenobarbital	2–3 mg/kg	IV	Bolus to effect	Bolus over 15–20 minutes. Half-life can be prolonged; decreases thermoregulatory control, respiratory drive, and blood pressure
Phenytoin	5–10 mg/kg loading; 5 mg/kg maintenance	IV	q4h for first 24 hours; then b.i.d. (?)	Seizure control
Magnesium sulfate†	0.05 mg/kg/hr loading dose; 0.025 mg/kg/hr maintenance‡	IV	Constant rate infusion for first hour and for maintenance	Discontinue if muscle tremors or hypotension occur. Treat for 24–48 hours after hypoxic insult
Gabapentin	8 mg/kg	p.o.	b.i.d. to t.i.d.	Seizure control

* *IV,* Intravenous.

† To make 0.1 gm/ml solution, add 20 ml 50% $MgSO_4$ to 80 ml 0.9% NaCl.

‡ Loading dose = 25 ml/hr of 0.1 gm/ml solution for 1 hour. Maintenance dose = 12 ml/hr of 0.1 gm/ml solution.

Probably the most important therapeutic interventions are aimed at maintaining cerebral perfusion, which is achieved by careful titration of intravenous fluid support, neither too much nor too little (see Fluid Therapy in Neonates) and judicious administration of inotropes and pressors to maintain adequate perfusion pressures (see Pressor and Inotrope Therapy in Neonates). Cerebral interstitial edema is only truly present in the most severe cases[139,140]; in most cases the lesion is intracellular edema and most of the classic agents used to treat cerebral interstitial edema (e.g., mannitol) are minimally effective treating cellular edema. Occasionally the author uses thiamine supplementation in the intravenous fluids to support metabolic processes, specifically mitochondrial metabolism and membrane Na^+,K^+-ATPases, involved in maintaining cellular fluid balance.[141,142] This therapy is rational and inexpensive but unproven in efficacy. Only if cellular necrosis and vasogenic edema are present are drugs such as mannitol and dimethyl sulfoxide indicated, and again these cases are usually the most severely affected. In the author's clinic, practitioners rarely have used dimethyl sulfoxide in neonates for the last several years and have recognized no change in outcome by discontinuing its use. When the practitioners use intravenously administered dimethyl sulfoxide, they do so within the first hour after an acute asphyxial insult and use it primarily for its hydroxyl radical scavenging effects and its theoretical modulation of postischemic reperfusion injury.[143]

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Naloxone has been advocated for treating HIE in human beings and in foals,[144–146] perhaps based on a study suggesting that postasphyxia blood-brain barrier disruption was related causally to poor neurologic outcome in a lamb model of HIE and that naloxone prevented disruption and neurologic dysfunction among those survivors with an intact blood-brain barrier.[145] However, other studies have demonstrated that naloxone exacerbates hypoxic-ischemic brain injury in 7-day-old rats subjected to unilateral common carotid artery ligation and

hypoxia. Moreover, systemic acidosis and cellular edema were no different in naloxone-treated animals compared with animals treated with saline solution. The authors concluded that high doses of naloxone in fact may reduce the resistance of the fetus to hypoxic stress.[146] The use of naloxone in human neonatal resuscitation remains controversial, for whether the contradictory effects are related to a reduction in acute neuronal swelling by osmotic effects or by a more direct receptor-mediated mechanism is currently unknown.[147] Naloxone is most effective in resuscitation of compromised human infants born to mothers addicted to drugs. Some practitioners are using γ-aminobutyric acid adrenergic agonists to manage HIE in foals, based on evidence showing neuroprotection when used in ischemia alone and combined with NMDA antagonists.[148–150] The author currently has no experience with these compounds and cannot comment regarding their efficacy in foals. Regional hypothermia also is being investigated as a potential therapy for global hypoxia/ischemia; published data are consistent with the theory that cooling must be continued throughout the entire secondary phase of injury (about 3 days) to be effective.[151] Experimentally, this approach has resulted in dramatic decreases in cellular edema and neuronal loss; its practical application remains to be demonstrated.

Despite a lack of consensus regarding the use of magnesium to treat infants with HIE, the author has used magnesium sulfate infusion as part of the therapy for selected foals with HIE for the past several years. The rationale is based primarily on the evidence demonstrating protection in some studies and a failure of any one study to demonstrate significant detrimental effects. The clinical impressions of the author to date suggest that the therapy is safe and may decrease the incidence of seizure in patients. The author administers magnesium sulfate as a constant rate infusion over 1 hour after giving a loading dose. The author has continued the infusion for up to 3 days without demonstrable negative effect beyond some possible trembling. Given the current evidence, a 24-hour course of treatment may be effective and all that is necessary. Postasphyxial treatment certainly may be beneficial in foals with HIE, and maternal magnesium therapy may be beneficial in certain high-risk pregnancy patients.

Foals with PAS often have a variety of metabolic problems including hypo- or hyperglycemia, hypo- or hypercalcemia, hypo- or hyperkalemia, hypo- or hyperchloremia, and varying degrees of metabolic acidosis. Although one needs to address these problems, one should not forget the normal period of hypoglycemia that occurs postpartum and should not treat aggressively so as to avoid worsening the neurologic injury. Foals suffering from PAS also have frequent recurrent bouts of hypoxemia and occasional bouts of hypercapnia. Intranasally administered oxygen is generally needed in these cases as a preventative therapy and as direct treatment, for the appearance of the abnormalities can be sporadic and unpredictable. Additional respiratory support, particularly in those foals with centrally mediated hypoventilation and periods of apnea or abnormal breathing patterns, include caffeine (per os or per rectum) and positive pressure ventilation. Caffeine is a central respiratory stimulant and has minimal side effects at the dosages used (10 mg/kg loading dose; 2.5 mg/kg as needed).[152] The author purchases whatever oral form of caffeine is available at the local convenience store or drug store and administers it dissolved in warm water per rectum. Foals treated with caffeine have an increased level of arousal and are more reactive to the environment. Adverse effects generally are limited to restlessness, hyperactivity, and mild to moderate tachycardia. Mechanical ventilation of these patients can be rewarding and generally is required for less than 48 hours. One must monitor and maintain blood pH within the normal range. Metabolic alkalosis can develop in some of these foals and requires clinician tolerance of some degree of hypercapnia. pH is important in evaluation and consideration of alternatives for treatment. If the respiratory acidosis is not so severe as to affect the patient adversely (generally >70 mm Hg), and the pH is within normal limits, the foal may tolerate hypercapnia.[153] The goal is to normalize pH. Foals with respiratory acidosis as compensation for metabolic alkalosis do not respond to caffeine. Metabolic alkalosis in critically ill foals frequently is associated with electrolyte abnormalities, creating differences in strong ion balance. One handles this pH perturbation best by correcting the underlying electrolyte problem.

Maintaining tissue perfusion and oxygen delivery to tissues is a cornerstone of therapy for PAS to avoid additional injury. One should maintain the oxygen-carrying capacity of the blood; some foals require transfusions to maintain a packed cell volume greater than 20%. Adequate vascular volume is important, but one should take care to avoid fluid overload in the foal. Early evidence of fluid overload is subtle accumulation of ventral edema between the front legs and over the distal limbs. Fluid overload can result in cerebral edema, pulmonary edema, and edema of other tissues, including the gastrointestinal tract. This edema interferes with 1394
normal organ function and worsens the condition of the patient. One maintains perfusion by supporting cardiac 1395
output and blood pressure by judicious use of intravenous fluid support and inotrope/pressor support. The author does not target therapy to a specific systolic, mean, or diastolic pressure but monitors urine output, mentation, limb perfusion, gastrointestinal function, and respiratory function as indicators that perfusion is acceptable. For these patients to require pressor therapy is not unusual, but in some cases the hypoxic damage is sufficiently severe to blunt the response of the patient to the drugs.

The kidney is a target for injury in patients with PPH, and for renal compromise to play a significant role in the demise of these foals is not unusual. Clinical signs of renal disease are generally referable to disruption of normal control of renal blood flow and tubular edema leading to tubular necrosis and renal failure. These foals have signs of fluid overload and generalized edema. One must balance urine output and fluid therapy in these cases to prevent additional organ dysfunction associated with edema. Although evidence has accumulated that neither dopamine nor furosemide play a role in protecting the kidney or reversing acute renal failure, these agents can be useful in managing volume overload in these cases.[154–156] The aim is not to drive oliguric renal failure into a high-output condition but rather to enhance urine output.

Overzealous use of diuretics and pressors in these cases can result in diuresis requiring increased intravenous fluid support and can be counterproductive. The author's approach is more conservative. Low doses of dopamine administered as a constant rate infusion of 2 to 5 μg/kg/min are usually effective in establishing diuresis by natriuresis. One should avoid large doses of dopamine (>20 μg/kg/min) because high doses can produce systemic and pulmonary vasoconstriction, potentially exacerbating PPH.[157] One can administer a bolus (0.25 to 1.0 mg/kg) or constant rate infusion (0.25 to 2.0 mg/kg/hr) of furosemide, but once furosemide diuresis is established, one must evaluate electrolyte concentrations and blood gas tensions frequently because potassium, chloride, and calcium losses can be considerable and because significant metabolic alkalosis can develop from strong ion imbalances. The author does not aim for urine production rates of 300 ml/hr, as has been presented by other authors as a urine output goal for critically ill equine neonates.[158] Rather the author looks for urine output that is appropriate for fluid intake and does not attempt to drive urine output to an arbitrary goal by excessive fluid administration or pressor use. Although the average urine output for a normal equine neonate is about 6 ml/kg/hr (~300 ml/hr for a 50-kg foal), these values were obtained from normal foals drinking a milk diet with a large free water component.[98–100] The urine of normal newborn foals is dilute, reflecting the large free water load they incur by their diet. Expecting critically ill foals to produce such large volumes of urine, particularly those on restricted diets or receiving total parenteral nutrition, is an exercise in futility, and manipulating fluid, pressor, or diuretic therapy in attempt to meet an artificial goal is inappropriate. Fluid therapy in the critically ill neonate is discussed later in this chapter.

One final caveat regarding renal dysfunction in PAS is that one should perform therapeutic drug monitoring when it is available. Many antimicrobial agents used to manage these cases, most notably the aminoglycosides, depend on renal clearance. Aminoglycoside toxicity occurs in the equine neonate and exacerbates or complicates the management of renal failure originally resulting from primary hemodynamic causes. The author monitors aminoglycoside concentrations for 30-minute peak and 23- to 24-hour trough values in these

cases and adjusts dosage and frequency of drug administration based on these results. The author considers a trough value of less than 2 µg/dl as desirable for gentamicin and amikacin.

Foals with PAS suffer from a variety of problems associated with abnormalities within the gastrointestinal tract.[159] Commonly they have ileus, recurrent excessive gastric reflux, and gas distention. These problems are exacerbated by constant feeding in the face of continued dysfunction and continued hypoxia. Frequently, enteral feeding cannot meet their nutritional requirements, and partial or total parenteral nutrition is required. One must give special attention to passive transfer of immunity (see Failure of Passive Transfer) and glucose homeostasis in these cases. Although some practitioners use prokinetic agents as therapy for ileus in these cases, the author's approach is again more conservative. Appearance of damage to the gastrointestinal tract can be subtle and lag behind other clinical abnormalities for days to weeks. Low-grade colic, decreased gastrointestinal motility, decreased fecal output, and low weight gain are among the most common clinical signs of gastrointestinal dysfunction in these case, but more severe problems, including necrotizing enterocolitis and intussusception, have been associated with these cases. The return to enteral feeding must be slow in many of these cases. A currently debated topic is constant versus pulsed enteral feeding.[160–162] The author uses pulsed feeding through an indwelling small-gauge feeding tube. In many foals these tubes stay in place for weeks and cause no problems as the foals are returned to their dams for sucking or are trained to drink from a bottle or bucket.

Foals with PAS are also susceptible to secondary infection. Treatment of recognized infection is covered under sepsis in this chapter. If infection is recognized in these patients after hospitalization, one should give attention to the likelihood of nosocomial infection and should direct antimicrobial therapy based on known nosocomial 1395
pathogens in the NICU and their susceptibility patterns until culture and sensitivity results become available. 1396
One should make repeat determinations of immunoglobulin G (IgG) concentration; additional intravenous plasma therapy may be required. Nosocomial infections are often rapidly overwhelming, and acute deterioration in the condition of a foal with PAS should prompt a search for nosocomial infection.

The prognosis for foals with PAS is good to excellent when the condition is recognized early and aggressively treated in term foals. Up to 80% of these neonates survive and go on to lead productive and useful athletic lives.[20–23] The prognosis decreases with delayed or insufficient treatment and concurrent problems such as prematurity and sepsis.

19.4.4 PREMATURITY/DYSMATURITY/POSTMATURITY

In human NICUs the survival rates of low-gestation-length infants has increased dramatically since the 1980s concurrent with improvements in obstetric and neonatal care. The now routine, well-validated use of antenatal steroid and artificial surfactant therapies has contributed greatly to the enhanced survival of this patient population, although the use of these particular therapies is not common or frequently indicated in the equine NICU.[163,164] However, with improved care, outcomes in the equine NICU population have improved also, with survival of premature patients in many NICUs exceeding 80%.[21] In the equine population, gestation length is much more flexible than in the human population; however, the definition of the term *prematurity* needs reexamination. Traditionally, *prematurity* is defined as a preterm birth of less than 320 days of gestation in the horse. Given the variability of gestation length in the horse, ranging from 310 days to more than 370 days in some mares, a mare with a usual gestation length of 315 days possibly could have a term foal at 313 days, whereas a mare with a usual gestation length of 365 days may have a premature foal at 340 days, considered the normal gestation length. Foals that are born postterm but are small are termed *dysmature;* a postmature foal is a postterm foal that has a normal axial skeletal size but is thin to emaciated. Dysmature foals may have been

classified in the past as small for gestational age and are thought to have suffered placental insufficiency, whereas postmature foals are usually normal foals that have been retained too long in utero, perhaps because of an abnormal signaling of readiness for birth, and have outgrown their somewhat aged placenta. Postmature foals become more abnormal the longer they are maintained, also may suffer from placental insufficiency, and are represented best by the classic foal born to a mare ingesting endophyte-infested fescue.[165] Box 19-3 compares the characteristics of premature/dysmature foals with those of postmature foals.

The causes of prematurity/dysmaturity/postmaturity include the causes of high-risk pregnancy presented in Box 19-1. Additional causes include iatrogenic causes such as early elective induction of labor based on inaccurate breeding dates or misinterpretation of late-term colic or uterine bleeding as ineffective labor. Most causes remain in the category of idiopathic, with no discernible precipitating factor. Despite lack of an obvious cause, 1396

premature labor and delivery does not just happen, and even if undetermined, the cause may continue to affect 1397 the foal in the postparturient period. All body systems may be affected by prematurity, dysmaturity, and postmaturity, and thorough evaluation of all body systems is necessary.

19.4.4.1

BOX 19-3 CLINICAL CHARACTERISTICS OF PREMATURE/DYSMATURE AND POSTMATURE FOALS

19.4.4.1.1

Premature/Dysmature

Low birth weight

Small frame; thin

Poor muscle development possible

Flexor laxity common

Periarticular laxity

Hypotonia more common

High chest wall compliance

Low lung compliance

Short, silky hair coat

Domed forehead

Floppy ears; poor cartilage development

Weak suck reflex

Poor thermoregulation

Gastrointestinal tract dysfunction

Delayed maturation of renal function: low urine output

Entropion with secondary corneal ulcers

Poor glucose regulation

19.4.4.1.2 Postmature

Normal to high birth weight

Large frame; thin

Poor muscle development possible

Flexor contraction common

Hypertonia more common

Long hair coat

Fully erupted incisors

Weak suck reflex

Poor thermoregulation

Gastrointestinal tract dysfunction

Delayed maturation of renal function: low urine output

Poor glucose regulation

Delayed time to standing

- Hyperreactive
- Poor postural reflexes

Adapted from Palmer JE: Prematurity, dysmaturity, postmaturity. Proceedings of the International Veterinary Emergency Critical Care Symposium, San Antonio, Tex., 1998. pp 722-723.

Respiratory failure is common in these foals, although the cause usually is not surfactant deficiency. Immaturity of the respiratory tract, poor control of respiratory vessel tone, and weak respiratory muscles combined with poorly compliant lungs and a greatly compliant chest wall contribute to respiratory failure in these cases. Most require oxygen supplementation and positional support for optimal oxygenation and ventilation. One must extend effort to maintain these “floppy foals” in sternal recumbency. Some foals may require mechanical ventilation. These foals also require cardiovascular support but are frequently unresponsive to commonly used pressors and inotropes: dopamine, dobutamine, epinephrine, and vasopressin. Careful use of these drugs and judicious intravenous fluid therapy are necessary. The goal should not be one of achieving specific pressure values (e.g., mean arterial pressure of 60 mm Hg) but of adequate perfusion. Renal function, reflected in low

urine output, is frequently poor initially in these cases because of delay in making the transition from fetal to neonatal glomerular filtration rates.[166] The delay can result from true failure of transition or from hypoxic/ischemic insult. One should approach fluid therapy cautiously in these cases; initial fluid restriction may be in order to avoid fluid overload. Many premature/dysmature/postmature foals have suffered a hypoxic insult and have all of the disorders associated with PAS, including HIE. Treatment is similar to that of term foals with these problems. These foals also are predisposed to secondary bacterial infection and must be examined frequently for signs consistent with early sepsis or nosocomial infection.

The gastrointestinal system of these foals is not usually functionally mature, which may result from a primary lack of maturity or from hypoxia. Dysmotility and varying degrees of necrotizing enterocolitis are common. One commonly encounters hyperglycemia and hypoglycemia. Hyperglycemia generally is related to stress, increased levels of circulating catecholamines, and rapid progression to gluconeogenesis, whereas hypoglycemia is associated with diminished glycogen stores, inability to engage gluconeongenesis, sepsis, and hypoxic damage.[167] Immature endocrine function is present in many of these foals, particularly regarding the hypothalamic-pituitary-adrenal axis, and contributes to metabolic derangements.[168,169] One should delay enteral feeding when possible until the foal is stable regarding metabolic and cardiorespiratory parameters. On intiating enteral feeding, one should provide small volumes initially and slowly increase the volume over several days.

One frequently encounters musculoskeletal problems, particularly in premature foals, that include significant flexor laxity and decreased muscle tone. Postmature foals frequently are affected by flexure contracture deformities, most likely because of decreased intrauterine movement as they increase in size. Premature foals frequently exhibit decreased cuboidal bone ossification that predisposes them to crush injury of the carpal and tarsal bones if weight bearing is not strictly controlled. Physical therapy in the form of standing and exercise is indicated in the management of all these problems, but one should take care to ensure that the patient does not fatigue or stand in abnormal positions. Bandaging of the limbs is contraindicated because this only increases laxity, although light bandages over the fetlock may be necessary to prevent injury to that area if flexor laxity is severe. The foals are predisposed to angular limb deformity and must be observed closely and frequently for this problem as they mature.[170]

The overall prognosis for premature/dysmature/postmature foals remains good with intensive care and good attention to detail. Many of these foals (up to 80%) survive and become productive athletes.[21] Complications associated with sepsis and musculoskeletal abnormalities are the most significant indicators of poor athletic outcome.

19.4.5 SEPSIS

The last 20 years have seen an explosion of new therapeutic agents purportedly useful for treating sepsis. Unfortunately, clinical trials investigating these new therapies have failed to demonstrate a positive effect, have shown negative results, or have resulted in diametrically opposed study results, one showing a benefit and another showing no benefit or a detrimental effect. On a positive note, the survival rate of foals being treated for sepsis has improved. Work was done regarding foal diseases and their treatment in the 1960s, but the field did not attract much serious attention until the 1980s. Since that time almost every major veterinary college and many large private referral practices have constructed NICUs or their equivalent. Next to hypoxic ischemic asphyxial syndromes, sepsis is the number one reason for presentation and treatment at these facilities. Neonatal septicemia of the horse has been the subject of three international workshops,[171–173] and a

perinatology lecture covering some aspect of neonatal sepsis has been presented at almost every large continuing education meeting attended by equine veterinarians.

Concensus criteria conferences[1] in the early 1990s defined sepsis and septic shock for human beings.[174,175] *Sepsis* was defined as the systemic response to infection manifested by two or more of the following conditions as a result of infection: a) temperature >38° C or <36°; b) heart rate >90 beats/min; c) respiratory rate >20 breaths per minute or Pa_{CO_2} <32 torr; and d) white blood cell count >12,000 cell/μl, <4,000 cell/μl, or >10% immature (band) forms. *Septic shock* was defined as sepsis induced hypotension or the requirement for vasopressors/ionotropes to maintain blood pressure despite adequate fluid resuscitation along with the presence of perfusion abnormalities that may include lactic acidosis, oliguria, or acute alteration in mental status. These definitions are broadly acceptable and applicable to neonatal sepsis in foals, and many of the treatment modalities in human medicine have been applied in some manner to the equine neonatal patient. Additional definitions that have come into vogue that are actually useful at times, include the following: SIRS, the systemic inflammatory response system; MODS, multiple organ system dysfuction; and MOFS, multiple organ failure syndrome. (SIRS is sick, MODS is sicker, and MOFS is dying.) The compensatory response syndrome (CARS) ideally balances SIRS and keeps it from becoming detrimental. If balance is achieved, recovery is possible. Imbalance progresses to septic shock, MODS, and MOFS. In horses, MODS is manifested most commonly as renal failure, hepatic failure, central nervous system dysfunction, and disseminated intravascular coagulation. Managing the septic patient involves early recognition of all the potential alphabet combinations and supporting the patient or intervening in the face of multiple clinical consequences, termed *CHAOS* (*C*ardiovacular compromise; *H*omeostasis; *A*poptosis; *O*rgan dysfunction; *S*uppression of the immune system).[176] Inflammatory mediators are involved in all these processes and can be beneficial or detrimental, depending on timing and opposing responses. Neutrophils, platelets, lymphocytes, macrophages, and endothelial cells are involved, and the implicated inflammatory molecules grow daily in numbers.

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Sepsis in the foal initially can be subtle, and the onset of clinical signs varies depending on the pathogen involved and the immune status of the foal. For the purposes here, the discussion is limited to bacterial sepsis, but the foal also is susceptible to viral and fungal sepsis, which can appear similar to bacterial sepsis. Failure of passive transfer (FPT) of immunity can contribute to the development of sepsis in a foal at risk.[177,178] Testing for and treating FPT has received attention in the veterinary literature. It remains true, however, that foals presented to NICUs that have an ultimate diagnosis of sepsis have FPT.[16,19] The current recommendation is that foals have IgG levels greater than or equal to 800 mg/dl for passive transfer to be considered adequate. Other risk factors for the development of sepsis include any adverse advents at the time of birth, maternal illness, or any abnormalities in the foal. Although the umbilicus frequently is implicated as a major portal of entry for infectious organisms in the foal, the gastrointestinal tract may be the primary site of entry.[179] Other possible portals of entry include the respiratory tract and wounds.

Early signs of sepsis include depression, decreased suck reflex, increased recumbency, fever, hypothermia, weakness, dysphagia, failure to gain weight, increased respiratory rate, tachycardia, bradycardia, injected mucous membranes, decreased capillary refill time, shivering, lameness, aural petechia, and coronitis. If sepsis is recognized early, patients with sepsis may have a good outcome, depending on the pathogen involved. Gram-negative sepsis remains the most commonly diagnosed, but increasingly gram-positive septicemia is being recognized.[180] Foals in intensive care units and at referral hospitals have an additional risk of nosocomial infection. An attempt to isolate the organim involved early in the course of the disease becomes important. If possible, one should obtain blood cultures, and if localizing signs are present, one should obtain samples as deemed appropriate.

Cultures should be aerobic and anaerobic. Recently, work has been done evaluating real-time polymerase chain reaction technology in sepsis in the foal as a means of identifying causative organisms.[181,182] Until one obtains antimicrobial sensitivity patterns for the pathogen involved, one should initiate broad-spectrum antimicrobial therapy (Table 19-5). Intravenously administered amikacin and penicillin are good first-line choices, but one should monitor renal function closely. Other first-line antimicrobial choices might include high-dose ceftiofur sodium or ticarcillin/clavulanic acid. One should treat failure of passive transfer if present. One should provide intranasal oxygen insufflation at 5 to 10 L/min even if hypoxemia is not present to decrease the work of breathing and provide support for the increased oxygen demands associated with sepsis.[183] Should arterial blood gas analysis reveal significant hypoventilation, one may administer caffeine orally or per rectum to increase central respiratory drive. Mechanical ventilation may be necessary in cases of severe respiratory involvement such as with acute lung injury or acute respiratory distress syndrome. If the foal is hypotensive, one may administer pressor agents or inotropes by constant rate infusion (Table 19-6). Inotrope and pressor therapy generally is restricted to referral centers where these drugs can be given as constant rate infusions and blood pressure can be monitored closely. Some practitioners use nonsteroidal antiinflammatory agents and, in specific circumstances, corticosteroids. Use of these drugs should be judicious because they may have several negative consequences for the foal including renal failure and gastric/dunodenal ulceration.[184–186]

Nursing care is one of the most important aspects of treating septic foals. Foals should be kept warm and dry. 1398
They should be turned at 2-hour intervals if they are recumbent. Feeding septic foals can be a challenge if 1399
gastrointestinal function is abnormal, and total parenteral nutrition may be needed. If at all possible, foals should be weighed daily and blood glucose levels monitored frequently. Some foals become persistently hyperglycemic on small glucose infusion rates. These foals may benefit from constant rate low-dose insulin infusions (Table 19-7). Recumbent foals must be examined frequently for decubital sore development, the appearance of corneal ulcers, and for heat and swelling associated with joints and physis.

TABLE 19-5 Antimicrobial Drugs Dosages Used in Foals in the Neonatal Intensive Care Unit

DRUG	DOSE	ROUTE	FREQUENCY	COMMENTS
Amikacin sulfate	25–30 mg/kg	IV*	s.i.d.	Requires therapeutic drug monitoring: peak >60; trough <2
Ampicillin trihydrate	20 mg/kg	p.o.	t.i.d.	Poor absorption noted in foals >2–3 weeks of age.
Sodium ampicillin	50–100 mg/kg	IV	q.i.d.	—
Amoxicillin trihydrate	20–30 mg/kg	p.o.	t.i.d.	Poor absorption noted in foals >2–3 weeks of age.
Cefotaxime	50–100 mg/kg	IV	q.i.d.	—
Cefuroxime (Ceftin)	30 mg/kg/day	p.o.	b.i.d., t.i.d.	Total daily dose is divided into 10 mg/kg t.i.d. or 15 mg/kg b.i.d.
Cephalexin	10 mg/kg	p.o.	q.i.d.	—
Ceftiofur (Naxcel)	10 mg/kg	IV	q.i.d.	Give slowly over 20 minutes as double-diluted volume in 0.9% saline.
Chloramphenicol palmitate	50 mg/kg	p.o.	q.i.d.	Public health concerns
Chloramphenicol succinate	10–25 mg/kg	IV	q.i.d.	Public health concerns
Enrofloxacin	2.5–10 mg/kg	IV, p.o.	s.i.d.	Chondropathy and arthropathy reported in foals.
Erythromycin stearate	20–30 mg/kg	p.o.	t.i.d. to q.i.d.	Avoid warm temperatures and high humidity. Colitis reported in dams of foals receiving this drug.
Erythromycin lactiobionate	~5 mg/kg	IV	Every 4–6 hours	—
Gentamicin sulfate	8.8 mg/kg	IV	s.i.d.	Requires therapeutic drug monitoring: peak >40; trough <2
Imipenem	10–20 mg/kg	IV	q.i.d.	Seizures reported as adverse reaction.
Metronidazole	15 mg/kg 25 mg/kg	p.o., as needed	q.i.d. b.i.d.	Anorexia can occur.
Potassium penicillin Sodium penicillin	20,000–50,000 U/kg	IV	q.i.d.	Give potassium penicillin slowly over 5 to 10 minutes.
Rifampin	5 mg/kg	p.o.	b.i.d.	Always administer with second antimicrobial because of rapid development of resistance.

Ticarcillin	50–100 mg/kg	IV	q.i.d.	—
Ticarcillin and clavulonic acid (Timentin)	50–100 mg/kg	IV	q.i.d.	—
Trimethoprim-sulfonamide	30 mg/kg	p.o.	b.i.d.	—
Fluconazole	8 mg/kg loading; 4 mg/kg	p.o.	b.i.d.	—
Adapted from Palmer JE: *Neonatal drug doses*. Proceedings of the sixth International Veterinary Emergency Critical Care Symposium, San Antonio, Tex., 2000.				

* *IV,* Intravenous.

TABLE 19-6 Inotrope and Pressor Medications Used in the Neonatal Intensive Care Unit[*]

DRUG	DOSE	ROUTE	COMMENT
Dopamine	3–20 μg/kg/min	IV-CRI[†]	Follow the rule of 6: 6 × mass of foal (kg) = number of milligrams to add to 100 ml saline (1 ml/hr = 1 g/kg/min)
Dobutamine	3–40 μg/kg/min	IV-CRI	
Epinephrine[‡]	0.2–2 μg/kg/min	IV-CRI	
Norepinephrine[‡]	0.2–3 μg/kg/min	IV-CRI	
Data fom Connoly Neonatal Intensive Care Unit, Kennet Square, Penn.			

* One should use these medications to effect and should monitor blood pressure during their use.

† *IV-CRI,* Intravenously at constant rate infusion.

‡ For epinephrine and norepinephrine, apply a “rule of 0.6” where 0.6 × mass of foal (kg) = number of mg drug to add to 100 ml saline so 1 ml/hr = 0.1 μg/kg/min.

The prognosis for foals in the early stages of sepsis is fair to good. Once the disease has progressed to septic 1399
shock the prognosis decreases, although short-term survival rates are as good as those in human critical care 1400
units. Long-term survival and athletic outcomes are fair. Racing breed foals that make it to the track perform similarly to their age-matched siblings.[21]

TABLE 19-7 Miscellaneous Drugs Used in the Neonatal Intensive Care Unit

DRUG	DOSE	ROUTE	FREQUENCY	COMMENTS
Aminophylline	2–3 mg/kg	IV[*]	b.i.d. to q.i.d.	Monitor theophylline levels. Therapeutic: 6–12 μg/l Toxic: >20 μg/l
Caffeine	10 mg/kg loading dose 2.5 mg/kg maintenance	Orally as needed	s.i.d., b.i.d.	Steady state level: 5–20 μg/l Toxic: >50–75 μg/l
Dimethyl sulfoxide	1 g/kg	IV	Once	Administer as 5% to 10% solution; dimethyl sulfoxide is hypertonic.
Heparin	40–100 U/kg	SQ/IV	b.i.d.	—
Insulin (protamine zinc)	0.15 U/kg	IM/SQ	b.i.d.	—
Insulin (regular)	0.00125–0.2 U/kg/hr	IV	Constant rate Infusion	Pretreat lines: insulin adsorbs to lines.

Adapted from Palmer JE: *Neonatal drug doses*. Proceedings of the International Veterinary Emergency Critical Care Symposium, San Antonio, Tex., 2000.

* *IV,* Intravenous; *SQ,* subcutaneous; *IM,* intramuscular.

19.5 Other Diseases Causing Weakness In Foals

19.5.1 BOTULISM

Botulism is a neuromuscular disease of foals characterized by flaccid paralysis.[187] Although the disease is discussed in detail elsewhere in this text, the form most commonly observed in foals, the toxicoinfectious form, deserves some specific comments. The causative organism is *Clostridium botulinum,* an anaerobic organism. Although affected adults usually acquire the disease by ingestion of preformed toxin elucidated from the organism, in the foal less than 8 months of age the organism can survive and multiply in the gastointestinal tract and produce necrotic foci within the liver, giving the foal constant exposure to newly formed toxin. The horse is exquisitely sensitive to the toxin, and only small quantities of toxin are required to produce clinical signs and death in affected animals. The ε-toxin of *C. botulinum* binds to the presynaptic membrane of motor neurons and prevents transmission of impulses by blocking the release of acetylcholine from the presynaptic vessicles. This block produces the clinical signs of muscle weakness, manifested in foals as trembling (shaker foals) or acute recumbency.[188] Pupillary dilation, dysphagia, tremors, recumbency, and terminal respiratory distress caused by respiratory muscle paralysis occur. Foals can be found acutely dead. In endemic areas (the Northeast and mid-Atlantic regions of United States), for these foals to be evaluated first as having colic is not unusual.

Treatment aims to neutralize the toxin by administration of botulinum antitoxin and to provide antimicrobial treatment of the infection with penicillin, metronidazole, and/or oxytetracycline.[189,190] At a minimum, feeding of milk replacer via indwelling nasogastric tube at 20% of the body weight of the foal per day divided into

every 2-hour meals is required. Many of these foals require respiratory support (in the form of intranasal oxygen insufflation), because of respiratory muscle paralysis. Respiratory acidosis is present on arterial blood gas analysis in most of these foals because of hypoventilation and lateral recumbency, but they can tolerate some degree of hypercapnia ($Pa{CO_2}$ ~70 mm Hg) if the pH is normal and oxygenation ($Pa{O_2}$ >70 mm Hg; percent oxygen saturation of hemoglobin, >90%) is adequate. Metabolic alkalosis can accompany the respiratory acidosis, but this is a compensatory change and resolves once gas exchange is normalized. Some of these patients require mechanical ventilation, which may be lifesaving. One may discontinue mechanical ventilation as clinical signs resolve and the respiratory muscles gain strength. Nursing care is important, and these foals should be turned every 2 hours. They should be maintained in sternal recumbency if possible and kept warm and dry. With good nursing care, good nutritional support, and adequate respiratory support, the prognosis for these foals is good. The limiting factor in the prognosis for life is often financial.[190] Foals that recover from the acute stage of this disease eventually fully recover.

Botulism is an expensive disease to treat and is also an entirely preventable disease.[189,190] All pregnant mares in endemic areas should be vaccinated against *C. botulinum*. Vaccination does not prevent all cases of botulism, particularly if the foal has failure of passive transfer or acquires the disease after maternal immunity wanes and before its own vaccination. 1400

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19.5.2 NUTRITIONAL MUSCULAR DYSTROPHY (WHITE MUSCLE DISEASE)

Nutritional muscular dystrophy or white muscle disease is a vitamin E/selenium–responsive muscle disease of horses of all ages probably caused by a dietary deficiency of selenium and vitamin E.[191] The condition occurs most commonly in geographic areas with low selenium levels in the soil, generally the northeastern, northwestern, Great Lakes and mid-Atlantic regions of the United States.

Two forms of the disease are described in foals: the fulminant form, in which the foal is found acutely dead, and the subacute form. In the fulminant form, death usually is attributed to myocardial lesions resulting in cardiovascular collapse. The subacute form is characterized by dysphagia and gait abnormalities primarily caused by stiffness of the muscles of locomotion. Paralysis, if present, is not flaccid as in botulism. Abnormal function of respiratory muscles may complicate the clinical situation. Aspiration pneumonia may be present following problems associated with swallowing; the tongue and pharyngeal muscles frequently are affected in the early stages of disease.[191] Foals with severe disease may have widespread muscle necrosis leading to hyperkalemia, which can be severe and result in death of the foal. Serum activities of the muscle enzymes creatine kinase and aspartate aminotransferase may be greatly increased. Diagnosis is confirmed at necropsy or ante mortem by determination of decreased vitamin E, selenium, and glutathione peroxidase concentrations in the blood of the foal before supplementation. Myoglobinuria and acute renal failure are not uncommon in these foals.

Treatment of foals with nutritional muscular dystrophy is primarily supportive. One should address all metabolic abnormalities. Some foals require intranasal oxygen insufflation. Affected foals are unable to suck effectively, and one should provide enteral (via an indwelling nasogastric tube) or parenteral nutritional support. Because of the high likelihood of aspiration pneumonia, one should administer broad-spectrum antimicrobial therapy parenterally. The patient should be kept quiet and should be stimulated minimally. Affected foals should receive parenteral (intramuscular) vitamin E and selenium supplementation. Selenium is toxic in large doses. The prognosis for severely affected foals is guarded. For less severely affected foals the prognosis is good with appropriate treatment. The disease is preventable by ensuring that mares receive sufficient vitamin E and selenium while pregnant and by supplementing foals with parenteral injections of

vitamin E and selenium at birth in endemic areas. A more complete discussion of the pathophysiology of this disease and the nutritional management is presented elsewhere in this text.

19.6 Diseases Causing Abnormal Mentation Or Other Neurologic Signs In Foals (Other Than Perinatal Asphyxia Syndrome)

19.6.1 HEPATIC ENCEPHALOPATHY

Primary liver disease is uncommon in the foal and occurs primarily as a sequela to sepsis. Clinical signs of severe liver disease may include depression, ataxia, and seizures. In affected foals, increases in serum liver enzyme activities and concentrations of ammonia and bile acids frequently can be identified. The mechanism(s) underlying hepatoencephalopathy are not delineated clearly, although increased excitatory neurotransmitters, or compounds that mimic their activity, are implicated. Hepatoencephalopathy is discussed in more detail elsewhere in this text.

Tyzzer's disease (*Clostridium piliformis* infection) rarely causes primary liver disease in foals from 4 to about 40 days of age. This disease is almost uniformly fatal. The incubation period is short, and the mare is thought to be the carrier.[192–196] Clinical signs range from acute death to depression, fever, and pronounced icterus. The feces of affected foals may appear white to grey because of the lack of bile. Clinicopathologic abnormalities include leukopenia, hyperfibrinogenemia, metabolic acidosis, and hypoglycemia.[197,198] Liver lesions at postmortem are characterized microscopically by multiple foci of necrosis. One usually can demonstrate variable numbers of elongated, slender intracytoplasmic bacilli within hepatocytes bordering the necrotic foci. Infiltration of the portal triads with inflammatory cells and biliary duct hyperplasia and degeneration are observable. The bacillus also occurs in association with myocardial lesions. Lesions in the intestine are characterised by mucosal necrosis with inflammatory cell infiltration, increased mucus production, submucosal lymphoid hyperplasia, and submucosal hemorrhage. Necrosis of lymphoid follicles, congestion, and hemorrhage can be present in the spleen and mesenteric lymph nodes.[196] Affected foals may have a profound metabolic acidosis that is unresponsive to treatment. The clinical course is short, and most affected foals die within a few hours of developing neurologic signs.

Primary liver disease has been reported in association with ferrous sulfate administration in a probiotic compound.[199] The lesion was massive hepatocellular necrosis and liver failure. The product is no longer commercially available. Portosystemic shunt is rare in the foal but has been reported in foals as young as 3 months of age.[200–202] 1401

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19.6.2 INFECTIOUS CAUSES

Most infectious causes of neurologic abnormalities in foals are associated with sepsis. Although rarely reported, *Halicephalobus gingivalis (deletrix)* infection has been reported in three foals; in one case the foal was 3 weeks of age.[203,204] Possibly transmission in these cases was transmammary; the dam in one case died 1 year later with confirmed *H. deletrix* infestation of her udder. *Listeria monocytogenes* has been reported as a cause of neurologic disease in foals.[205] Recently, *Sarcocystis neurona* was identified as the causative agent of central nervous system disease in a foal, and equine herpes myeloencephalitis has been diagnosed in individual foals and in herd outbreaks involving foals.[206,207] *Neospora* also was reported in one foal recently.[208] *Rhodococcus*

equi abscesses can form in the central nervous system or cause neurologic signs associated with compression, as with vertebral body abscesses.[209–211]

19.6.3 OTHER DISEASES

Cerebellar hypolasia, occipitoatlantoaxial malformation, and agenesis of the corpus callosum with cerebellar vermian hypoplasia have been reported in foals.[212–217] Ivermectin toxicity and moxidectin toxicity have been reported.[218,219] Electrolyte abnormalities such as extreme hypo- or hypernatremia may result in neurologic manifestations of disease.[220,221] Cervical stenotic myelopathy and degenerative myelopathy also have been reported in foals, although the age at onset is usually more than 4 months.[222] Idiopathic epilepsy of Arabian foals usually is associated with another infectious disease and is thought to be temporary and self-limiting.

19.7 Immunologic Diseases Of Foals

19.7.1 FAILURE OF PASSIVE TRANSFER

Causes, diagnosis, and treatment of FPT of immunity are covered in detail elsewhere in this text. Failure of passive transfer occurs when a foal fails to ingest a significant quantity of good-quality colostrum. Failure of passive transfer may occur by several mechanisms: failure of the foal to suck from the dam for any reason and failure of the dam to produce sufficient quantity of quality colostrum. Box 19-4 presents causes of FPT. Several methods are available for measuring IgG concentration in blood; the most reliable are enzyme-linked immunosorbent assay and single radial immunodiffusion technology-based tests.[223–229] Foals usually are tested at 24 hours of age, but one may test the foal earlier if colostrum ingestion has occurred and a concern exists regarding the passive transfer of immunity status of the foal, recognizing that additional increases in IgG concentration may occur with additional time.[230,231] The concentration of IgG in the blood of the foal has been used as an indicator of the adequacy of passive transfer, but the actual blood concentration at which FPT is diagnosed has been challenged in recent years.[232–234] Foals with sepsis commonly have a serum IgG concentration of less than 800 mg/dl.[16,19] Foals with FPT are more likely to die from sepsis.[177,178,235–237] One should consider the IgG concentration only as a marker for adequacy of colostral absorption. All the measured IgG is unlikely to be directed against the specific pathogen affecting any particular neonate, and IgG is not the only immune protection afforded the foal by colostrum. Many factors that confer local and more general immunity to the newborn are present in colostrum; these include growth factors, cytokines, lactoferrins, CD14, leukocytes, and other yet to be described proteins.[240–244] By considering IgG a marker of adequacy for passive transfer, similar to γ-glutamyltransferase in calves, the clinician can make choices for replacement that are more beneficial to the patient.[245] After one identifies FPT in a foal, treatment depends on the current condition of the foal and its local environment. Foals not presently ill and on well-managed farms with low population density and low prevalence of disease may not require treatment if their IgG concentration is between 400 and 800 mg/dl. Critically ill neonates with FPT in an equine NICU are by definition ill and in an environment with high 1402
disease prevalence. These patients require immediate treatment of FPT and frequent reassessment of their 1403
passive immunity status. Critically ill foals often fail to demonstrate the expected increase in blood IgG concentration based on grams of IgG administered per kilogram of body mass compared with healthy, colostrum-deprived foals.[235,246,247] Sick foals also demonstrate a more rapid decline in IgG concentration than do healthy foals because they use and catabolize available protein.

19.7.1.1 BOX 19-4 CAUSES OF FAILURE OF PASSIVE TRANSFER

19.7.1.1.1 Maternal Causes

Premature lactation

- Placentitis
- Twins
- Premature placental separation

Poor colostral quality

- Maiden mares
- Older mares

Failure of lactation

- Aglactia
- Fescue toxicosis

19.7.1.1.2 Foal Causes

Failure to ingest colostrum

- Weakness
- Prematurity
- Musculoskeletal deformity
- Perinatal asphyxia syndrome

Failure to absorb colostrum

- Prematurity

Necrotizing enterocolitis

One may treat foals with FPT by oral or intravenous administration of various products containing IgG. One can attempt oral administration of additional colostrum or IgG-containing products such as plasma, serum, or lyophilized colostrum in foals less than 12 to 24 hours of age.[248–250] Depending on the age of the foal and the maturity and function of the gastrointestinal tract, this treatment may be effective. Many NICUs and large breeding farms maintain colostrum banks for this purpose. One should administer plasma intravenously if the foal is not expected to absorb additional colostrum or if the enteral route is unavailable. Commercially available hyperimmune plasma products designed for use in foals are available and can be stored frozen. Plasma and banked colostrum should be stored in a non–frost-free freezer to minimize protein loss associated with freeze-thaw cycling.[251] One should administer plasma through special tubing with an in-line filter and should monitor patients closely for transfusion reactions.[252] One may use serum and concentrated IgG products, but the practitioner should be aware that many of these products focus on IgG retention and not on other factors associated with passive transfer of immunity. One should measure IgG concentration after transfusion and provide additional plasma as necessary. Administration of plasma to critically ill foals without FPT may be beneficial through provision of other factors present in the plasma. In these situations, fresh frozen plasma or fresh plasma may be best, particularly if transfusion of clotting proteins is desired.

19.7.2 NEONATAL ISOERYTHROLYSIS

Neonatal isoerythrolysis is a hemolytic syndrome in newborn foals caused by a blood group incompatibility between the foal and dam and is mediated by maternal antibodies against foal erythrocytes (alloantibodies) absorbed from the colostrum. The disease most often affects foals born to multiparous mares and should be suspected in foals less than 7 days of age with clinical signs of icterus, weakness, and tachycardia. A primiparous mare can produce a foal with neonatal isoerythrolysis if she has received a prior sensitizing blood transfusion or has developed placental abnormalities in early gestation that allowed leakage of fetal red blood cells into her circulation. Many are the causes of jaundice in newborn foals, including sepsis, meconium impaction, and liver failure, but these usually can be differentiated readily from neonatal isoerythrolysis by measuring the packed cell volume, which is usually less than 20% in foals with neonatal isoerythrolysis.

Foals with neonatal isoerythrolysis are born clinically normal then become depressed and weak and have a reduced suckle response within 12 to 72 hours of birth. The rapidity of onset and severity of disease are determined by the quantity and activity of absorbed alloantibodies. Affected foals have tachycardia, tachypnea, and dyspnea. The oral mucosa is initially pale and then becomes icteric in foals that survive 24 to 48 hours. Hemoglobinuria may occur. Seizures caused by cerebral hypoxia are a preterminal event.

The salient laboratory findings are anemia and hyperbilirubinemia. Most of the increased bilirubin is unconjugated, although the absolute concentration of conjugated bilirubin generally is increased well above normal. Urine may be red to brown and is positive for occult blood.

19.7.2.1 Cause and Pathogenesis

The natural development of neonatal isoerythrolysis has several prerequisites. First, the foal must inherit from the sire and express an erythrocyte antigen (alloantigen) that is not possessed by the mare. Blood group incompatibility between the foal and dam is not particularly uncommon, but most blood group factors are not

strongly antigenic under the conditions of exposure through previous parturition or placental leakage. Factor Aa of the A system and factor Qa of the Q system are highly immunogenic, however, and nearly all cases of neonatal isoerythrolysis are caused by antibodies to these alloantigens. The exception is in the case of mule foals in which a specific donkey factor has been implicated.[253–255] Mares that are negative for Aa or Qa or both are considered to be at risk for producing a foal with neonatal isoerythrolysis. The risk involves approximately 19% and 17% of Thoroughbred and Standardbred mares, respectively. Second, and perhaps most important, the mare must become sensitized to the incompatible alloantigen and produce antibodies to it. The mechanism for this is not known in many instances but generally is believed to result from transplacental hemorrhage during a previous pregnancy involving a foal with the same incompatible blood factor.[255]

Sensitization via transplacental contamination with fetal erythrocytes earlier in the current pregnancy is possible, but an anamnestic response is generally necessary to induce a pathogenic quantity of alloantibodies.[256] Ten percent of Thoroughbred mares and 20% of Standardbred mares have antibodies to the Ca blood 1403
group antigen without known exposure to erythrocytes.[255] Some common environmental antigen is 1404
postulated possibly to lead to production of anti-Ca antibodies. Data suggest that these natural antibodies may suppress an immune response to other blood group antigens because mares negative for Aa that have anti-Ca antibodies often do not produce antibodies to Aa of the erythrocytes in their foals that also contain Ca antigen. This antibody-mediated immunosuppression is thought to result from the destruction of fetal cells before the dam mounts an immune response to other cell surface antigens. Natural alloantibodies have not been associated with neonatal isoerythrolysis in horses.

After the mare becomes sensitized to the erythrocytes of her foal, alloantibodies are concentrated in the colostrum during the last month of gestation. Unlike the human neonate, which acquires alloantibodies in utero and thus is born with hemolytic disease, the foal is protected from these antibodies before birth by the complex epitheliochorial placentation of the mare. Thus the final criterion for foal development of neonatal isoerythrolysis is ingestion in the first 24 hours of life of colostrum-containing alloantibodies specific for foal alloantigens. Immunoglobulin-coated foal erythrocytes are removed prematurely from circulation by the mononuclear phagocyte system or are lysed intravascularly via complement. The rapidity of development and severity of clinical signs are determined by the amount of alloantibodies that was absorbed and their innate activity. Alloantibodies against Aa are potent hemolysins and generally are associated with a more severe clinical syndrome than antibodies against Qa or other alloantigens. The highest alloantibody titers are likely to be produced by mares that were sensitized in a previous pregnancy and then subsequently reexposed to the same erythrocyte antigen during the last trimester of the current pregnancy. Prior sensitization of a mare by blood transfusion or other exposure to equine blood products may predispose to neonatal isoerythrolysis.[256]

19.7.2.2 Diagnosis

One can make a tentative diagnosis of neonatal isoerythrolysis in any foal that has lethargy, anemia, and icterus during the first 4 days of life. Blood loss anemia caused by birth trauma is attended by pallor. Icterus caused by sepsis or liver dysfunction would not be associated with anemia. One must base the definitive diagnosis of neonatal isoerythrolysis on demonstration of alloantibodies in the serum or colostrum of the dam that are directed against foal erythrocytes. The most reliable serodiagnostic test for neonatal isoerythrolysis is the hemolytic cross-match using washed foal erythrocytes, mare serum, and an exogenous source of absorbed complement (usually from rabbits).[5] Although this test is impractical in a practice setting, a number of qualified laboratories routinely perform this diagnostic service. The direct antiglobulin test (Coombs' test)

may demonstrate the presence of antibodies on foal erythrocytes; however, false negatives occur frequently. Most human or veterinary hematology laboratories can perform routine saline agglutination cross-match between mare serum and foal cells. Because some equine alloantibodies act only as hemolysins, agglutination tests may be falsely negative. Most field screening tests of colostrum have not proved to be reliable enough for practical use.

19.7.2.3 Treatment

If one recognizes neonatal isoerythrolysis when the foal is less than 24 hours old, one must withhold the dam's milk and feed the foal an alternative source of milk during the first day of life. One can accomplish this by muzzling the foal and feeding it via nasogastric tube. The minimum necessary amount of milk is 1% of body mass every 2 hours (e.g., a 50-kg foal should receive 500 ml or 1 pint of mare's milk or milk replacer every 2 hours). The udder of the mare should be stripped regularly (at least every 4 hours) and the milk discarded. In most instances, clinical signs are not apparent until after the foal is 24 hours old, when colostral antibodies have been depleted or the absorptive capacity of the foal's intestine for immunoglobulin has diminished. Withholding milk at this point is of minimal benefit.

Supportive care to ensure adequate warmth and hydration is paramount. The foal should not be stressed and exercise must be restricted. Confining the mare and foal to a box stall is a best. Intravenous fluids are indicated to promote and minimize the nephrotoxic effects of hemoglobin and to correct any fluid deficits and electrolyte and acid-base imbalances. Antimicrobials may be necessary to prevent secondary infections.

One should monitor foals carefully for the necessity of blood transfusion, although transfusion should be used only as a lifesaving measure. When the packed cell volume drops below 12%, blood transfusion is warranted to prevent life-threatening cerebral hypoxia. Erythrocytes from the dam are perfect in terms of nonreactivity with the blood of the foal; however, the fluid portion of the blood of the mare has to be removed completely from the cells to prevent administration of additional harmful alloantibodies to the foal. One can pellet the erythrocytes of the dam from blood collected in acid-citrate-dextrose solution by centrifugation or gravity and then aseptically draw off the plasma by suction apparatus or syringe and replace it with sterile isotonic (0.9%) saline. One thoroughly mixes the cells with the saline and then repeats the centrifugation or sedimentation, followed by aspiration and discarding of the saline. One should perform this washing process at least three times. One then can suspend the packed erythrocytes in an equal volume of 1404

1405 isotonic saline for administration. Erythrocyte washing by centrifugation is more desirable than gravity sedimentation because antibody removal is more complete and packed cell preparations can be prepared more quickly (each gravity sedimentation requires 1 to 2 hours). Packed red blood cells are advantageous in overcoming the problem of volume overload.

When equipment or conditions do not allow the safe use of dam erythrocytes, an alternative donor is necessary. Because the alloantibodies absorbed by the foal generally are directed against Aa or Qa and because the latter are highly prevalent among most breeds of horses, a compatible blood donor is difficult to identify. The odds of finding a donor without Aa or Qa are higher in Quarter Horses, Morgans, and Standardbreds than in Thoroughbreds and Arabians. Previously blood-typed individuals negative for Aa and Qa and free of alloantibodies are optimal. One should give 2 to 4 L of blood or 1 to 2 L of packed erythrocytes over 2 to 4 hours. These allogeneic cells have a short life span and represent a large burden to the neonatal mononuclear phagocyte system, which may cause increased susceptibility to infection. In addition, these cells sensitize the foal to future transfusion reactions. One must measure all potential harm against the benefit in each situation.

If a mule foal is the patient, one should not use blood from a female previously bred to a donkey. In cases in which transfusion will be delayed, one cannot identify a compatible donor, or the packed cell volume is so low as to be life-threatening (hemoglobin <5 mg/dl), one may administer polymerized bovine hemoglobin products at a dose of 5 to 15 ml/kg.[257] One may use dexamethasone (0.08 mg/kg) to treat peracute neonatal isoerythrolysis if the packed cell volume is less than 12% and transfusion may be delayed or is not fully compatible, but dexamethasone has detrimental effects on blood glucose regulation in the neonate, and because the antibody in question is of maternal origin, corticosteroid therapy in immunosuppressive doses probably is not indicated. Intranasal oxygen insufflation (5 to 10 L/min) may be beneficial. Most foals with neonatal isoerythrolysis have adequate passive transfer of immunity, but antimicrobial therapy is indicated to protect against secondary sepsis resulting from the compromised condition of the foal. Supportive care and good nursing care, including keeping the foal warm and quiet are essential. One should expect the packed cell volume to decline again 4 to 7 days after transfusion.[258]

19.7.2.4 Client Education

The prognosis for neonatal isoerythrolysis in foals depends on the quantity and activity of absorbed antibodies and is indirectly proportional to the rate of onset of signs. In peracute cases the foal may die before the problem is recognized, whereas foals with slowly progressive signs often live with appropriate supportive care.

Like most diseases, neonatal isoerythrolysis is much more effectively prevented than treated.[259] Any mare that has produced a foal with neonatal isoerythrolysis should be suspect for the production of another affected foal; thus one should provide all subsequent foals with an alternative colostrum source and discard the colostrum of the dam unless she is bred to a stallion with known blood type compatibility. Mares negative for Aa and Qa alloantigens are most at risk of producing affected foals, thus they should be identified by blood-typing. Subsequently, breeding of these mares may be restricted to Aa- and Qa-negative stallions, thus eliminating the possibility of producing an affected foal. In breeds with a high prevalence of Aa or Qa alloantigens (e.g., Thoroughbreds and Arabians), a stallion negative for these and suitable based on other criteria may be difficult to identify. If these “at risk” mares are bred as desired, their serum should be screened in the last month of pregnancy for the presence of erythrocyte alloantibodies. One must test mares with low or equivocal titers closer to the time of parturition. If one detects alloantibodies, the colostrum of the dam should be withheld and the foal then should be provided with an alternative colostrum source. Maternal alloantibodies to Ca do not appear to mediate neonatal isoerythrolysis in foals and actually may be preventive by removing potentially sensitizing cells from the circulation[56]; therefore one should not deprive foals of colostrum from mares possessing anti-Ca antibodies, even when Ca is present on their erythrocytes. Rarely, the antigens De, Ua, Pa, and Ab have been associated with neonatal isoerythrolysis in foals; however, to consider mares without these alloantigens to be at risk for neonatal isoerythrolysis is not practical.

19.7.3 IMMUNE-MEDIATED THROMBOCYTOPENIA AND NEUTROPENIA

These syndromes recently have been recognized and described within the veterinary literature, although they have been recognized widely in human neonatology for many years.[260–263] Affected foals demonstrate these hematologic abnormalities within the first week of life, and the mechanism is similar to neonatal isoerythrolysis following ingestion of maternal antibody directed against the platelet or the neutrophil. In general, affected foals are healthy but may demonstrate bleeding tendencies if thrombocytopenia is severe or they may be more susceptible to sepsis. One confirms the diagnosis by appropriate testing for platelet- and neutrophil-associated

antibody.[264] One must rule out other causes of neonatal thrombocytopenia and neutropenia, particularly sepsis. Foals born to the mare in the future seem likely to be at risk for developing similar problems, and one should 1405

1406 treat future foals as one treats neonatal isoerythrolysis foals: prevent sucking from the dam and provide an alternate source of passive immunity in the form of banked colostrum or intravenous plasma. One should provide an alternative nutritional source, such as foal milk replacer, to the foal for the first 48 hours of life and should muzzle the foal while it is in the company of its dam for that period of time. Treatment is primarily supportive, but in the case of severe thrombocytopenia, transfusion of platelet-enriched fresh plasma may be indicated. Granulocyte colony-stimulating factor has been used in foals with neutropenia, but substantial efficacy has yet to be demonstrated. Broad-spectrum antimicrobial therapy may be prudent in cases of alloantibody-associated neutropenia. Treatment with immunosuppressive doses of corticosteroids is probably unwarranted, given the increased risk of infection, because the antibody in question is of maternal origin.

Other specific diseases of the immune system of foals, severe combined immunodeficiency, selective IgM deficiency, transient hypogammaglobulinemia, agammaglobulinemia, and other unclassified immunodeficincies are covered in detail elsewhere in this text.

19.8 Diseases Of The Respiratory Tract

19.8.1 RESPIRATORY DISTRESS

The neonate can experience respiratory distress immediately after birth because of several congenital respiratory tract or cardiac anomalies. Chief among these causes are bilateral choanal atresia, stenotic nares, dorsal displacement of the soft palate caused by anatomic deformity or neurologic impairment, accessory or ectopic lung lobes, lung lobe hypertrophy, lung lobe dysplasia, cardiac anomalies with right-to-left shunting, and miscellaneous causes such as subepiglotic cysts and severe edema of the larynx.[264–271] One must evaluate and treat these situations immediately and should consider them true emergencies.

One readily can recognize foals with airway occlusion by the lack of airflow through the nostrils despite obvious attempts to breathe and by respiratory stridor. These foals may demonstrate open-mouth breathing and their cheeks may puff outward when they exhale. One foal with congenital bilateral choanal atresia was recognized during extrauterine intrapartum resusucitation because of an inability to pass a nasotrancheal tube. One can establish an effective airway by orotracheal intubation in these cases under most circumstances, but some foals require an emergency tracheostomy. One diagnoses the underlying problem by endoscopy or radiography in most cases. Treatment of choanal atresia and cystic structures is surgical, whereas severe laryngeal edema and laryngeal paralysis frequently respond to medical management. Until the underlying problem is resolved in these cases, one should administer broad-spectrum antimicrobial therapy and feed the foal by intubation or total parenteral nutrition. One can give colostrum, but these foals frequently develop aspiration pneumonia if allowed to suck from their dams, so intravenously administered plasma also may be necessary to provide sufficient passive immunity.

19.8.2 RESPIRATORY DISEASES ASSOCIATED WITH HYPOXEMIA IN THE NEONATE

Arterial blood gas determinations are the most sensitive indicator of respiratory function readily available to the clinician. The most readily available arteries for sampling are the metatarsal arteries and the brachial arteries. Portable arterial/venous blood gas analyzers now are making arterial blood gas analysis more practical in the field, and the technique is no longer reserved for large referral practices. Managing a critically ill equine neonate without knowledge of arterial blood gas parameters is veritably impossible. Pulse oximetry is useful,

but these monitors only measure oxygen saturation of hemoglobin. Desaturation can occur rapidly in critically ill neonates. The utility of these monitors in the foal has yet to be demonstrated clearly, particularly in cases of poor peripheral perfusion.[272] The most common abnormalities recognized with arterial blood gas analysis are hypoxemia with normo- or hypocapnia and hypoxemia with hypercapnia. *Hypoxemia* is defined as decreased oxygen tension of the arterial blood (decrease PaO_2), and *hypoxia* is defined as decreased oxygen concentration at the level of the tissue, with or without hypoxemia. Hypoxia results from hypoxemia, decreased perfusion of the tissue bed in question, or decreased oxygen-carrying capacity of the blood resulting from anemia or hemoglobin alteration.

Five primary means by which hypoxemia may develop are (1) low concentration of oxygen in the inspired air such as in high altitude or in an error mixing ventilator gas; (2) hypoventilation; (3) ventilation/perfusion mismatch; (4) diffusion limitation; and (5) intrapulmonary or intracardiac right-to-left shunting of blood. Hypoxemia is not an uncommon finding in neonates but must be evaluated in terms of the current age of the foal and its position.[15,273–276] One also must consider the difficulty encountered in obtaining the sample because severe struggling can affect the arterial blood gas results. Table 19-8 presents normal arterial blood gas parameters for varying ages of foals. The normal foal has a small shunt fraction (~10%) that persists for the first few days of life and contributes slightly to a blunted response to breathing 100% oxygen compared with the adult. Hypoxemia frequently occurs in foals with prematurity, PAS, and sepsis, although other conditions 1406
also result in hypoxemia in the neonate. In the early stage of sepsis associated hypoxemia, $PaCO_2$ may be within 1407
normal limits or decreased if the foal is hyperventilating for any reason. If the lung is involved significantly in the underlying pathologic condition, such as with severe pneumonia, acute lung injury, or acute respiratory distress syndrome, increased $PaCO_2$ may well be present, representing respiratory failure.[277]

TABLE 19-8 Normal Arterial Blood Gas Values for Foals[*]

GESTATIONAL AGE	POSTNATAL AGE	POSITION	pH	$PaCO_2$ (mm Hg)	PaO_2 (mm Hg)
Term[†]	2 minutes	Lateral	7.31 ± 0.02	54.1 ± 2.0	56.4 ± 2.3
	15 minutes		7.32 ± 0.03	50.4 ± 2.7	57.5 ± 3.6
	30 minutes		7.35 ± 0.01	51.5 ± 1.5	57.0 ± 1.8
	60 minutes		7.36 ± 0.01	47.3 ± 2.2	60.9 ± 2.7
	2 hours		7.36 ± 0.01	47.7 ± 1.7	66.5 ± 2.3
	4 hours		7.35 ± 0.02	45.0 ± 1.9	75.7 ± 4.9
	12 hours		7.36 ± 0.02	44.3 ± 1.2	73.5 ± 3.0
	24 hours		7.39 ± 0.01	45.5 ± 1.5	67.6 ± 4.4
	48 hours		7.37 ± 0.01	46.1 ± 1.1	74.9 ± 3.3
	4 days		7.40 ± 0.01	45.8 ± 1.1	81.2 ± 3.1
Premature[‡]	0.5–11 hours	Lateral	7.21 ± 0.05	55.3 ± 3.6	53.7 ± 1.5

* Reported values are assumed to not be temperature corrected. Values are mean ± SEM.

† Stewart JH, Rose RJ, Barko AM: Respiratory studies in foals from birth to seven days old, *Equine Vet J* 16:323, 1984.

‡ Rose RJ, Rossdale PD, Leadon DP: Blood gas and acid-base status in spontaneously delivered term-induced and induced premature foals, *J Reprod Fert Suppl* 32:521, 1982.

Hypoxemia usually is treated with intranasal humidified oxygen insufflation at 4 to 10 L/min. Hypercapnia is not a simple matter to treat. One must try to distinguish between acute and chronic hypercapnia. Acute hypercapnia usually is accompanied by a dramatic decrease in blood pH of 0.008 pH units for each 1 mm Hg increase in $PaCO_2$. This acidemia can promote circulatory collapse, particularly in the concurrently hypoxemic and/or hypovolemic patient. The effects of more chronic CO_2 retention are less obvious because the time course allows for adaptation. The pH change is less, about 0.003 pH units per 1 mm Hg increase in $PaCO_2$, because it is balanced by enhanced renal absorption of bicarbonate by the proximal renal tubule. Most foals with acute respiratory distress are in the acute stages of respiratory failure, but chronic adaptation begins to occur within 6 to 12 hours and is maximal in 3 to 5 days. One will note an increase in bicarbonate, particularly if the acidemia is primarily respiratory in origin. Intravenous administration of sodium bicarbonate to correct respiratory acidosis/acidemia should be done cautiously in these foals because CO_2 retention may only be increased. Also, one should remember that 1 mEq of sodium is administered with each mEq of bicarbonate and hypernatremia has been seen in foals treated exuberantly with sodium bicarbonate. Foals with hypercapnia of several days' duration also may develop a blunted respiratory drive to increased CO_2. In these foals, oxygen administration, although essential to treat hypoxemia, may further depress ventilation and further decrease pH. This effect is caused by a loss of hypoxic drive following oxygen therapy. One should consider these foals candidates for mechanical ventilation if the $PaCO_2$ is greater than 70 mm Hg or is contributing to the poor condition of the foal, such as causing significant pH changes. If hypercapnia is caused by central depression of ventilation, as frequently occurs in foals with PAS, one can administer caffeine (10 mg/kg loading dose; then 2.5 mg/kg as needed) per rectum or orally in foals with normal gastrointestinal function. Other clinicians may recommend continuous rate infusions of doxapram hydrochoride (Dopram; 400 mg/total dose at 0.05 mg/kg/min) for these foals. If this therapy fails, one should consider mechanical ventilation. Mechanical ventilation of foals with central respiratory depression is rewarding and may be necessary only for a few hours to days. A special category is the foal with botulism exhibiting respiratory failure caused by respiratory muscle paralysis. These foals do well with mechanical ventilation, although the duration of mechanical ventilation is more prolonged, frequently more than 1 week. Foals with primary metabolic alkalosis usually have compensatory respiratory acidosis. Treatment of hypercapnia is not necessary in these cases because it is in response to the metabolic condition. These foals do not respond to caffeine, and they should not be ventilated mechanically if this is the only disorder present.

19.8.2.1 Bacterial Pneumonia

In the neonate, bacterial pneumonia usually results from sepsis or aspiration during sucking. Foals with sepsis can develop acute lung injury or acute respiratory distress syndrome as part of the systemic response 1407
to sepsis, and this is frequently a contributor to the demise of foals in septic shock. The best way to diagnose 1408
bacterial pneumonia is by cytologic examination and culture of a transtracheal aspirate, but blood culture may aid in early identification of the causative organism and allow for early institution of directed antimicrobial therapy. A second frequent cause of bacterial pneumonia in the neonate is aspiration caused by a poor suck reflex or dysphagia associated with PAS, sepsis, or weakness. One must take care to ensure that aspiration is not iatrogenic in foals being bottle fed. Auscultation over the trachea while the foal is sucking helps identify occult aspiration. One should suspect occult aspiration pneumonia in any critically ill neonate that is being bottle fed or is sucking on its own that has unexplained fever, fails to gain weight, or has a persistently increased fibrinogen level.

Older foals develop bacterial pneumonia, frequently following an earlier viral infection.[278] Bacterial pneumonia is discussed in depth elsewhere in this text, but a few comments specific to the foal are necessary. One should auscultate and percuss the thorax of the foal, but results may not correlate closely with the severity of disease. The most commonly isolated bacterial organism in foal pneumonia is *Streptococcus zooepidemicus,* and one may isolate it alone or as a component of a mixed infection.[278–280] Transtracheal aspirate for culture and cytologic examination is recommended because mixed gram-positive and gram-negative infections are common, and antimicrobial susceptibility patterns can be unpredictable. One should split the obtained aspirate and submit samples for bacterial culture, virus isolation, and cytologic examination. Additional diagnostics include radiography, ultrasonography, and serial determination of white blood cell counts (with differential) and blood fibrinogen concentrations. Treatment includes administration of appropriate antimicrobial therapy. Some foals may benefit from nebulization with saline or other local products. Ascarid larval migration through the lung can mimic bacterial pneumonia.[281] In these cases the foal may not respond to antimicrobial therapy and should be dewormed with ivermectin. Deworming the mare within 1 month of parturition and frequent deworming of the foal prevent ascarid migration pneumonia in most foals.

A special category of bacterial pneumonia in foals is *Rhodococcus equi* bronchopneumonia. This pneumonia of young foals was described first in 1923.[282] The organism originally was known as *Corynebacterium equi* and is a gram-positive pleomorphic coccobacillus usually less than 1 μm in diameter and 2 μm in length. The organisms frequently are associated in L- and V-shaped clusters that have been termed *Chinese character formations. R. equi* has an acid-fast staining characteristic under some growing circumstances because of the presence of mycolic acid in its cell wall, similar to *Mycobacterium* and *Nocardia* species. Mycolic acid promotes granuloma formation. The organism is able to multiply in and destroy macrophages as it prevents phagosome lysosome fusion.[283,284] Much attention has been paid to this organism in recent years, given its propensity to produce enzootic and epizootic outbreaks of disease. The organism is thought to be primarily an opportunistic pathogen, and it lives in the soil of most geographic areas. Foals are affected most frequently between the ages of 1 and 6 months, when maternally derived immunity has begun to wane. The disease is insidious, and foals may have significant pulmonary involvement before developing noticeable clinical signs.

Phagocytosis of *R. equi* by equine macrophages is not associated with a functional respiratory burst and, at least in human beings, the L-arginine–NO pathway is not required for intracellular killing of this organism.[285,286] Optimal binding of *R. equi* to mouse macrophages in vitro requires complement and is mediated by Mac-1, a leukocyte complement receptor type 3 (CR3, CD11b/CD18).[287] Opsonisation of *R. equi* with specific antibody is associated with increased phagosome-lysosome fusion and enhanced killing of *R. equi,* suggesting that the mechanism of cellular entry is important.[283] Neutrophils from foals and adult horses are fully bactericidal, and killing of *R. equi* is enhanced considerably by specific opsonizing antibody.[288]

The ability of *R. equi* to induce disease in foals likely depends on host and microbial factors. Knowledge of the virulence mechanisms of *R. equi* was speculative until the discovery of the virulence plasmid.[289] As opposed to most environmental *R. equi* organisms, isolates from clinically affected foals typically contain 85- to 90-kb plasmids encoding an immunogenic virulence-associated protein (VapA) that is expressed on the bacterial surface in a temperature-regulated manner.[290] Plasmid-cured bacteria lose their ability to replicate and survive in macrophages and are cleared from the lungs within 2 weeks of intrabronchial challenge without producing pneumonia.[291] However, expression of VapA alone is not sufficient to restore the

virulence phenotype. Six other genes have approximately 40% overall amino acid identity with VapA, and the identification of multiple genes with considerable homology suggests these genes constitute a virulence-associated gene family in *R. equi*.[292] Other candidates for virulence factors include capsular polysaccharides and cholesterol oxidase, choline phosphohydrolase, and phospholipase C exoenzymes ("equi factors"), but their roles have not been defined clearly.

The primary manifestation of disease caused by *R. equi* infection is severe bronchopneumonia with granuloma, abscess formation, or both. Up to 50% of foals diagnosed with bronchopneumonia also have 1408
extrapulmonary sites of infection.[293] As the pneumonia progresses, clinical signs may include decreased 1409
appetite, lethargy, fever, tachypnea, and increased effort of breathing characterized by nostril flaring and increased abdominal effort. Cough and bilateral nasal discharge are inconsistent findings. A smaller percentage of affected foals may have a more devastating, subacute form. These foals may be found dead or have acute respiratory distress with a high fever and no previous history of clinical respiratory disease.

Hyperfibrinogenemia is the most consistent laboratory abnormality in foals with *R. equi* pneumonia. Neutrophilic leukocytosis (>12,000 cells/μl), with or without monocytosis, is common.[294] Thoracic radiography is a useful diagnostic aid, frequently revealing a prominent alveolar pattern with poorly defined regional consolidation and/or abscessation. Ultrasonography is a helpful diagnostic tool when the disease involves peripheral lung tissue. Although a number of serologic tests have been described, serologic diagnosis of *R. equi* infections is controversial and difficult because exposure of foals to this organism at a young age leads to production of antibody without necessarily producing clinical disease.[295,296] Serologic tests may be more useful at the farm level to detect overall exposure than at the individual level. Bacteriologic culture combined with cytologic examination of a tracheobronchial aspirate remains the most definitive method for accurate diagnosis of *R. equi* pneumonia. However, foals without clinical disease exposed to contaminated environments may have *R. equi* in their tracheae from inhalation of contaminated dust; therefore one should interpret culture results in the context of the overall case presentation.[297] Culture results in one study were as sensitive as polymerase chain reaction–based assays and offered the advantage of allowing in vitro antimicrobial susceptibility testing.[298] However, polymerase chain reaction is likely to be a useful tool, and results from a second trial suggest the assay is more sensitive and specific than culture of tracheobronchial aspirates for diagnosis.[299]

The combination of erythromycin and rifampin has become the treatment of choice for *R. equi* infections in foals, and the combination reduces the likelihood of resistance to either drug. The recommended dosage regimen for rifampin is 5 mg/kg every 12 hours or 10 mg/kg every 24 hours orally. The recommended dose of estolate or ethylsuccinate esters of erythromycin is 25 mg/kg every 8 or 12 hours orally.[300] Recently, azithromycin has been recommended for treatment of *R. equi* infection at a dosage of 10 mg/kg orally every 24 hours for 5 to 7 days and then every other day.[301] Alternatively, clarithromycin at 7.5 mg/kg every 12 hours orally, in combination with rifampin, may be therapeutically effective. Severely affected foals may require intranasal oxygen insufflation, intravenous fluid support, and nutritional support. Treatment generally continues for 4 to 10 weeks until all clinical and laboratory evidence of infection is resolved. Although well tolerated by most foals, erythromycin can result in soft feces. This diarrhea is generally self-limiting and does not require cessation of therapy, but one should monitor affected foals carefully. An idiosyncratic reaction characterized by severe hyperthermia and tachypnea has been described in foals treated with erythromycin during periods of hot weather.[302] Affected foals should be moved to a colder environment and treated with antipyretic drugs and alcohol baths if necessary. *Clostridium difficile* enterocolitis has been reported in the

dams of nursing foals treated with erythromycin given orally.[303] The dam is exposed to active erythromycin by coprophagy or by drinking from a communal water source where the foal has "rinsed" its mouth.

Prevention of *R. equi* pneumonia on farms with recurrent problems is problematic. The most clearly demonstrated prophylactic measure to date has been the administration of plasma that is hyperimmune to *R. equi* to foals within the first week of life and then again when maternal immunity begins to wane at around 30 days of age.[304–311] No effective vaccination protocols for the dam or foal have been described to date. Farm management is important in preventing disease, and control measures include frequent manure removal, avoidance of overcrowded conditions, and planting of dusty or sandy soils.[304]

The prognosis for *R. equi* bronchopneumonia is fair to good in foals with the more chronic form of the disease. Foals with acute respiratory distress have a more guarded prognosis, as do foals with sites of significant extrapulmonary infection. The long-term prognosis for survival for foals with *R. equi* bronchopneumonia is good, and many foals perform as expected as athletes.[312]

19.8.2.2 Viral Pneumonia

The most commonly identified causes of viral pneumonia in foals are equine herpesviruses 1 and 4 (EHV-1 and EHV-4), equine influenza, and equine arteritis virus (EVA). Equine herpesvirus 1 is probably the most clinically important, but outbreaks of EVA in neonates have occurred and are devastating.[27,313–318] Adenovirus is reported sporadically and as a problem in Arabian foals with severe combined immunodeficiency.[319–321]

In the neonate, infection with EHV-1 or EVA is almost uniformly fatal and antemortem diagnosis is difficult, even once an outbreak on a particular farm is identified. Several factors appear common to foals with EHV-1, including icterus, leukopenia, neutropenia, and petechial hemorrhage, but these problems also are identified in foals with severe sepsis.[315,322,323] The antiviral drug acyclovir (10 to 16 mg/kg orally or per rectum 4 to 5 times per day) has been used in cases of EHV-1 in neonates, with some evidence of efficacy in mildly affected foals or foals affected after birth.[323] If viral pneumonia is a possibility, one should collect 1409
blood and tracheal aspirates at presentation for bacterial and virus isolation. The lungs of foals with EHV-1 1410
or EVA are noncompliant, and pulmonary edema may be present. Mechanical ventilation of these cases may prolong life, but death is generally inevitable because of the magnitude of damage to the lungs. Foals suspected of having EHV-1 or EVA should be isolated because they may be shedding large quantities of virus and pose a threat to other neonates and pregnant mares. Foals with EVA generally are born to seronegative mares, and intravenous treatment with plasma with a high titer against EVA may prove beneficial because passive immunity appears to have a large role in protection against this disease in neonates.[318,324]

Older foals and weanlings may be affected by herpesviruses. Disease is usually mild, although a fatal pulmonary vasculotropic form of the disease has been described recently in young horses.[325,326] The clinical signs of disease are indistinguishable from influenza and include a dry cough, fever, and serous to mucopurulent nasal discharge, particularly if secondary bacterial infection occurs. Rhinitis, pharyngitis, and tracheitis may be present. Treatment of affected foals is primarily supportive. Foals also may become infected with EHV-2. The predominant clinical signs are fever and lymphoid hyperplasia with pharyngitis.[327,328] Diagnosis is by virus isolation.

19.8.3 OTHER CAUSES OF RESPIRATORY SIGNS IN FOALS

Rib fractures have been recognized in 3% to 5% of all neonatal foals and can be associated with respiratory distress.[87] Potential complications of rib fractures include fatal myocardial puncture, hemothorax, and pneumothorax. Rib fractures frequently are found during physical examination by palpation of the ribs or by auscultation over the fracture sites. One can confirm the diagnosis by radiographic and ultrasonographic evaluation. Often multiple ribs are affected on one side of the chest. Specific treatment is generally unnecessary, but direct pressure on the thorax should be avoided in all cases. Some specific patients may benefit from surgical stabilization of some fractures, particularly those fractures overlying the heart.

Pneumothorax can occur spontaneously or following excessive positive pressure ventilation[329] or following tracheostomy surgery or trauma. Any foal being ventilated mechanically that suddenly has respiratory distress and hypoxemia should be evaluated for pneumothorax. Diagnosis is by auscultation and percussion of the thorax, but one can confirm the diagnosis with radiographic and ultrasonographic evaluation of the thorax. Needle aspiration of air from the pleural space also confirms the diagnosis. Treatment is required in cases in which clinical signs are moderate to severe or progressive and involves closed suction of the pleural space. Subcutaneous emphysema can complicate treatment of this problem.

Idiopathic or transient tachypnea has been observed in Clydesdale, Thoroughbred, and Arabian breed foals. In human infants, transient tachypnea can be related to delayed absorption of fluid from the lung, perhaps because of immature sodium channels.[330] In foals, tachypnea generally occurs when conditions are warm and humid and is thought to result from immature or dysfunctional thermoregulatory mechanisms. Clinical signs of increased respiratory rate and rectal temperature develop within a few days of birth and may persist for several weeks. Treatment involves moving the foal to a cooler environment, body clipping, and provision of cool water or alcohol baths. These foals frequently are treated with broad-spectrum antimicrobial drugs until infectious pneumonia can be ruled out.

A syndrome of bronchointerstitial pneumonia and acute respiratory distress has been described in older foals and appears to be a distinct entity from acute respiratory distress syndrome in neonatal foals in association with sepsis.[331] The underlying cause has not been identified, but the genesis is probably multifactorial with several potential pathogens being implicated. Affected foals have acute respiratory distress with significant tachypnea, dyspnea, nostril flare, and increased inspiratory and expiratory effort. Auscultation reveals a cacophony of abnormal sounds including crackles and polyphonic wheezes in all lung fields. Loud bronchial sounds are audible over central airways, and bronchovesicular sounds are lost peripherally. Affected foals are cyanotic, febrile, and unwilling to move or eat. Foals may be found acutely dead. Laboratory abnormalities include leukocytosis, hyperfibrinogenemia, and hypoxemia with hypercapneic acidosis. Foals can be dehydrated severely and have coagulation changes consistent with disseminated intravascular coagulation. Hypoxic injury to other organs, primarily the kidneys and liver, can occur. Chest radiographs reveal a prominent interstitial pattern overlying a bronchoalveolar pattern that is distributed diffusely throughout the lung. This syndrome is a respiratory emergency. Treatment is broad-based and includes administration of oxygen, nonsteroidal antiinflammatory agents, broad-spectrum antimicrobial therapy, nebulization, judicious intravenous fluid therapy, nutritional support, and corticosteroid therapy. One must manage hyperthermia in the foal. Corticosteroid therapy appears to have been lifesaving in most of the reported surviving foals. Because this syndrome is associated with high environmental temperatures in some areas, prevention involves control of ambient temperatures, not transporting foals during hot weather, and keeping foals out of direct sun on hot days, particularly foals being treated with erythromycin for suspected or confirmed *R. equi* infection.[332] 1410

19.9 Diseases Of The Urinary Tract

19.9.1 UROPERITONEUM

Uroperitoneum has been recognized as a syndrome in foals for more than 50 years.[333,334] Classically, affected foals are 24 to 36 hours old at the time clinical signs first are recognized.[334–336] Previous reports had a proportionately larger affected male than female population.[334,335,337] The hypothesis was that colts were more at risk because their long, narrow, high-resistance urethra was less likely to allow bladder emptying, resulting in rupture of a full bladder during parturition when high pressures were applied focally or circumferentially around the bladder.[333] More recent reports suggest that such extreme sex bias may have been an artifact of small case numbers in the early reports.

Rupture or disruption of any structure of the urinary tract can occur. The dorsal wall of the bladder has been reported to be a frequent disruption site, with the ventral wall less likely to be involved.[336] The urachus appears to be the next most commonly affected structure. A few cases of ureteral and urethral defects have been reported.[336,337] Sepsis does not appear to favor one site over the others.[338]

The pathophysiology of uroperitoneum is not yet understood fully. The high pressure exerted on a full bladder during parturition once was thought to be the main cause. Full bladder and obstruction caused by a partial umbilical cord at parturition, strenuous exercise, and external trauma have been reported as causes.[339] A few reports describe smooth and noninflamed edges of torn tissue, suggesting the possibility of congenital bladder wall defects.[338,340,341] Sepsis leading to urinary tract rupture and uroperitoneum may occur in foals hospitalized for a variety of unrelated problems. The onset of clinical signs of uroperitoneum may be insidious in these foals, and diagnosis may be less obvious.[338]

Clinical signs associated with uroperitoneum in the neonatal foal typically include straining to urinate, dribbling urine, and a stretched-out stance. Weakness, tachycardia, tachypnea, and not sucking well are also common. A distended abdomen may be evident, and one may feel a fluid wave on ballottement of the abdomen. Occasionally, urine accumulates in the scrotum and should not be confused with hernia. Foals also may show signs of sepsis, including fever, injected mucous membranes, diarrhea, and disease of other body systems.

Laboratory findings vary depending on the duration of the uroperitoneum and on the presence and severity of sepsis. Classic findings include hyperkalemia, hyponatremia, and hypochloremia arising from equilibration of urine electrolytes and water with blood across the peritoneal membrane.[335–337] The usual foal diet of milk, which is high in potassium and low in sodium, promotes the electrolyte abnormalities. Foals that develop uroperitoneum while receiving intravenous fluids may not have classic electrolyte imbalances at the time clinical signs are recognized.[338] Increased serum creatinine concentration is often present, whereas blood urea nitrogen concentrations occasionally, but not consistently, are increased.[335–337] Metabolic acidosis and hypoxemia may be present. Some patients also have serum hypoosmolality.[335] One should test foals for failure of passive transfer. One of the most sensitive laboratory tests for uroperitoneum is the ratio of peritoneal to serum creatinine. A ratio greater than or equal to 2:1 is considered diagnostic of uroperitoneum. One should collect peritoneal fluid and test it for creatinine concentration, as well as for cytologic findings, culture, and sensitivity. Cytologic evaluation of peritoneal fluid is necessary to identify concurrent peritonitis or other gastrointestinal compromise. One should perform an electrocardiogram on initial evaluation of a foal with

suspected uroperitoneum because hyperkalemia may result in bradycardia, increased duration of the QRS complex, a shortened Q-T interval, increased P-wave duration, prolonged P-R interval, or atrioventricular conduction disturbances. Other possible cardiac sequelae to hyperkalemia include cardiac arrest, third-degree atrioventricular block, ventricular premature contractions, and ventricular fibrillation.[337,340]

For any foal exhibiting signs of dypsnea, tachypnea, or hypoxemia, one should have thoracic radiographs taken *before* induction of anesthesia to rule out pleural effusion, pneumonia, or acute respiratory distress syndrome, which could complicate ventilation and oxygenation during anesthesia and the postoperative period. Ultrasonography has become the tool of choice in the diagnosis of uroperitoneum and is a useful tool available to the practitioner.[342] One can image free peritoneal fluid readily, and tears within the bladder are readily visible. The empty bladder with a significant defect, in a fluid-filled abdomen, will collapse on itself and often have a U shape. One also can visualize urachal and urethral lesions. Six of eight foals in one study had urinary tract lesions identified sonographically, and all 31 foals of another study underwent sonographic evaluation, and a significant correlation between ultrasonographic findings and location of the lesion at surgery existed.[336,338]

Initial treatment aims to stabilize the patient and correct any electrolyte and acid-base abnormalities and provide fluid volume replacement. One should use 0.9% or 0.45% saline with 5% dextrose until laboratory data are available. A potassium concentration of greater than 5.5 mEq/L can be life threatening. One can manage hyperkalemia by peritoneal drainage to decrease whole-body potassium stores using teat cannulae, Foley catheters, large-gauge (16 or 14) intravenous catheters, or human peritoneal dialysis catheters. Fluid 1411
replacement at least should equal the amount of fluid removed from the abdomen to prevent acute hypotension 1412
caused by expansion of previously collapsed capillary beds. Abdominal drainage also helps ventilation and decreases the work of breathing by decreasing pressure on the diaphragm. One may administer calcium gluconate, glucose, sodium bicarbonate, or insulin intravenously to decrease serum potassium concentrations. These maneuvers do not correct the whole-body potassium overload, however, and once therapy is discontinued, hyperkalemia can reappear until the urine is removed from the abdomen. One should correct hyponatremia slowly. Because of the real possibility of concurrent sepsis, one should obtain blood cultures *before* preoperative administration of antimicrobials. Broad-spectrum coverage (penicillin and amikacin or ceftiofur sodium) is recommended until culture results become available. One should perform therapeutic drug monitoring when using aminoglycoside therapy. However, the peak value may be depressed because of the increased volume of distribution represented by the volume of urine in the abdomen, so one should not make dose adjustment based on a low peak until obtaining a new peak after surgical correction of the uroperitoneum. One should treat foals with failure of passive transfer with adequate volumes of intravenously administered plasma.

After one has addressed the metabolic abnormalities, one may consider surgical management. Medical management using an indwelling Foley catheter has been described.[343] Preoperative medical stabilization reduces anesthetic risk. Safer inhalant agents such as isoflurane also have decreased risk. Removal of the internal umbilical remnant at the time of surgery is usual. One should consider culturing any removed umbilical remnant and submitting the remnant for histopathologic evaluation. Recurrence of urinary tract rupture can occur. Sepsis, hypoxemia, pneumonia, peritonitis, and acute respiratory distress syndrome complicate the management of uroperitoneum. Many affected foals are persistently oxygen dependent for several days following surgical correction, and one should perform serial arterial blood gas analyses before discontinuing intranasal oxygen supplementation.

Prognosis is associated closely with concurrent illness, especially septicemia. Uncomplicated uroperitoneum from a defect in the bladder has a good prognosis. If the location of the lesion is other than the bladder, the prognosis is not as favorable.[337] Foals with septicemia have a much poorer prognosis.[338,339]

19.9.2 ACUTE RENAL FAILURE

Acute renal failure most often occurs as a complication of prenatal asphyxial syndrome, sepsis, or aminoglycoside therapy. Acute renal failure also has been reported following oxytetracycline administration in foals.[344] The dose of oxytetracycline commonly used to treat flexural deformities in foals is approximately 10 times the antimicrobial dose. Many foals treated in this manner also have suffered some degree of perinatal asphxia, which also damages the kidney, because of prolonged parturition precipitated in part by the flexural deformity. Evaluation of renal function in these foals *before* the administration of the first dose of oxytetracycline and continued monitoring of serum creatinine concentrations before administering subsequent doses of this nephrotoxic compound would seem reasonable. Hemodialysis has been used as therapy in one of these cases, but prevention is important because these foals may fail to respond to usual therapy for oliguric renal failure and are euthanized.[344]

19.9.3 CONGENITAL RENAL DISEASE

The most commonly reported congenital deformity of the kidney of the foal is renal hypoplasia and dysplasia, which may have a heritable component.[345,346] Renal arteriovenous malformations have been reported also.[347] Ectopic ureters and fenestrated ureters have been described in the foal.[348–350] Congenital renal defects, among others, were reported in three weak, recumbent neonatal foals born to mares being treated for equine protozoal myeloencephalitis.[351] Mares received sulfadiazine or sulfamethoxazole-trimethoprim, pyrimethamine, folic acid, and vitamin E orally. The foals were anemic, leukopenic, azotemic, hyponatremic, and hyperkalemic. Serum folate concentrations were lower than those reported in the literature for clinically normal brood mares. Treatment was unsuccessful. Necropsy revealed lobulated kidneys with thin cortices and a pale medulla. The authors postulated that oral administration of sulfonamides, 2,4-diaminopyrimidines (pyrimethamine with or without trimethoprim), and folic acid to mares during pregnancy is related to congenital defects in newborn foals.

19.10 Umbilical Disorders

The umbilicus serves as the conduit for nutrition and gas exchange between the dam and the fetal foal. The urine from the foal is expelled via this structure into the allantoic cavity. The author has recognized cases of in utero bladder distention in the fetus that were associated with multiple twists decreasing urine flow or focal stenosis creating the same effect. Foals born with this condition did not have bladder rupture associated with parturition but did have other severe abnormalities that eventually resulted in their demise, primarily premature delivery with failure to adapt to extrauterine life (P.A. Wilkins, J.E. Palmer, and F.T. Bain, unpublished data). At birth the umbilicus breaks, leaving a small external remnant and a large internal remnant. The umbilicus long has been regarded as the primary site of entry of pathogens into the neonate, although this has been challenged recently. 1412

1413 Treatment of the umbilicus after birth involves dipping it (preferably just the most distal component) with various caustic compounds. The most current recommendation is to treat the umbilicus with dilute chlorhexidine, povidone-iodine, or dilute iodine solutions for just a few times following birth. Exuberant treatment of the umbilical stump with caustic solutions can lead to scalding of the ventral abdomen and may promote patency of

the urachus. The ultrasonographic appearance and measurements of the umbilical arteries, urachus, and umbilical vein of foals from 6 hours to 4 weeks of age have been described in detail.[342] A 7.5-MHz sector scanner transducer placed across the midline of the ventral portion of the abdominal wall of the foal works best because of the superficial location of these structures. The mean (± SD) diameter of the umbilical vein was 0.61 ± 0.20 cm immediately cranial to the umbilical stalk, 0.52 ± 0.19 cm midway between the umbilicus and liver, and 0.6 ± 0.19 cm at the liver. The urachus and umbilical arteries of normal foals have a mean total diameter of 1.75 ± 0.37 cm at the bladder apex. The umbilical arteries scanned along either side of the bladder have a mean diameter of 0.85 ± 0.21 cm. One can use these measurements and the ultrasonographic appearance of the internal umbilical structures from clinically normal foals as references to diagnose abnormalities of the umbilical structures in neonatal foals.[352,353] The most common abnormalities of these structures are focal abscess formation, hematoma, and urachal tear.

19.10.1 HERNIA

Herniae traditionally have been thought to develop from failure of closure at the umbilical stump after birth. However, the closure of the body wall defect at the umbilicus was studied in relation to the development of umbilical herniae in a large group of normal foals followed from birth until 5 months of age or from birth until 11 months of age.[354] At birth, approximately half of these foals had a defect in the body wall at the umbilicus that was termed a *palpable umbilical ring*. In 18 foals this defect disappeared within 4 days, but in one foal the ring did not close and a hernial sac with abdominal contents was palpable. This foal was considered to be the only foal to have a truly congenital umbilical hernia. Twelve foals developed an umbilical hernia between 5 and 8 weeks of age. The prevalence of umbilical herniae was much higher than in other studies, possibly because of the prospective nature of the study. Based on this study, the large majority of umbilical herniae would appear not to result from failure of closure but rather to be acquired after birth. One should consider the palpable ring structure within the body wall at the umbilicus a variant of normal in the foal and should not call it a hernia until the foal is at least 1 month of age.

In one study of 147 horses treated for umbilical herniae over a 13½-year period, only 8.8% developed complications in association with umbilical defects.[355] Six horses had intestinal incarceration; the incarceration was reduced manually in 3 horses before admission and resolved without treatment in 2 others. The hernia was surgically reduced in 1 horse. Herniorrhaphy was performed on 4 of the 5 horses in which the incarceration did not require surgical reduction, and the fifth was managed conservatively. The study confirmed that complications of umbilical herniae are rare in horses; however, when they do develop, they may be one of various forms, some of which are insidious in onset. The primary differential diagnosis for an external swelling in the umbilical stump region is an external abdominal abscess, which will be firm, variably painful, warm, and nonreducible. Ultrasonographic evaluation readily can confirm either possibility.

19.10.2 OTHER CONGENITAL ABNORMALITIES

One report describes a 3-day-old foal that died from intestinal strangulation caused by a remnant of vitelline vein that extended between the umbilicus and the portal vein.[356]

19.10.3 PATENT URACHUS

Patent urachus frequently is recognized in the abnormal neonate, probably because of the increased recumbency and decreased movement of these patients. Cauterization of a patent urachus is no longer recommended except

in cases that persist for long periods of time (>1 month) after the foal becomes more active. Surgical resection may provide relief in some foals, but most cases resolve without treatment if given enough time. Foals with a patent urachus may posture and strain frequently to urinate, some of this may be associated with irritation or local infection of the urachus. One can alleviate this by administration of broad-spectrum antimicrobial therapy such that the drug has a high concentration in the urine (e.g., trimethoprim-sulfa drug combinations) and by oral administration of phenazopyridine hydrochloride (Pyridium), a dye that anesthetizes the urinary tract epithelial surfaces (see Table 19-7). This dye turns the urine orange and stains everything yellow-orange that it or the urine touches but can provide a great deal of relief to foals with this problem.

19.10.4 UMBILICAL REMNANT INFECTION

The umbilicus has been considered the traditional point of entry of bacteria into the septic neonate, and septic foals have been referred to as having "navel ill" and "joint ill" in the past. Although current thought suggests that the gastrointestinal tract may be the route of entry in most septic neonates, infection of the umbilicus— 1413
1414
termed *omphalitis,* or *omphalophlebitis* if the vessels are involved—still occurs as a single focus of infection or along with more generalized infection. External signs, such as swelling, heat, pain, ventral edema, or purulent discharge may be present in some foals, but more usually external signs are minimal and one suspects infection because of infection in another site (e.g., an infected joint), fever, or otherwise unexplained increased blood fibrinogen concentration. One confirms the diagnosis by ultrasonographic evaluation of the internal umbilical remnant. Any of the umbilical structures may be involved. A complete description of the evaluation is available within the relevant veterinary literature, but the examination is performed best with the foal standing using a 7.5-MHz probe with a standoff.[353] The usual finding is that the affected structure is larger than expected. A fluid-filled core and echogeneic shadows consistent with gas may be apparent in some cases. Interpretation requires some experience, and the examiner should be familiar with variants of normal, such as gas shadows associated with a patent urachus and enlarged vessels caused by hematoma formation, so that treatment is not initiated inappropriately.

Two options for treatment are surgical and medical. Medical treatment is preferable in cases in which the lesion is well localized and small and in foals with a medical condition that is not amenable to anesthesia and surgical intervention. One should institute broad-spectrum antimicrobial therapy, and one may need to continue therapy for 2 to 3 weeks. Most affected foals respond to medical therapy. Frequent reevaluation of the abnormality is necessary, every 5 to 7 days initially, and one should measure blood fibrinogen concentrations at reevaluation because they should stabilize and decrease with effective treatment. Failure to respond to therapy within 10 days to 2 weeks suggests that an empiric change in the antimicrobial used may be necessary. In foals that are refractory to medical management or where the lesion is large, surgical excision of the entire umbilical remnant may be desirable.

19.11 Diseases of The Gastrointestinal Tract

19.11.1 COLIC IN THE NEONATE

Colic in the foal can be difficult to diagnose accurately because one cannot perform an examination per rectum. However, many diagnostic aids, most importantly ultrasonography, are available to help differentiate medical from surgical causes of abdominal discomfort in the foal.

19.11.2 OBSTRUCTION

Intestinal accidents of all types described in adult horses, with the possible exception of enteroliths, occur in foals. Intussusception, volvulus, displacement, diaphragmatic hernia, and intra- and extraluminal obstruction have been reported in foals. Abdominal ultrasonographic and radiographic evaluation greatly aids diagnosis. Treatment is primarily surgical. Foals with PAS and intestinal dysmotility are at increased risk of intussusception and displacement, and Miniature breed foals appear to be at increased risk for fecolith and enterolith formation.

19.11.3 MECONIUM RETENTION/IMPACTION

Meconium retention or impaction is a common cause of abdominal discomfort in newborn foals. Most foals defecate shortly after their first meal. The usual practice for most owners or veterinarians attending the birth of a foal is to administer an enema to aid this process. In the past, phosphate-based commercially available enemata (Fleet) were used frequently, but if used excessively these types of enemata can create problems of their own, including rectal irritation and hyperphosphatemia. The best enema is warm soapy water made with a mild soap such as liquid Ivory soap that can be administered through soft rubber tubing using gravity flow. Foals with significant meconium retention become colicky within the first few hours of life as gas accumulates within their bowel. Frequently, one can palpate the meconium through the abdominal wall. Additional diagnostics can include abdominal ultrasonography and radiography, particularly if one must rule out other, more serious types of colic. These foals assume a classic stance with an arched back. One must differentiate this stance from the stance assumed by foals with uroperitoneum, which is more extended. Foals with meconium retention have had simultaneous ruptured bladder, however, so the clinician must be sure to evaluate the foal fully for both problems. Foals that do not respond rapidly to enema administration need additional treatment, which can include giving mineral oil (2 to 4 ounces) by nasogastric tube. One can treat persistent meconium retention resulting in significant abdominal distention by muzzling the foal to prevent further milk intake and administering intravenous fluids at an appropriate maintenance rate. If continuous rate infusion is possible, 5% to 10% dextrose is the preferred fluid to use to provide calories to the foal. One should not use dextrose as a bolus fluid. More aggressive treatment would include administration of retention enemata made using acetylcysteine, which serves as an irritant and increases secretion. Extreme cases of meconium retention may require surgical intervention, but this is usually not necessary and most cases resolve with medical management alone within 12 to 24 hours. Some foals require pain managment. One should avoid nonsteroidal antiinflammatory drugs in the neonate because of their effects on renal function and gastric mucosal blood flow 1414
(see Gastric Ulcers). Many foals respond well to butorphanol administered intramuscularly at a dose of 3 to 5 1415
mg to an average 50-kg foal. Intranasal oxygen insufflation is beneficial in foals with significant abdominal distention.

One should evaluate foals with meconium impaction/retention for evidence of PAS because intestinal dysmotility is common in PAS. Colostrum is a laxative, and these foals also may suffer from failure of passive transfer, with meconium retention resulting from the lack of adequate colostrum. These foals are also at risk of sepsis because the mucosal intestinal barrier probably has been disrupted and translocation of bacteria can occur. One should obtain blood cultures on these foals and should monitor them closely for signs of sepsis.

19.11.4 CONGENITAL DEFECTS

Atresia within the gastrointestinal system of the foal occurs infrequently, but clinical signs are characteristic.[357] Acute colic occurs within the first few hours and is accompanied by abdominal distention similar to meconium retention. Three primary types of atresia are described in the foal: membrane atresia, cord atresia, and blind-end atresia. Antemortem diagnosis of atresia, short of abdominal exploratory surgery, is aided by the lack of meconium staining of the rectum or any administered enema fluids. Additional diagnostic tests may include administration of a barium enema for a radiographic study, colonoscopy, and abdominal ultrasonography. Abdominocentesis is usually normal until bowel rupture is imminent or has occurred. One can make affected foals more comfortable by muzzling them to prevent further milk intake and by supplying them with fluids and nutrition intravenously. If one attempts surgical correction, one first should initiate broad-spectrum antimicrobial therapy and determine passive transfer status. Frequently, these foals are hypoxemic because of the abdominal distention, and oxygen supplementation is desirable.

19.11.5 LETHAL WHITE SYNDROME

Solid white foals born to overo-overo matings of American Paint Horses may suffer from congential aganglionosis of the ileum, cecum, and colon. These foals present similarly to foals with meconium impaction or atresia in that colic develops shortly after birth and involves progressive abdominal distention with feeding. The inherited defect is in the endothelin receptor gene.[358–361] No effective treatment exists, but the clinician should be aware that not all white foals of this mating are affected, and some simply may have meconium retention, so a short period of treatment may be warranted.

19.11.6 NECROTIZING ENTEROCOLITIS

Necrotizing enterocolitis is considered the most common acquired gastrointestinal emergency of human infants.[362,363] The 1500 to 2000 infants that die every year from this disease in the United States and the large number of infants who develop short gut syndrome from this disease only represent the tip of the iceberg of the problems necrotizing enterocolitis causes. The widespread fear of necrotizing enterocolitis among neonatologists and pediatric surgeons has contributed in large part to the use of the intravenous route rather than the gastrointestinal tract for nourishing these infants for long periods. The pathogenesis of necrotizing enterocolitis is unknown but may result from a disturbance of the delicate balance among gastrointestinal perfusion, enteric organisms, and enteral feeding. Risk factors for necrotizing enterocolitis in human infants include prematurity, hypoxic-ischemic insult, and formula or breast milk feedings. The clinical spectrum of necrotizing enterocolitis is multifactoral and ranges from temperature instability, apnea, lethargy, abdominal distention, bilious residuals, septic shock, disseminated intravascular coagulation, and death. Medical management is usually adequate treatment for necrotizing enterocolitis. In the neonatal foal, necrotizing enterocolitis is probably one of the most underrecognized causes of gastrointestinal dysfunction and in the past has been attributed only to infection with anaerobic organisms including *Clostridium perfringens* type C and *C. difficile*.[364] Although a specific form of enteritis is associated with intestinal infection by these organisms, most necrotizing enterocolitis is associated with prematurity or PAS in the infant and the foal.

One should suspect necrotizing enterocolitis in any foal that is having difficulty tolerating oral feeding, demonstrating signs of ileus, or having episodes of colic and in any foal with occult blood or frank blood in the stool. Foals exhibiting any of these clinical signs should not be fed orally if possible and should receive

parenteral nutrition until gastrointestinal function returns to near normal. The mucosal barrier of the intestine is unlikely to be fully intact, and these foals are at risk for sepsis from bacterial translocation. One should institute broad-spectrum antimicrobial therapy in these foals and, if any evidence of coordinated gastrointestinal motility is apparent, should administer sucralfate orally as a protectant.

19.11.7 GASTRIC ULCERS

Gastric ulcer disease has been recognized in foals, and lesions vary in anatomic distribution, severity, and cause. In clinically normal neonatal foals (<30 days of age), gastric ulcers and mucosal desquamation have been documented.[365–368] Because of these reports and other early reports of death following ruptured clinically silent ulcers in neonatal foals, for years many clinicians felt it necessary to treat critically ill neonates with antiulcer medication prophylactically.[369–371] Recently, this paradigm has been challenged. 1415

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The pathophysiology of gastric ulcer disease is described most reasonably as an imbalance in protective and aggressive factors.[372–374] These protective factors are responsible for maintaining a healthy gastrointestinal tract by promoting adequate mucosal blood flow, adequate mucus and bicarbonate production, prostaglandin E_2 production, epithelial growth factor production, gastric afferent innervation, epithelial cell restitution, and gastroduodenal motility. Probably the most important factor is maintenance of mucosal blood flow. Hypoxia, NO, prostaglandins, and gastric afferent innervation influence mucosal blood flow. The aggressive factors include gastric acid, bile salts, pepsin, and enzymes. Few specific causes have been found for gastric ulcer disease in foals. Excessive administration of nonsteroidal antiinflammatory drugs can result in ulceration of the glandular and squamous epithelium because of an inhibition of prostaglandin production, which leads to a decrease in mucosal blood flow and an increase in acid production. Nonsteroidal antiinflammatory drugs also can impair the healing of lesions and rarely are indicated in neonatal equine medicine.[372,373]

In the critically ill neonate the suspected cause of gastric ulcers has shifted away from an excessive amount of intraluminal gastric acid toward gastric mucosal ischemia caused by hypoxia, low blood flow conditions, or both.[375] Perforating gastric ulcers are more likely a manifestation of necrotizing enterocolitis than of excessive gastric acid. Shock, sepsis, or trauma can result in gastric mucosal ischemia, allowing for the disruption of epithelial cell integrity and permitting damage by aggressive factors or providing an environment suitable for the establishment of bacteria colonization.[375,376] Impairment of mucosal blood flow also may result in reperfusion injury, allowing the formation of gastric ulcers. In the sick neonatal foal (<7 days of age) a wide variability in the intragastric pH has been documented depending on the type of disease, severity, and milk intake frequency and volume, suggesting that in the critically ill equine neonate, ulcer prophylaxis using histamine antagonists or proton pump inhibitors is not only unnecessary but unlikely to work.[377]

Clinically significant gastric ulcers can occur in the squamous, glandular, or both portions of the stomach as a primary problem or resulting from another problem. Clinical signs include diarrhea, abdominal pain, restlessness, rolling, lying in dorsal recumbency, excessive salivation, and bruxism. In the neonatal foal the only clinical signs present may be depression or partial anorexia until a more catastrophic event, such as perforation, occurs. Some lesions in the gastric mucosa extend from the pylorus into the proximal duodenum and can result in stricture of the pylorus and proximal duodenum. These foals are usually older (>1 month of age) and have a greater volume of reflux. Bruxism and ptyalism are also more prominent in these older foals.

The most sensitive and specific method for diagnosing gastric ulcers is visualization by endoscopic examination.[365] Unfortunately, the use of gastric endoscopy has led to recognition of relative nonlesions and

ulcers resulting from other problems and of clinically significant disease states. The clinician should not stop simply when ulceration of the stomach is recognized with endoscopy but should examine that patient fully for other potential sources of the clinical signs. Other diagnostic tests may help in determining the severity of the ulcers, including fecal occult blood or gastric blood assessments, contrast radiography, abdominal ultrasound, and abdominocentesis. Endoscopy of the foal stomach carries an additional risk of exacerbating colic in the short term, unless the examiner ensures that as much introduced air as possible is evacuated from the stomach at the end of the procedure.

The presence of a brown gastric reflux fluid may indicate the presence of bleeding ulcers. Blood in the feces of the neonate is more consistent with a diagnosis of necrotizing enterocolitis, which can be associated with gastric ulcers. Contrast radiography is useful if one suspsects delayed gastric emptying or pyloric or duodenal stricture in older foals. If a stricture has occurred, one will note a delay in complete emptying of barium from the stomach (>2 hours).[367] Abdominal ultrasound may be useful to visualize free abdominal fluid and gastric or small intestinal distention if one suspects a perforation. One can visualize portions of the descending duodenum, and a thickened duodenum should increase the index of suspicion for duondenal stricture. Abdominocentesis also may confirm perforation.

Traditional therapy for gastric ulceration includes mucosal adherents, histamine type 2 receptor antagonists, proton pump inhibitors, and antacids.[378] The most widely used mucosal adherent is sucralfate, which is a hydroxy aluminum salt of sucrose. The main therapeutic action of sucralfate is to bind to the negatively charged particles in the ulcer crater.[378,379] At a pH less than 2, sucralfate is converted to a sticky viscous gel, which adheres to the ulcer crater and remains adhered for 6 hours, but at a higher pH, sucralfate remains in a suspension. Sucralfate is still effective because it inhibits pepsin and buffers hydrogen ions. Other important actions of sucralfate include stimulating production of prostaglandin E, which maintains mucosal blood flow; increasing bicarbonate secretion; stimulating mucous secretion; decreasing peptic activity; and binding epidermal growth factor. The histamine type 2 receptor antagonists include cimetidine, ranitidine, and
famotidine. These compounds block the interaction of histamine with the histamine type 2 receptor on the 1416
parietal cell, resulting in inhibition of gastric acid secretion. Clinically normal neonatal foals have a highly 1417
acidic gastric fluid that is influenced by sucking. Intravenous and oral administration of ranitidine increases intragastric pH in normal foals but critically ill neonatal foals have a blunted response to ranitidine administration.[377,380] One possible conclusion reached from these studies is that in critically ill neonatal foals, gastric ulcers may not be caused by an increased intraluminal gastric acidity.

The most commonly used proton pump inhibitor is omeprazole. This drug has not as yet been approved for use in foals under 30 days of age. Omeprazole inhibits the secretion of hydrogen ions at the parietal cell by irreversibly binding to the H^+,K^+-ATPase proton pump of the cell. Most of the lesions in older foals were healed after daily administration of omeprazole for 28 days according to one report.[381] Table 19-9 summarizes the therapeutic agents for treating gastric ulcers in foals.

Prophylactic treatment of critically ill neonates for gastric ulcers has been standard therapy for years because of the evidence of clinically silent ulcers. This approach may not be appropriate for several reasons. An increased incidence of nosocomial pneumonia and systemic sepsis is associated with high gastric pH in human patients in intensive care.[382–384] Patients in intensive care units treated prophylactically with histamine type 2 receptor antagonists are more likely to develop pneumonia during ventilation therapy and gastric colonization with potentially pathogenic bacteria or yeast.[382,385] An acidic environment appears to protect against airway colonization by bacteria of intestinal origin and bacteria translocated across the gastrointestinal tract. Pathogenesis of ulcers in the neonatal foal most likely does not involve increased intraluminal gastric acid but

instead may be caused by decreased mucosal perfusion associated with shock, hypoxia, and hypoxic/ischemic insult to the gastric mucosa. A recent report revealed that gastric ulcer disease in equine NICU patients is independent of pharmacologic prophylaxis.[386] In this study, despite decreased treatment, the incidence of gastric ulcers found in these foals at necropsy had decreased significantly. The decrease was attributed to overall improvement in management of these cases. Similarly, in a human intensive care unit, the incidence of stress ulcers decreased independent of the use of prophylaxis.[375,387] Early treatment of sepsis, sufficient oxygenation, improved monitoring, institution of enteral feedings, and improved nursing care may contribute to the reduction in gastric ulcers in the neonatal patient. Use of histamine type 2 receptor antagonist and proton pump inhibitors apparently may not be necessary; however, in some instances sucralfate may be useful. Sucralfate reduced the rate of bacterial translocation in a rat model during hemorrhagic shock and also may prohibit the generation of acute gastric mucosal injury and progression to ulcer formation induced by ischemia-reperfusion.[388,389] In a human medical intensive care unit, airway colonization by new pathogens occurred more frequently in patients receiving agents that increased gastric pH than in those receiving sucralfate.[382,390] In the critically ill neonatal foal, risk factors for gastric ulceration have not been identified clearly, although foals treated routinely with nonsteroidal antiinflammatory drugs may be at increased risk for gastric lesions. Prophylactic treatment for gastric ulcers in critically ill neonates may not be necessary, and one should consider carefully the pros and cons of their use *before* their administration.

TABLE 19-9 Therapeutic Agents for Treating Gastric Ulcers in Foals

DRUG CATEGORY	DRUG	DOSE	ROUTE	FREQUENCY
Mucosal protectant	Sucralfate	10–20 mg/kg	p.o.	t.i.d. to q.i.d.
Histamine type 2 receptor antagonist	Cimetidine	10–20 mg/kg	p.o.	q4h
	Ranitidine	6.6 mg/kg	IV[*]	q4h
		5–10 mg/kg	p.o.	b.i.d. to q.i.d.
		0.8–2.2 mg/kg	IV	q.i.d.
Proton pump inhibitor	Omeprazole	4 mg/kg	p.o.	s.i.d.
		1–2 mg/kg	p.o.	s.i.d. (prophylaxis)
Antacids	Milk of Magnesia	2–4 oz	p.o.	s.i.d. to b.i.d.
	Maalox	240 ml	p.o.	q4h

Adapted from Barr B: Gastric ulcer prophylaxis in the critically ill equine neonate. In Wilkins PA, Palmer JE, editors: *Recent advances in equine neonatal care,* Ithaca, NY, 2001, International Veterinary Information Service (A0413.1101).

* *IV,* Intravenous.

19.11.8 DIARRHEAL DISEASES OF FOALS

19.11.8.1 Foal Heat Diarrhea

Foal heat diarrhea is a mild, self-limiting form of diarrhea that occurs in foals between 5 and 14 days of age, about the time of the "foal heat" in the dam. The definitive cause of foal heat diarrhea has yet to be described, 1417

but the condition may be associated with dietary changes or changes in gastrointestinal function that occur around that time. This form of diarrhea is not caused by *Stongyloides westeri* infestation as previously thought.[391] Foals with foal heat diarrhea are not systemically ill and should not require therapy. One should evaluate fully any foals with diarrhea at this time for other possible causes of diarrhea, particularly if they are unwell or exhibit anorexia or dehydration. 1418

19.11.8.2 Viral Diarrhea

Viral diarrhea occurs most commonly in large groups of mares and foals that are housed together. Rotavirus is an isolate from the feces of up to 40% of foals with diarrhea worldwide, alone or with another pathogen.[392,393] The virus infects and denudes the microvilli, resulting in increased secretion combined with decreased absorption. The virus interferes with disaccharidase function and alters the function of the intestinal sodium-glucose cotransport proteins. The initial clinical signs are anorexia and depression, with profuse watery diarrhea occurring shortly thereafter. Severely affected foals may become significantly dehydrated and have electrolyte abnormalities, primarily hyponatremia and hypochloremia with metabolic acidosis. These foals generally require intravenous fluid support, whereas less severely affected foals may require only symptomatic therapy. Definitive diagnosis is by detection of the virus in the feces of foals with diarrhea. However, none of the available tests are particularly sensitive, and the virus also may be found with other intestinal pathogens. Recently, vaccination of pregnant mares has been suggested as a means of prevention, with preliminary results suggesting efficacy.[394,395] Although a definitive role for adenovirus has not been established in the foal, adenovirus is a common co-isolate from foals with rotaviral diarrhea.[396] A specific equine coronavirus recently has been identified from an immunocompetent foal with diarrhea, and a second report of cornavirus diarrhea was published recently.[397,398] One case report suggests a parvovirus caused diarrhea in the foal.[399]

Treatment of viral diarrhea in foals is primarily supportive. Intravenous fluid and parenteral nutritional support may be necessary in severe cases. Very young foals may benefit from intravenous plasma administration and broad-spectrum antimicrobial coverage to limit bacterial translocation. One can administer sucralfate orally in these cases as a gastrointestinal protectant and to discourage bacterial translocation. Foals with moderate to severe metabolic acidosis may benefit from sodium bicarbonate administration if their ventilatory function is normal. One administers sodium bicarbonate at half the calculated deficit (0.5 × standard base excess × body mass in kilograms) as an isotonic solution at the maintenance fluid rate. One should reevaluate sodium and bicarbonate (or standard base excess) concentrations regularly. Nonspecific therapy of diarrhea is discussed elsewhere in this text.

19.11.8.3 Bacterial Diarrhea

Diarrhea is frequently the primary presenting complaint in foals with sepsis, so one should rule out this differential diagnosis in foals less than 1 week of age. One should evaluate all neonatal foals with diarrhea for possible sepsis and should include a blood culture whenever possible.

Clostridium perfringens and *C. difficile* are recognized increasingly as serious pathogens of the foal.[400–403] Foals with either pathogen generally have abdominal pain, dehydration, and profuse watery diarrhea. Some foals may have red-tinged or frankly bloody feces, which carries a poorer prognosis. Most foals with this type of diarrhea require intensive care or, at the minimum, intravenous fluid administration. Outbreaks of this type of diarrhea on farms occasionally occur, and the suggestion is that the dam has a role in transmission of

the bacteria. Diagnosis is by recognition of the offending organism by Gram stain of the feces, by bacterial isolation from the feces, and by detecting the presence of toxins associated with the organisms. Specific treatment includes oral administration of metronidazole and broad-spectrum antimicrobial coverage as prophylaxis for bacterial translocation associated sepsis in younger foals. Foals with severe blood loss in their feces may require transfusion of whole blood.

Salmonella spp., *Escherichia coli, Bacteroides fragilis,* and *Aeromonas hydrophila* have been implicated in diarrhea in foals. *Salmonella* generally is associated with septicemia in foals, and although some convincing evidence exists for a role for *E. coli* in foal diarrheal disease, the extent of *E. coli* as a pathogen of the gastrointestinal tract in foals has yet to be described fully.[371,404–407]

Proliferative enteropathy is a transmissible enteric disease caused by *Lawsonia intracellulare*.[408,409] Most foals have been weaned *before* the appearance of clinical signs of depression, rapid and significant weight loss, subcutaneous edema, diarrhea, and colic. Poor body condition with a rough hair coat and a pot-bellied appearance are common in affected foals. Clinicopathologic abnormalities included hypoproteinemia, leukocytosis, anemia, and increased serum creatine kinase concentration. Postmortem reveals characteristic intracellular bacteria within the apical cytoplasm of proliferating crypt epithelial cells of the intestinal mucosa. Antemortem diagnosis of equine proliferative enteropathy is based on clinical signs,
hypoproteinemia, and the exclusion of other common enteric pathogens. Fecal polymerase chain reaction 1418
analysis may be positive for the presence of *L. intracellulare,* and affected foals develop antibodies against *L.* 1419
intracellulare.[410] Treatment with erythromycin estolate alone or combined with rifampin for a minimum of 21 days is recommended with additional symptomatic treatment when indicated.

19.11.8.4 Protozoal Diarrhea

Cryptosporidium spp. cause gastroenteritis and diarrhea in many animal species and are not host-specific. *Cryptosporidium* has been implicated as the casual agent of diarrhea in foals, but the organism is isolated from the feces of diarrheic foals and normal foals with the same frequency and concentration, making a clear role for the organism difficult to elucidate.[411–413] Diarrhea caused by *Cryptosporidium* in other species and that described for foals is generally self-limiting, with a clinical course of between 5 to 14 days. Immunosuppressed patients, including foals compromised by concurrent disease, are thought to be at increased risk for complications resulting from infection with this organism.[411,412] Treatment is symptomatic. Cryptosporidiosis is a disease with zoonotic potential, and one should take appropriate precautions, including use of gloves and frequent hand washing, if organisms are identified in the feces of any patients so as to prevent spread to other patients and personnel. *Eimeria leukarti, Trichomonas equi,* and *Giardia equi* have been identified in the feces of normal horses and horses with diarrhea. Transmission studies have failed to produce reliable clinical signs, and the prevalence and significance of these organisms in the genesis of foal diarrhea remain unknown.

19.11.8.5 Parasitic Diarrhea

Strongyloides westeri is a common parasitic infection of foals.[392,414] Transmission is transmammary, and patent infection is recognizable in the foal by 8 to 12 days of age. This nematode previously was associated anecdotally with foal heat diarrhea, but the association has not been demonstrated clearly. The diarrhea is generally mild and is treated effectively by deworming with benzimidazole or ivermectin anthelmintics.[391]

Strongylus vulgaris fourth-stage larvae cause diarrhea in young foals during migration through the arterioles of the cecum and descending colon. Clinical signs may resemble thromboembolic colic.[414] The prepatent period is about 6 months, and diagnosis is based on clinical examination, clinicopathologic changes, and farm deworming history. Patients with diarrhea associated with this parasite may have peripheral leukocytosis, neutrophilia, eosinophilia, and hypoproteinemia. Appropriate deworming with ivermectin (label dose), fenbendazole (10 mg/kg/day orally for 5 days), or thiabendazole (440 mg/kg/day orally for 2 days) is recommended, with the last two drug dosages being larger than the label dose.

Cyathostomiasis, or diarrhea resulting from the sudden emergence of encysted cyathostome larvae, is an unusual cause of diarrhea in the foal.

19.12 Neonate Therapy

19.12.1 FLUID THERAPY IN NEONATES

The clinician managing critically ill neonates must recognize that intravenous fluid therapy simply cannot be scaled down from adult management approaches. Fluid management of the ill neonate, particularly over the first few days of life, must take into consideration that the neonate is undergoing a large transition from the fetal to the neonatal state and that important physiologic changes are taking place.[166] These transitions include shifts in renal handling of free water and sodium and increased insensible losses because of evaporation from the body surface area and the respiratory tract. The newborn kidney has a limited ability to excrete excess free water and sodium, and the barrier between the vascular and interstitial space is more porous than that of adults. Water and sodium overload, particularly in the first few days of life, can have disastrous long-term consequences for the neonate.[416,417] In the equine neonate, excess fluid administration frequently manifests as generalized edema formation and excessive weight gain, frequently equivalent to the volume of excess fluid administered intravenously. In cases in which antidiuretic hormone secretion is inappropriate, as in some foals with PAS, generalized edema may not form, but the excess free water is maintained in the vascular space. This syndrome of inappropriate antidiuretic hormone secretion is recognized in the foal that gains excessive weight not manifested as edema generally, with decreased urine output and electrolyte abnormalities such as hyponatremia and hypochloremia.[418] The foal manifests neurologic abnormalities associated with hyponatremia. The serum creatinine concentration varies in these cases, but urine always is concentrated compared with the normally dilute, copious amounts of urine produced by foals more than 24 hours of age on a milk diet. If measured, serum osmolarity is less than urine osmolarity. The treatment for this disorder is fluid restriction until weight loss occurs, electrolyte abnormalities normalize, and urine concentration decreases. If the clinician is unaware of this differential diagnosis, the neonate can be assumed mistakenly to be in renal failure, and the condition can be exacerbated by excessive intravenous fluid administration in an attempt to produce diuresis.

The problem of appropriate fluid management in critically ill neonates has been recognized by medical physicians for years and has resulted in changes in fluid management of these patients. The approach taken has 1419
been one of fluid restriction, in particular sodium restriction but also free water restriction, and has resulted in 1420
improved outcome and fewer complications, such as patent ductus arteriosus and necrotizing enterocolitis.[416,417] The calculations used for maintenance intravenous fluid support in these patients takes into consideration the ratio of surface area to volume and partially compensates for insensible water losses. Maintenance fluids are provided as 5% dextrose to limit sodium overload and provide sufficient free water to

restore intracellular and interstitial requirements. The calculation for maintenance fluid administration is as follows:

First 10 kg body mass	100 ml/kg/day
Second 10 kg body mass	50 ml/kg/day
All additional kilograms of body mass	25 ml/kg/day

As an example, the average 50-kg foal would receive 1000 ml/day for the first 10 kg of body mass, 500 ml/day for the next 10 kg of body mass, and 750 ml/day for the remaining 30 kg of body mass for a total of 2250 ml/day. This translates to an hourly fluid rate of about 94 ml/hr.

One should adjust the fluid and sodium requirements for ongoing losses exceeding the maintenance requirements. These losses can take the form of diarrheal losses and excessive urine output, such as those with glucose diuresis and renal damage resulting in an increased fractional excretion of sodium. The normal fractional excretion of sodium in neonatal foals is less than that of adult horses, usually less than 1% (J.E. Palmer, unpublished data). In the critically ill foal the sodium requirement can be met with as little as 140 mEq of sodium per day, about that administered in a single liter of normal equine plasma. One can address sodium deficits by separate infusion of sodium-containing fluids, although this may not be necessary if one considers the sodium being administered in other forms, including drugs administered as sodium salts and any constant rate infusions (pressors, inotropes, etc.) that are being provided as solutions made with 0.9% sodium chloride.

The author has used this approach to fluid therapy in her NICU for the last few years and believes that the percentage of foals suffering from generalized edema and related problems has decreased. If one takes this approach to fluid therapy, one should take the weight of the patient once daily, or even twice daily, and monitor the fluid intake and output as closely as practical. One should evaluate any larger than anticipated weight gains or losses. One should not expect urine output to approach the reported normal of 300 ml/hr for a 50-kg foal because the free water administered is limited, unless the patient is experiencing diuresis (glucosuria, resolution of the syndrome of inappropriate antidiuretic hormone secretion, resolution of previous edematous state, renal disease). One should obtain the urine specific gravity several times daily and should determine fractional excretion of sodium at regular intervals. If the volume of urine produced by the patient is measured accurately, one can determine sodium losses accurately and can obtain creatinine clearance values. One should obtain blood pressure measurements at regular intervals throughout the day because hypotension can be a problem in these patients, particularly in septic foals and foals suffering from PAS, and one may need to increase fluid therapy to maintain adequate vascular volume. Patients with hypotension may need inotrope and pressor support.

19.12.2 PRESSOR AND INOTROPE THERAPY IN NEONATES

Inotrope and pressor therapy generally is restricted to referral centers where these drugs can be administered as constant rate infusions and blood pressure can be monitored closely. Blood pressure can be monitored directly or indirectly by the use of cuffs placed on the base of the tail. Both techniques have advantages and disadvantages. Although direct blood pressure measurements are considered the gold standard and are generally more accurate, the difficulty in placing and maintaining arterial catheters and lines in these patients severely restricts the utility of this method. Indirect techniques can be inaccurate and are affected by cuff size and placement. However, indirect techniques are easier to use in the NICU and can be useful if trained staff are using the equipment. In the author's NICU, once practitioners identify the appropriate cuff size, they dedicate that cuff to that patient for the duration of the hospitalization to decrease variability caused by using different

cuffs. One should monitor the blood pressure of all recumbent patients at regular intervals, and trends upward or downward should prompt the clinician to make necessary adjustments.

Foals suffering from PAS and sepsis are the patients most at risk for significant hypotension and perfusion abnormalities. Perfusion is maintained by supporting cardiac output and blood pressure with judicious use of intravenous fluid support and inotrope/pressor support. The author does not aim for any specific target systolic, mean, or diastolic pressure. Instead the author monitors urine output, mentation, limb perfusion, gastrointestinal function, and respiratory function as indicators that perfusion is acceptable. For NICU patients to require inotrope and pressor therapy is not unusual, but in some cases hypoxic and septic damage is sufficiently severe to blunt the response of the patient to the drugs. One must approach each patient as an individual, and no single inotrope/pressor protocol will suffice for all patients.

Dobutamine is a β-adrenergic inotrope that is frequently used as first choice therapy in NICU patients. Its effects are β_1 at the lower dose range. Neonates have a limited ability to increase stroke volume in an effort to 1420

maintain cardiac output, and one may observe tachycardia in these patients as heart rate increases to maintain 1421
cardiac output and vascular pressure. Dobutamine is useful after patients are volume replete for support of cardiac output. The dose range is between 2 to 20 μg/kg/min provided as a constant rate infusion.

Dopamine has dopaminergic activity at low doses, β_1 and β_2 activity at moderate doses, and α_1 activity at high doses. Dopamine causes norepinephrine release, which has lead to the suggestion that this is its major mode of action at higher doses. At doses greater than 20 μg/kg/min, intrapulmonary shunting, pulmonary venous vasoconstriction, and reduced splanchic perfusion may occur. Dopamine also produces natriuresis at lower doses through a direct effect on renal tubules. For these reasons, dopamine has fallen out of favor at some referral institutions.

Norepinephrine has α_1 and β_1 activity but variable β_2 activity, resulting in potent vasopressor effects; it has inotropic and chronotropic effects, but its chronotropic effect usually is blunted by vagal reflexes slowing the heart rate induced by the increase in blood pressure. In many critical care units, norepinephrine has become a pressor of choice and frequently is used along with dobutamine. Evidence suggests that splanchic perfusion is maintained better with norepinephrine than with some other pressors.[419] The dose range is 0.2 to 2.0 μg/kg/min, although larger doses have been used when necessary in certain patients.

Epinephrine has α_1, α_2, β_1, and β_2 activity; β activity predominates and results in increased cardiac output and decreased peripheral resistance at low doses. Epinephrine has been associated with hyperglycemia, hypokalemia, lipolysis, increased lactate concentration, and increased platelet aggregation. The effect on renal function is controversial. Use of epinephrine usually is limited to those patients not responding to other pressors.

Vasopressin (antidiuretic hormone) is a pressor gaining a great deal of attention in the critical care literature. Vasopressin appears to be depleted from the neurohypophysis in septic shock,[420] and short-term administration of vasopressin spares conventional vasopressor use, in addition to improving some measures of renal function.[421] Low-dose vasopressin infusion increases mean arterial pressure, systemic vascular resistance, and urine output in patients with vasodilatory septic shock that are hyporesponsive to catecholamines. These data indicate that low-dose vasopressin infusions may be useful in treating hypotension in patients with septic shock.[422] The author has been using low-dose vasopressin in patients in her NICU for the past few years and has the clinical impression that blood pressure is defended more readily using this agent in concert with other management strategies. The author commonly uses low-dose vasopressin constant rate infusion with dobutamine constant

rate infusion as the initial inotrope/pressor therapy in cases requiring pressure defense, although no prospective studies are yet available regarding this drug in veterinary medicine.

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19.13 19.1—Musculoskeletal Disorders of Foals

Elizabeth M. Santschi

The advances in medical care of equine neonates in the last 20 years have resulted in the survival of many foals that previously would have died from sepsis, asphyxia, and prematurity; and the successful management of their musculoskeletal system can be a major challenge. Major factors adding to the challenge are the immaturity of components of the musculoskeletal system and the demands placed on them by a growing and active foal. Additional pressures to treat orthopedic conditions in foals have come from an overall increase in the demand for quality health care for animals, advances in medical science, and in some breeds the increasing value of the juvenile equine athlete. Equine veterinarians that encounter pediatric orthopedic problems are only beginning to get the information needed to make appropriate treatment decisions.

19.13.1 NEONATAL MUSCULOSKELETAL SYSTEM

The equine neonate has specific differences in structure and physiology from adults that one must consider when designing an optimal therapeutic or management strategy. Few investigations have focused on the equine neonatal musculoskeletal system,[1–6] but a large body of clinical information exists, and one can make cautious extrapolations from work in other species.[7] Neonatal equine bones have accelerated modeling and remodeling processes[5] that result in accelerated fracture healing and an increased susceptibility to deformation caused by excessive loading. Contralateral limb varus deformities of the growth centers (most commonly distal radius and metacarpus/metatarsus) are common in overloaded limbs. The increased plasticity of the skeletal structure also is mirrored in the soft tissue support system, for these units become flaccid within 2 weeks of immobilization.[4] This laxity is important, because it further compromises the use of the fractured limb and can last as long as the coaptation was in place. Additional divergences from adult physiology include musculoskeletal immaturity (generalized or focal) and immune system differences. Finally, foals are lighter and can tolerate and will assume recumbency more readily than adults. The net results of these differences are that one must consider the use of external coaptation carefully, fractures heal quickly, one must consider damage to the contralateral limb from overstress, reducing weight bearing is possible, and infection is always lurking.

19.13.2 TRAUMA

19.13.2.1 Long Bone Fractures

Stresses can affect the musculoskeletal system of the foal at any time, including in utero. Although rare, reports describe in utero fractures (K. Sprayberry, personal communication, 2003) that result in foal locomotor problems and even maternal uterine damage from sharp bone ends. The cause is presumably from vigorous muscular activity of the foal, but one cannot rule out direct trauma. The fractures result in foal lameness and can increase the likelihood of dystocia and caused colic in one mare when the broken bones damaged the uterus. Treatment depends on how long the fracture has been present and on the fracture location and configuration, but if the fracture is repairable, internal fixation probably is necessary. Fractures 1431
occurring during foaling result from aggressive obstetric manipulation (mandibles) or chest compression. 1432
One should stabilize unstable mandibular fractures. Appendicular fractures usually do not occur during parturition because of the robust character of the bones of the foal.

After birth, foals are susceptible to external trauma from many sources. The dilemma is that younger foals with fractures are more likely to heal but also are more likely to develop contralateral limb problems because of excessive weight bearing and affected limb flexor tendon laxity if the limb is immobilized fully. As a result, internal fixation is often the best choice for neonatal fractures to keep the fractured limb in use.

19.13.2.2 Small Bone and Avulsion Fractures

Proximal sesamoid bone fractures result from hyperextension of the fetlock joint. Foals are lame after the fracture, but the lameness can be mild and often diminishes quickly. Soft tissue swelling occurs over the sesamoids. Fractures are usually simple, can occur uniaxially or biaxially, and can be apical, midbody, or basilar. Fractures can occur in any joint and can affect multiple sesamoids in one foal. However, they most commonly are single forelimb fractures[8] and in Thoroughbreds are most frequent in the left front medial proximal sesamoid (J.P. Morehead, personal communication, 2003). Of particular interest to neonatologists

is that proximal sesamoids fractures often occur in recovered neonatal patients that are allowed too much exercise too soon. Foals from the NICU need a gradual introduction to pasture turnout to allow their musculoskeletal system to adjust. Mares are often in need of turnout, but in the interest of their foals, they must wait.

Treatment of proximal sesamoid fractures in foals is stall confinement with support bandaging. Healing occurs, albeit with some distortion of the shape of the sesamoid. Severely displaced fragments result in large and misshapen sesamoids, and surgery may be considered for these foals, because restriction of fetlock flexion can occur after conservative therapy.

Third phalangeal fractures are also common in foals. These foals have a lameness that worsens with hoof compression. Hoof abscesses are uncommon in young foals but should be considered. Most commonly, radiographs reveal nonarticular small fractures on the wings on the third phalanx. The fractures are associated with hard ground and exercise. The fractures heal with stall confinement, and unlike adults, leave no discernable radiographic fibrous union.

Avulsion fractures of the proximal insertion of the peroneus tertius and the origin of the long digital extensor tendon have been reported.[9,10] Both soft tissue structures attach to the extensor fossa of the distal femur. The two affected foals had lameness of a hindlimb associated with swelling, pain, and crepitation. Radiographs revealed multiple avulsion fractures of the extensor fossa. Because of the intraarticular fragments in the femoropatellar joint, and the fear of later degenerative joint disease, fragments were removed arthroscopically. Both foals were juveniles at last follow-up; one foal was considered normal, and one had a mild residual lameness.

19.13.2.3 Tendon and Ligament Damage

Tendon and ligament damage is uncommon in neonates probably because of their low body weight. Extensor tendon damage following flexural deformities is the most common tendon problem and is discussed in Congential Flexural Deformities of Foals. Gastrocnemius ruptures are one of the most devastating problems and have occurred after forced extraction because of a breech presentation, severe flexor tendon laxity, and tarsal contracture. Loss of gastrocnemius function usually results in a non–weight-bearing limb, although an intact superficial digital flexor tendon may make some weight bearing possible. Complete loss of support is difficult to treat successfully. Coaptation of the limb is logical but difficult to obtain. Schroeder-Thomas splints have been used but are difficult to manage. Tube casts also are used but must be changed frequently, and cast sores are inevitable (L.R. Bramlage, personal communication, 2003). The prognosis for athletic function is guarded.

Treatment for ligamentous injuries is usually some form of coaptation, although surgical repairs have been performed when coaptation was unworkable.[11] Coaptation in proper limb alignment allows the ligaments to heal and should be used if the injury will destabilize a joint and cause damage to growing epiphyses or cuboidal bones. One can achieve coaptation with casts or splints under a bandage. Casts are initially a greater expense, and cast sores and their resulting white hairs are a risk, but the rigid immobilization and the lack of the requirement for daily adjustment makes them preferable. Important to musculotendinous health is some measure of weight bearing to avoid laxity after coaptation removal, which one can achieve by using tube casts and splints that allow weight bearing. Following coaptation, bandaging and a gradual return to exercise are recommended for ligamentous injuries.

19.13.3 LUXATING PATELLAE

Patellar luxation can affect foals in one or both hindlimbs, and the luxation can vary from a laxity in the medial attachments to complete luxations that cannot be replaced in the patellar groove of the distal femur.[12,13] Medial luxations have not been reported. Clinical signs vary from a slight discontinuous motion during stifle flexion to an inability to stand. Many foals have a crouching stance on the affected limb because of an inability to extend 1432
the stifle. The pathophysiology of patellar luxations is unknown. Congenital bilateral luxations are common in 1433
Miniature horse foals and are believed to be genetic. Luxations are rarer in other breeds and are occasionally traumatic. The affected limbs are usually not grossly abnormal except for effusion of the femoropatellar joint and the luxation. A shallow trochlear groove has been reported to be a cause of patellar luxation, but objective evidence is lacking.

One should evaluate foals for the ability to stand. Once the appropriate supportive care is provided, if a foal cannot stand, euthanasia is recommended. Most bilateral luxations in horses fit in this category. However, Miniature horse foals often can stand sufficiently to nurse despite bilateral luxations, and one may consider treatment. Treatment consists of replacing and stabilizing the patella and sometimes surgically deepening the patellar groove. Delaying surgical repair until the foal is approximately 30 days old is recommended to avoid neonatal problems, allow the musculoskeletal system to mature, and provide good anchors for suture. Some surgeons worry that delay may cause further femoropatellar developmental abnormalities, but in a small number of cases, this has not been an issue. The prognosis for Miniature horse foals appears to be good because of their low body weights and modest performance expectations. Too few reports about the correction of unilateral luxations in light horses exist to make a definitive statement about prognosis except that success and failure have been experienced.[12,13]

19.13.4 CONGENITAL FLEXURAL DEFORMITIES

Congenital flexural deformities in foals can be classified as severe (rarely correctable), moderate (correctable with therapy), or mild (self-correctable). Examples of severe flexural deformities include arthrogryposis (deformities of multiple limbs and often the head and neck), severe carpal deformities (flexor angle of the carpus less than 90 degrees), and tarsal contractures (rare). Extraordinary methods have been used to correct severe deformities[14] but are often unsuccessful.

Mild flexural deformities are those that result in an upright conformation to the limb, but the foal can bear weight on the limb and load the flexor structures. These foals require no specific treatment and will self-correct with controlled exercise.

Moderate flexural deformities are those that make bearing weight on the limb and loading the flexor structures and ligaments difficult for the foal. When these deformities occur bilaterally (most common), the foals cannot rise to suckle or does so with great difficulty, and the lack of weight bearing worsens the flexural deformity. Examples of moderate flexural deformities include carpal and forelimb fetlock flexural deformities that usually occur together, hindlimb fetlock flexural deformities with coronopedal flexion or hyperextension, and the uncommon coronopedal flexural deformity alone.

Treatment of moderate flexural deformities aims to place the solar surface of the foot on the ground so that the weight of the foal can stretch the flexor structures. Splints are useful for restoring the limb to normal orientation but require attention to detail because the splints often exert an extreme amount of tension on the soft tissues,

and the skin of the foal is thin. Pressure sores are easy to create and at a minimum result in an extended convalescence.

The first step in splint application is to apply a separate heavy bandage to the limb, which should be reapplied as necessary because the bandage can slip and cause focal constriction. Commercial gauze over cotton bandage material works better than sheet cotton as a bandage. The splint is made of polyvinyl chloride pipe cut in half or thirds. Using 50% of the diameter of the pipe results in less splint rotation but is bulkier and leaves more splint exposed to cause trauma. One cuts off the corners of the splint and pads the ends with gauze or roll cotton covered with tape. Palmar or plantar placement of the splint is preferable, but severe deformities may require initial dorsal placement. As the limb straightens, one can bend the splint to tape the fetlock into the bend to extend it. One can tape the splint tightly to the limb over the bandage with nonelastic (white or duct) tape. This procedure requires at least two persons, one to extend the limb firmly and hold the limb and one to tape. One should leave the splint on for 8 to 12 hours and then remove it for 8 to 12 hours. One can reapply splints as necessary.

In addition to splints, some medications are of value for treating flexural deformities. Oxytetracycline (40 to 50 mg/kg) given intravenously appears to relax the soft tissues.[15] The mechanism of action is unknown, and the drug is most efficacious when given in the first 3 days of life. This dose is high but appears to be safe for healthy foals and can be repeated at 24-hour intervals. Foals should be normovolemic during tetracycline administration. One should use the drug with caution in foals with renal impairment. Foals should be urinating and have reasonable urinary parameters (serum urea nitrogen, creatinine, and urinalysis) before tetracycline use. Diarrhea is an uncommon sequela to tetracycline use. One should monitor the unaffected limbs closely because all limbs experience a relaxation of the palmar/plantar support.[15,16] Discontinuation of tetracycline therapy before affected limbs are normal but after they can bear weight is common because of worsening laxity in the "normal" limbs. One also can use phenylbutazone (4 mg/kg) for a short time when the splints are used. Some analgesia appears to help the foals use the limbs and stretch the soft tissues. One should not use phenylbutazone for long periods of time because of the potential of inducing gastric ulcers.

Surgical treatment of congenital flexural deformities rarely is indicated. Severely affected foals rarely respond 1433
favorably to surgery, and mildly affected foals do not need it. Surgery is most appropriate for foals with 1434
moderate flexural deformities that are neglected or have not responded to splinting and tetracycline. The most common surgical therapy performed for congenital flexural deformities is the inferior check ligament desmotomy for fetlock or coronopedal flexural deformities.

Ruptures of the extensor tendons commonly occur with congenital flexural deformities and result from the foal overloading the extensor tendons. No specific therapy for the ruptures is necessary. If the rupture is extensive, it can interfere with the ability to extend the fetlock and to place the foot flat. These foals then tend to knuckle over, even after correction of the flexural deformity. A firm fetlock bandage extends the digit and assists in foot placement until the extensor tendons heal.

19.13.5 CONGENITAL EXTENSION DEFORMITIES

Foals commonly are born with hyperextension deformities of the fetlock of varying degrees of severity. All but the worst deformities self-correct as muscle tone improves. A deeply bedded stall is all that is usually necessary to protect the soft tissues, but one can apply a light bandage to the coronary band and pastern if trauma is a problem. Severe deformities are more problematic but rare, so therapeutic recommendations are not available. Hyperextension of the carpus occasionally occurs and usually is treated conservatively. However, a tube cast to align the limb may be necessary to protect the dorsal surface of developing carpal bones.

19.13.6 CONFORMATIONAL DEVIATIONS

Neonatal foals exhibit three categories of forelimb conformational deviations: angulation, rotation, and carpal offset. Angular deviations most commonly are centered in the metaphysis and epiphysis, but their location is described by the closest joint, usually the carpus and fetlock. When the deviation of the distal limb is lateral to the long axis, the deviation is valgus, and when the deviation is medial, the deviation is varus. More than one joint can be affected, and although rare in neonates, valgus and varus can occur in different joints in one limb.

Rotational deformities appear to originate most commonly in the diaphysis or metaphysis of the radius or the metacarpus. In neonates the direction of rotation of the distal limb at both sites is almost exclusively outward. Associated angular and rotational deviations occur.[17] In neonates, limb deviations occur in foals with narrower chests and less developed pectoral muscles than in straight foals, and they appear to have an initial greater overall weakness in the musculoskeletal system because it first interacts with gravity, body mass, and ground reaction forces. However, after the first few days of life, the asymmetric loading of the growth centers does affect limb deviations. Angulation results from a compressive load that is asymmetric in a frontal plane but is uniform in the sagittal plane, and rotation occurs when the compressive load is asymmetric in both planes and the limb develops around an overloaded axis point. Considered this way, valgus and outward rotation deviations in young foals are coupled, as are varus and inward rotation in older foals. The loading asymmetry for valgus/outward rotation foals is accentuated as foals assume a base-wide posture that is more stable side-to-side but promotes a lateralization of the limb load. The specific effects of intermittent versus static loads, strain magnitude versus strain rate, and shear and hydrostatic stress on growing bones is only beginning to be understood. However, clinical experience supports the general observation that excessive cartilage compression is deleterious to bone growth.

Offset carpal conformation describes a joint that appears to deviate outwardly and then inwardly, all within the carpus. The deformity is thought to be centered at the radiocarpal joint, but the specific structural cause of offset has not been determined. This conformation is more common in older foals but occasionally occurs in neonates. The deviation is particularly common when incomplete ossification of the carpal bones is present.

The causes of conformational deviations are a matter of some debate. As always, the major factors are genetics or environment. Genetic influences include the assortment of alleles that controls bone form and growth and the assortment that modulates bone remodeling. Many in the horse industry believe that genetics is a strong determiner of limb conformation. Environmental influences are many and include the intrauterine environment, the postnatal limb load, nutrition, and bad luck. Suffice to say, the situation is complex, but one must consider biologic and mechanicobiologic influences when evaluating the growth of long bones.[18]

Several factors may contribute to the common occurrence of deviations in the carpus. First, the carpus is in the middle of the limb and is subject to the greatest bending forces. Second, the carpal anatomy is complex and perhaps is not understood completely. The carpus has seven cuboidal bones, two long bones, and two epiphyses (distal radial and lateral styloid); and cartilage surrounds all. The ligamentous support includes collateral ligaments, innumerable intracarpal ligaments, and a palmar carpal soft tissue ligament. The distal radial physis is not flat transversely, but undulates in the frontal and sagittal planes.[3] A separate center of ossification for the lateral styloid process is found at its palmar-lateral aspect. Because of this separate center of ossification, more cartilage and less bone are in the lateral aspect of the distal radial growth center, suggesting it may be more susceptible to growth alterations from load. 1434

Less common conformation deformities in young foals include hindlimb deformities, windswept conformation, diaphyseal deviations (usually of the metacarpus/metatarsus), gross congenital malformations such as agenesis and polydactyly, and acquired varus deformities of the carpus and fetlock. Hindlimb conformational deviations can manifest as tarsal and fetlock angular deformities and external limb rotation, usually centered above the tarsus. Windswept foals have limbs (usually both forelimb or both hindlimbs) that are curved in the same direction in the frontal plane. Diaphyseal deviations, agenesis, and polydactyly are rare and have various presentations. Acquired varus deformities are caused by excessive loading, which appears to be focused medially on the growth plates. 1435

19.13.6.1 Evaluation

One should evaluate the limbs to determine the location, extent, and potential cause of the deviation. Evaluation consists of observation and then palpation for heat, swelling, or ligament laxity. Ligamentous laxity of the medial carpal ligaments is an important cause of carpal valgus and should be evaluated carefully. Lameness is not a characteristic of uncomplicated angular limb deformities and suggests further evaluations are necessary. Radiography is indicated for foals with severe deviations (all tarsal valgus), ligamentous laxity, lameness, or joint effusions. Ultrasonography may be valuable for selected soft tissue evaluations.

19.13.6.2 Nonsurgical Treatments

Conservative therapy is by far the most commonly used therapy in foals less than 30 days of age.[19] Mild to moderate carpal valgus and external rotation of the carpus and fetlock are common and normal in neonates, particularly light breed horses. Most congenital limb deviations improve with age, if the developing musculoskeletal system is protected from overuse and abnormal loads. Approximately 90% of Thoroughbred foals with congenital carpal valgus self-correct. Those foals that do not most often have abnormal bone (incomplete ossification) with normal stress or normal bone with abnormal stress (ligamentous laxity or contralateral limb lameness). Correction continues for several months, and on average, foals reach their straightest conformation (regarding angulation) at approximately 10 months of age (E.M. Santschi, unpublished data).

TABLE 19-10 Exercise Recommendations for Foals With Uncomplicated Carpal Valgus*

TIME	VALGUS CATEGORY (APPROXIMATE ANGULAR DEVIATION) MILD (4°–10°)	MODERATE (11°–16°)	SEVERE (>16°)
Initial exercise	Paddock turnout	Stall confinement; playing while mare is walked in hand daily	Large stall confinement; limited walking in hand
After 1–2 weeks	Pasture turnout	Paddock turnout	Stall confinement; playing while mare is walked in hand
Maintenance	Pasture turnout	Pasture turnout	Paddock turnout

* Increasing levels of exercise are allowed as the angulation decreases; static and worsening deviations are indications for further evaluation.

Determination of the appropriate treatment for foals with angular limb deformities is based on the age of the foal, the severity and location of the deviation, and its causes. One must evaluate the entire foal and the affected limb. If the carpal collateral ligaments have no laxity and carpal incomplete ossification is not suspected, one may use an exercise program such as in Table 19-10, assuming that the foal has no contradicting additional problems. Exercise is essential for the robust development of almost every body system for neonates, and fresh air and good ventilation reduce the occurrence of respiratory disease. Appropriate limb loading along with growth and maturity is what straightens limbs, but excessive amounts of loading can be deleterious. For example, one should use exercise cautiously in foals with very asymmetric deviations. When one limb is much more deviated than the other, it appears to be loaded excessively and compromised more than if both limbs were affected similarly. And finally, limb deviations are additive. Foals with external rotation and carpal valgus improve more slowly than those with one type of deviation.

Incomplete ossification of cuboidal bones and focal ligamentous laxity are complicating matters of great potential impact on adult conformation. They generally manifest as a moderate to severe limb deviation. Physical examination indicates laxity because angular limb deviations are reducible. Radiographs are the best way to evaluate the extent of carpal bone ossification. Incomplete ossification of the cuboidal bones can be
focal or widespread. Focal immaturity is not common but can result in severe angulation. Generalized 1435
immaturity is more frequent and initially often manifests as an offset conformation with valgus angulation. 1436
When the foal becomes heavier, assumes a base-wide stance, and is allowed exercise, crushing of the bones of the lateral carpus (usually the lateral styloid process of the radius, the ulnar, the fourth and the intermediate facet of the third carpal bone) results in a permanent intracarpal valgus deviation. The same result occurs when significant medial carpal ligament laxity goes untreated.

In the forelimb, foals with collateral ligamentous laxity and moderate to severely immature cuboidal bones should have external coaptation placed on the affected limb to maintain axial orientation. Tube casts that allow weight bearing on the digit are preferred to splints. Ligamentous laxity in the carpus usually responds to tube casting for 7 to 10 days followed by bandaging and cautious exercise. The duration of similar coaptation necessary for immature carpal bones depends on the degree of immaturity and the speed with

which the bones mature. Because casts cannot be left on neonatal limbs for more than 7 to 10 days because of their fast growth, more than one cast may be necessary.

Treatment of tarsal valgus and rotational deformities is much less common than in the forelimbs because deviations are less common than in the forelimb, because some breeds prefer an outward position to the hindlimb, and perhaps because owners recognize it less frequently.[20] Hindlimbs generally are unaffected by ligamentous laxity, but tarsal incomplete ossification is common and often is associated with tarsal valgus. Treatment of tarsal incomplete ossification is important because tarsal crushing results in an unfavorable prognosis for athletic performance.[20,21] Hindlimbs require a slightly different approach to coaptation than forelimbs because of their anatomy. Foals can rise to stand if their forelimbs are fixed in extension but cannot do so if their hindlimbs are extended. The multiple bony protuberances of the hock make cast sores more likely than in the forelimb, so casts are problematic. Gutter splints are not useful because of the angle of the hock. Severely limiting exercise is part of allowing the tarsus to mature without cartilage crushing, but foals cannot always be recumbent. Extra small articulated anterior cruciate ligament splints for human beings (Playmaker Wraparound, dj Orthopedics, Vista, California) have given the best results. For small foals, a padded bandage is necessary under the splint, which is reversed to conform to the angle of the hock. The splints allow enough flexion in the hock for the foal to rise but appear sufficient when combined with stall rest to protect the cartilage from crushing. Splints are left on the hocks until the cuboidal bones have ossified as shown by radiography.

Fetlock conformational deviations in neonates that are treated best conservatively are rare. Outward rotation is the most common deviation but is thought to have minimal effect on the performance and improves with maturity. The only therapy used is to rasp the toe square to promote central breakover. Severe outward rotation can promote a fetlock valgus conformation, so one can use a medial hoof wall extension of epoxy to bring the limb load medially. The most commonly treated fetlock deviations are inward but usually occur in foals older than 30 days. However, if the deviation is noticed in neonates, one can use small lateral hoof wall extensions that generally are made of epoxy with fiberglass cloth embedded to prevent chipping.

Windswept foals are born with multiple deviations. Evaluating the foal as a whole is best rather than focusing on individual joints. Most of these foals become straight over time with conservative therapy.

19.13.6.3 Surgical Treatment

No surgical procedures are commonly accepted for direct treatment of rotational or carpal offset deviations, so angular deviations are described. Surgical procedures to correct carpal and fetlock valgus include periosteal transection and elevation and transphyseal bridging. Periosteal elevation is thought to accelerate growth on the concave side of the metaphysis, and transphyseal bridging is used to restrict the growth on the convex side of the physis. Studies indicate an approximately 80% improvement of carpal valgus foals after periosteal transection and elevation, but unfortunately they do not compare foals that had surgery with controls that did not.[22,23] Recently, some have suggested that most of the correction was unrelated to the surgery,[24] and one experimental study supports that conclusion.[25] As a result, at this time making firm recommendations about the indications for periosteal transection and elevation is difficult. However, periosteal transection and elevation has a low likelihood of complications and may be effective. The procedure is inexpensive and can be done in the field and therefore may be an option for clients with foals with carpal valgus in which a transphyseal bridging is undesirable or unnecessary. One indication is the very young foal born with a notably asymmetric epiphysis that results in a severe carpal valgus. This distal radial appearance is not particularly common, but the lack of ossification in the epiphysis can make a firm hold

with a transphyseal bridging difficult to achieve. However, one can use distolateral radial periosteal elevation at an early age in an attempt to accelerate correction of the valgus and protect developing carpal bones.

Often a degree of anxiety exists about correction of fetlock angulations because of the much shorter time period for physeal growth. Most fetlocks are in their final conformation by 60 days of age, so correction is best accomplished with earlier treatment, usually by 4 weeks of age. One can perform periosteal elevation on 1436
the medial (for varus deviations) or lateral (for valgus deviations) aspect of the distal metacarpus/metatarsus. 1437

The definitive treatment of limb angulation at a growth plate is transphyseal bridging. One should consider using the procedure at about 3 weeks of age for all moderate to severe fetlock deviations, at about 4 weeks for severe carpal deviations, and 6 to 8 weeks for mild fetlock deviations, moderate carpal deviations, and any worsening angular deformities. One must perform bridge removal when the limb straightens to prevent overcorrection.

Diaphyseal deviations are rare but can occur in varying degrees of severity. If the foal can bear weight on the limb, a conservative approach is indicated. One can consider periosteal elevation of the length of the concave surface of the long bone. If the foal cannot bear weight on the limb because of the severity of deviation, euthanasia is probably the best option. However, a revision osteotomy and internal fixation may be appropriate for selected foals.[26] Polydactyly is also rare and sometimes can be corrected surgically. The outcome is based on the degree of articular involvement.

19.13.7 ORTHOPEDIC INFECTIONS IN NEONATAL FOALS

Bacteria may invade the foal musculoskeletal system and cause orthopedic infection after delivery by the circulation, by direct extension from another system, or by direct inoculation. Hematogenous delivery is by far the most common and results in infection of synovial structures (joints, tendon sheaths, bursae) and bone. Extension from another site without hematogenous delivery is rare. Direct inoculation almost exclusively results from traumatic rather than surgical wounds.

Much is still to be learned about the pathophysiology of orthopedic infection, including the source of the infecting bacteria. The umbilicus commonly is accepted as a possible source of bacteria,[27] but many believe that the gastrointestinal and respiratory tracts are at least equally responsible. Associated conditions in foals with septic arthritis include failure of passive transfer, pneumonia, and enteritis.[28] The classification of orthopedic sepsis in foals into infection of bones and joints is probably irrelevant because most foals with septic arthritis also have infectious osteitis or osteomyelitis.[27,29] Septic arthritis is more readily recognizable because the reactivity of the synovium to the bacteria causes joint effusion and lameness and because early radiographic signs of bone infection in foals are equivocal.

Also unclear are the reasons for the apparent site predilection for orthopedic infection in foals. The femoropatellar joint and the tarsocrural joint are affected most frequently, followed by the carpal and fetlock joints, and finally an assortment of miscellaneous joints such as the elbow, shoulder, and hip.[28] The common association of osteomyelitis of the distal femoral, tibial, and metacarpal/metatarsal physes with a newly recognized septic arthritis suggests that the infection in that area started at the growth center (epiphysis, physis, or metaphysis). The localization of the apparent initial site of infection to the growth center has been suggested to result from “looping” metaphyseal vessels with sluggish blood flow that allow pathogens more time to escape the circulation.[29,30] However, transmission electron microscopy indicates that osteogenic cells and the

vascular endothelium are a continuous network in developing embryos,[31] indicating that the relationship between circulation and bone is more intimate than previously suspected.

A possible association between osteomyelitis and thickened or traumatized cartilage exists. Focal osteomyelitis lesions occur commonly at the bone cartilage junction[27,29] and particularly in areas where cartilage is attached at an angle to the long axis or where thickened.[29] An association also exists between incomplete ossification of the central and third tarsal bones and osteomyelitis.[32] Trauma to the metaphysis is a known predisposing cause of osteomyelitis in young bacteremic rabbits.[33] A trend exists for foals with more than one joint affected to be affected bilaterally in the same joint, rather than in random joints. This trend suggests that a “window” exists when a joint may be more susceptible to infection and that trauma to the developing cartilage may be a contributing factor. In neonates, cartilage is vascular,[34] and possibly small traumatic cartilage lesions with associated hemorrhage and exposure of bacterial binding sites might be the inciting cause for the location of infection.

The pathogens most commonly associated with septic arthritis in young foals are also those that frequently are implicated in neonatal sepsis. The most commonly isolated gram-negative organisms are *Escherichia coli* and other Enterobacteriaceae, *Actinobacillus equuli,* and *Salmonella* spp. Frequently isolated gram-positive organisms include *Streptococcus* spp., *Staphylococcus* spp., and *Rhodococcus equi*.[28] Anaerobic bacteria and fungi are rare but should be considered in refractory cases.

The diagnosis of orthopedic sepsis can be challenging. The most common clinical sign is lameness, followed by swelling around a joint or metaphysis. Joint effusion alone may cause the swelling, but edema is also common, especially if metaphyseal osteomyelitis is present. But effusion and edema can be difficult to detect because of the tissue surrounding the focus of infection in the shoulder, elbow, hip, and coffin joints. One should evaluate lame foals carefully by palpation to localize pain and swelling. If one can find no pain or swelling, one should obtain a complete blood count and fibrinogen level. Although a complete blood count is not always abnormal in 1437
foals with septic arthritis, abnormalities should raise the index of suspicion of infection. Elevations in 1438
fibrinogen are fairly common in septic arthritis,[28] and fibrinogen almost always is elevated if the infection involves bone. If hematologic values are normal, the lameness could be caused by trauma, but the foal should be monitored closely for improvement, and closer evaluation is indicated if improvement is not rapid.

An arthrocentesis is the diagnostic test of choice for confirmation of septic arthritis. One should perform joint puncture in a sterile fashion, and sedation is indicated to get an atraumatic tap. Short-term anesthesia is preferable when joints have effusion because one may perform joint lavage at the same time. Normal joint fluid should be clear to slightly yellow, should be viscous, and should contain less than 2500 nucleated cells per deciliter. The cell ratio should be roughly 50:50 polymorphonuclear and mononuclear. The total protein content should be less than 2.5 mg/dl. One should consider joints to be infected if the nucleated cell count is greater than 10,000 cells/dl. For joints falling between 2500 and 10,000 cells/dl, if the polymorphonuclear cell count is >90%, one should consider the joints infected. Cytologists are often reluctant to diagnose infection when nuclear degeneration or bacteria are not visible. This is overly conservative and results in delay in treating infections because bacteria and nuclear degeneration are rare in early cases of joint infection. Out of an abundance of caution, one should treat lame foals with suspicious joints as infected unless they are clearly normal. One should always culture joint fluid in an attempt to identify the offending organisms, but because of difficulties in culturing pathogens from joint fluid samples, absence of growth does not mean absence of infection. One obtains the best culture results if the foal has not been treated with antimicrobial agents beforehand. One should obtain as much joint fluid as possible for culture and should incubate it overnight in

blood culture media before plate inoculation. As always in potentially septic foals, blood culture may assist in the isolation of the organism.

Other orthopedic infections that do not involve the joint may be more difficult to detect. Often these are not apparent until infection breeches the joint and causes lameness. However, astute caretakers may notice early clinical signs such as mild lameness, fever, or edema centered at a growth center. Radiography and advanced imaging modalities such as magnetic resonance imaging are the best diagnostic tools for the localization of areas of osteitis and osteomyelitis. One should examine the area of concern carefully, giving particular attention to the growth centers and subchondral bone. Interpretation of radiographs may be difficult because these areas are complex and normally have irregular bone margins in the growing foal. If a normal contralateral joint is available, comparison radiographs may be useful. Because of the high metabolic turnover in growing foal bone, changes occur faster than with adults, so radiographs at the earliest sign of potential infection of bone and joint are recommended. If evidence of osteolysis is clear, aspiration of the area may yield material for culture.

The goals of treatment are to eliminate infection immediately and then resolve inflammation. Bacteria and products of inflammation elicited by infection are responsible for destruction of bone and cartilage. The ultimate aim of treatment is to protect the structures critical to athletic performance such as subchondral bone and cartilage in weight-bearing areas. Advances in the treatment of sepsis have resulted in hospital discharge rates of 78% for foals with septic arthritis, but their rate of high performers is 30%,[28] indicating a need for improvement. Equine veterinarians cannot replace what has been destroyed, so early identification and aggressive therapies are presently the best methods to improve performance rates.

One achieves the goals of treatment by physical removal of bacteria, products of inflammation, and debris and by medications to kill the bacteria and reduce inflammation. One should optimize the physiology and general health of the foal to assist this process; one should include other treatments and supportive therapies for septic foals, especially treatment of failure of passive transfer, in the therapeutic plan. Intravenous administration of antimicrobials (see Chapter 4) is the cornerstone of treatment of orthopedic infection, and if the drug is administered early in the course of infection and bacteria are susceptible, intravenous administration may be sufficient to eliminate the organisms. However, treatment of many foals does not begin until disease is advanced. If treatment begins after bacteria have had a chance to establish themselves, one should bring all appropriate methods to bear to end the infection.

Additional therapies for septic arthritis include joint lavage, arthrotomy (for drainage),[35,36] debridement (arthroscopically or arthrotomy),[37] intraarticular administration of antimicrobials, intravenous regional perfusion,[38] and antimicrobial beads.[39,40] One can use any sterile isotonic solution to flush a joint, and additives do not appear to give significant additional benefit. If radiographs do not indicate osteomyelitis, lavage, intraarticular antibiotics, and if possible, regional perfusion are recommended. If osteitis or osteomyelitis is present, debridement is indicated arthroscopically or via arthrotomy (one should culture the debris if the pathogen is unknown). If the joint is closed, one may use antibiotics intraarticularly. If the joint is left open to drain, regional perfusion is useful. Antimicrobial beads theoretically are best to use if the wound is closed, but they appear to give benefit even if the wound is open under a bandage. Because of concerns about 1438
the use of beads in a joint,[41] beads often are used in tissue defects and the surrounding tissues. The major goal 1439
is to remove material that is compromising healthy tissues and to obtain high concentrations of antimicrobials in infected tissues.

High antimicrobial concentrations are necessary because adhered bacteria are difficult to kill and may require many times the in vitro bacterial minimum inhibitory concentration. Intraarticular administration of

antimicrobials has been used for many years and has great value.[35] Regional perfusion of diluted antimicrobials recently has come into use and may be administered intraosseously[42] or intravenously. Intravenous perfusion is preferable because no special equipment is needed, but intraosseous perfusion may be valuable where intravenous access is impossible. The concept behind both procedures is to fill the venous vasculature in the area of the infection with antimicrobials diluted by a sterile balanced electrolyte solution. One isolates the anatomic area of interest using one or two tourniquets. The perfusate diffuses into all tissues and achieves much higher concentrations than are possible using intravenous therapy. This technique has shown excellent results as an adjunct therapy for orthopedic infection.[43] For foals, 12 to 20 ml total of perfusate containing 250 mg amikacin is useful for most single joint sites. Amikacin has given consistently good results without complication and is a good choice based on its concentration-dependent activity. One may use a higher volume for the stifle, but the thigh musculature makes an effective tourniquet difficult to achieve. Because of concerns that perfusion might dislodge bacteria and renew systemic sepsis, high concentrations of systemic antimicrobials are recommended at the time of the perfusion.

If joint lavage and intraarticular administration of antimicrobials are not sufficient to resolve infection, one may perform arthrotomy to assist the joint to drain. Passive and active drains add foreign material and so are not useful. Maintaining the joint under a sterile bandage is critical and can be difficult to do in proximal joints such as the stifle and elbow. Tie-over bandages can be useful in this application.

The best measure of success is the resolution of lameness and local inflammation. Radiographs may be helpful, but the most common sign of success is a failure of the infection to progress, rather than radiographic healing. One should continue intravenously administered antimicrobials for at least 1 week after the resolution of lameness. If an appropriate drug is available, one should give foals antimicrobials orally for at least 2 weeks more. A total of at least 4 weeks of antimicrobials is recommended for most foals with orthopedic infection.

Treatment failures usually result from an inability to kill bacteria adhered to isolated tissue (usually dead bone). Sometimes this failure is caused by incomplete debridement or an inability to access a known site of infection, but more frequently it is because infection has flourished in an unknown site. For this reason, multiple imaging modalities (radiographs, ultrasound, computed tomography, and magnetic resonance imaging) used multiple times are recommended for all refractory cases of septic arthritis.

Osteomyelitis not associated with a joint still involves a growth center. The ideal treatment for these infections is surgical debridement, systemic antimicrobial therapy, and some form of local antibiotic delivery.[44,45] Even in the face of large initial osseous defects, infection may resolve, the defect may heal, and the foal may regain normal limb anatomy and function with appropriate therapy.

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20 CHAPTER 20 TOXICOLOGIC PROBLEMS

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In today's society, any list of substances that are toxic or potentially toxic is probably incomplete. Industrial society is producing new and different compounds continuously that are potentially hazardous or fatal to human beings and many species of animals. Knowledge of toxic compounds and the mechanisms whereby they produce disease also is changing constantly, so certain substances that previously were thought to be inert now are known to affect the health of animals or human beings.

It behooves the veterinary clinician to be as informed as possible concerning the potentially toxic substances found in the environment. Not all toxins are distributed uniformly in nature (this is particularly true of toxic plants), so another reasonable assumption seems to be that in any given geographic area, certain toxicities are much more common than others.

Many factors influence the toxicity of a given substance, and exposition of these factors in great detail is not within the scope of this chapter. Books have been written on specific aspects of toxicity and all the different mechanisms that come into play relating to a specific substance causing harm to a specific animal at a specific point in time. The reader is referred to other sources for information regarding general toxicologic principles and measurements and quantification. One should note that factors such as age, species of animal, reproductive status, nutritional status, management, diet, and numerous other factors relating to the animal can influence the toxicity of a given substance. Other factors related to the compound itself—such as its bioavailability, its chemical form or structure, its concentration, and so forth—also can influence the toxicity of a substance at any point in time.

Most toxins do not damage a solitary tissue, organ, or organ system preferentially but frequently affect several organs or body systems at the same time. Although for clinical signs to be related predominately to a single organ system is not unusual, multiple organ involvement is the rule rather than the exception. This fact necessitates a thorough examination and evaluation of any animal presented for diagnosis of possible toxicosis. One should evaluate all body systems adequately in the animal that is suspected of having a toxicity.

The clinical manifestations of many toxicologic problems occur some time following initial exposure to the toxin. This delay can make diagnosis and treatment difficult. For this reason, many cases of suspected toxicity are treated empirically. If a specific antidote is available or indicated, however, one should use it in the treatment regimen. General rules of thumb regarding treatment of suspected toxicoses include the following:

1. Removal of the source from the environment, if possible
2. Removal of the toxin from the body of the animal, if possible
3. Cleaning the skin or contact surface with suitable agents if the route of exposure is superficial
4. Evacuation of the gastrointestinal tract by appropriate means if contamination was via ingestion
5. Maintenance of normal body functions and physiologic processes as much as possible by means of fluid administration and blood pH and electrolyte modification
6. Maintenance of body functions not affected by the toxin
7. Aiding elimination of the toxin source from the system of the animal as expeditiously as possible

8. Not causing damage to a secondary organ or system while treating the primary toxicosis

9. Preventing reexposure or recontamination of the animal by the toxic substance

In this chapter the toxins have been divided into broad categories of general clinical signs. A toxin is discussed most completely under the system to which the major clinical signs are referable. One must remember, however, that most toxins involve more than one organ system, so a number of toxins can be found in several categories. Specific antidotes are given where appropriate, and treatment aimed at symptomatic care is given at the end of each section.

20.1 Toxicoses Causing Signs Relating to the Gastrointestinal System

There is a wide range of toxic agents capable of inducing signs of gastrointestinal disease. Signs can vary from wild of chronic to peracute and life threatening. At times, determination that a toxicosis is the cause of a problem like diarrhea or colic can be difficult, time consuming, and relatively costly.

20.1.1 PLANTS

20.1.1.1 Oak (*Quercus* Species)

20.1.1.1.1 Clinical Signs

Oak blossoms, buds, leaves, stems, and acorns can be toxic to livestock. Most reports of toxicosis in livestock involve cattle and sheep, with a rare occurrence in the horse. Clinical signs attributed to acorn toxicosis in horses are acute onset of severe abdominal pain, rectal straining, hemorrhagic diarrhea, and pronounced intestinal borborygmi. One may note acorn parts in feces. Occasionally, horses are found dead, but other signs are hemoglobinuria and elevated heart and respiratory rates.

20.1.1.1.2 Pathophysiology

The toxicity of oak is attributed to tannins or their metabolites. Digallic acid is the major active metabolite produced by oak tannins. Following bacterial fermentation, digallic acid is converted to gallic acid and pyrogallol, both of which are considered toxic.[1] Pyrogallol and gallic acid are toxic to renal tubules and result in acute tubular necrosis, anuria, electrolyte abnormalities, and uremia.[1,2] Pyrogallol is also responsible for causing hemorrhagic gastroenteritis, subcutaneous hemorrhage, and hemolysis. Tannic acid itself is thought to result in increased vascular permeability, hemorrhage, and subsequent fluid loss into body spaces.[1]

20.1.1.1.3 Diagnosis

The diagnosis of oak toxicity is based on clinical signs and a history of exposure to the plant. Under adequate forage conditions, horses would seem to have a distaste for oak leaves and acorns, so most horses exposed to the plant do not develop toxicity. Toxicosis is more likely to result when abnormal conditions coupled with environmental factors result in large numbers of acorns being produced and becoming accessible to horses.

Laboratory findings compatible with oak toxicosis include dehydration or hemoconcentration to varying degrees, azotemia, hyperphosphatemia, hypocalcemia, and hypoproteinemia. Abnormal urine findings might include occult blood, proteinuria, and casts. An increase in gallic acid equivalent content in urine also has been used to support a diagnosis of oak toxicity.[1]

Necropsy findings suggestive of oak toxicosis include pericardial, thoracic, and peritoneal effusion; gastrointestinal and mesenteric edema; and pale and swollen kidneys that may bulge on cut surface. The intestinal tract may contain large quantities of acorn parts, and colonic ulceration has been reported.[1]

20.1.1.1.4 Specific Treatment and Management

No specific antidote for oak toxicity is available. Animals should be removed from further access to oak. Treatment of the acutely affected animal aims to maintain fluid and acid-base balance and to correct any electrolyte abnormalities. Balanced polyionic fluids given intravenously to promote diuresis are the basic therapy. One should supplement this therapy with calcium, bicarbonate, and other electrolytes as necessary. Anuric animals may gain additional benefit from furosemide at 1 mg/kg intravenously or dimethyl sulfoxide (DMSO) at 1 g/kg intravenously given as a 10% solution in addition to fluid therapy. One should attempt evacuation of the intestinal tract using mineral oil or other suitable laxative.

The prognosis for affected horses is guarded. A paucity of information exists concerning mortality rates in affected horses, but death caused by ingestion of acorns has been reported.[1]

20.1.1.2 Oleander *(Nerium oleander)*

20.1.1.2.1 Clinical Signs

In the literature, sudden death is the most common sign attributed to oleander poisoning. Other reports suggest that affected horses exhibit lethargy, inappetence, and occasional signs of abdominal pain.[3,4] Profuse, watery, catarrhal, or bloody diarrhea also may occur within a few hours of ingestion.[5] Cardiac irregularities, including alternating bradycardia and tachycardia, may be accompanied by a variety of arrhythmias.[5,6] The extremities of the horse may feel cold to the touch, and mucous membranes may appear blanched. Profuse sweating and muscle twitching are followed by weakness and death. Death may occur less than 12 hours following ingestion.

The green plant apparently is unpalatable to horses. Most toxicities occur when leaves have been incorporated into lawn clippings and offered to horses. Drying does not affect the toxicity of the leaves; therefore leaves incorporated into hay also may be toxic. According to reports, 0.005% of the horses body weight of green oleander is lethal to horses.[3] 1442

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20.1.1.2.2 Pathophysiology

Common oleander contains at least five cardiac glycosides that are found in all parts of the plant.[3,5,7] These glycosides (oleandrin, digitoxigenin, neriin, folinerin, rosagenin) inhibit the Na^+,K^+-ATPase (adenosinetriphosphatase) system, resulting in hyperkalemia, conduction abnormalities, and ventricular

arrhythmias. Which glycosides or metabolites cause specific symptoms is unclear because of undefined pharmacokinetics of the individual glycosides.[7]

20.1.1.2.3 Diagnosis and Treatment

Exposure to the plant along with the aforementioned clinical signs should alert the clinician to the possibility of oleander toxicity. The rapidity of onset of clinical signs or the finding of dead animals may preclude any effective treatment. One should initiate symptomatic therapy in those animals in which toxicosis is suspected. Evacuation of the intestinal tract by laxatives and enemas may be useful. Atropine and propranolol have been advocated, but one must use them wth extreme caution.[5,6] One should not use fluids containing calcium because they may augment the effects of the glycoside on the myocardium.[3]

20.1.1.3 Castor Bean *(Ricinus communis)*

20.1.1.3.1 Clinical Signs

Castor beans contain ricin, a protein phytotoxin that acts as a potent proteolytic enzyme with significant antigenic qualities.[6] A latent period ranging from hours to several days usually occurs before the onset of clinical signs in affected horses. The bean is apparently distasteful to horses, and intoxication most likely occurs when the bean inadvertently is mixed into the feed source.

The most commonly reported clinical signs of castor bean toxicosis described in the literature are varying degrees of abdominal pain, diarrhea, depression, incoordination, profuse sweating, and increased body temperature. One occasionally observes muscle twitching, convulsions, and prominent cardiac contractions. If the horse absorbs enough ricin, signs of shock and anaphylaxis predominate.[5,6] Death may ensue as soon as 24 to 36 hours following ingestion.

Ricin is reported to be toxic to horses. One reference cites a dosage of 0.1 μg/kg as a lethal dose,[6] and a second source indicates 25 g of castor beans is lethal.[5] No recent report of ricin or castor bean toxicosis could be found, but a published report in 1945 describes seven deaths attributed to castor bean toxicosis from a stable of 48 horses in London in 1931.[8] The exact number of affected horses was not reported. A recent review of the human literature suggests that castor bean (ricin) toxicosis in human beings is not nearly as lethal as reported in the early twentieth-century literature.[9] Whether this holds for horses is open to speculation.

20.1.1.3.2 Pathophysiology

The oil extract of the bean contains ricinoleic acid. Within the small intestine, ricinoleate acts to reduce net absorption of fluid and electrolytes and stimulates peristalsis.[10] The fibrous residue of the seed contains the water-soluble toxalbumin ricin. Ricin is absorbed from the gastrointestinal tract and is a potent inhibitor of protein synthesis. Ricin contains two polypeptide chains. Chain B, a lectin, binds to the cell surface to facilitate toxin entry into the cell. Chain A disrupts protein synthesis by activating the 60S ribosomal subunit. The red blood cell agglutinating properties of ricin are independent of these toxic effects.[7]

20.1.1.3.3

Diagnosis

One makes the diagnosis by a combination of history of exposure to the plant, clinical signs, and the identification of seeds in feed material or feces. Radioimmunoassay for ricin content in urine is available from certain laboratories.[7]

20.1.1.3.4

Treatment

Ricin has no specific antidote. Initial therapy aims to combat shock, alleviating abdominal pain, and evacuating the bowel. Maintenance of fluid and electrolyte balance is important. Various sedatives and analgesics may be useful to control abdominal pain, if present. Oral administration of laxatives and protectants such as mineral oil and charcoal is indicated. Antihistamines also have been recommended.[6]

20.1.1.4

Pokeweed *(Phytolacca americana)*

20.1.1.4.1

Clinical Signs

No reports of pokeweed intoxication in horses could be found in the literature. However, one text reports that horses show signs of gastrointestinal irritation and abdominal discomfort as the primary clinical signs. The plant also produces a burning sensation of the oral mucous membranes and may cause a hemolytic crisis. Fatalities caused by pokeweed ingestion are said to result from respiratory failure and convulsions.[11]

20.1.1.4.2

Pathophysiology

The plant contains phytolaccine, a powerful gastrointestinal irritant, which in human beings causes symptoms ranging from a burning sensation of the alimentary tract to severe hemorrhagic gastritis. Five nonspecific mitogens that have hemagglutinating and mitotic activity have been isolated. These substances vary in concentration in the plant throughout the growing season. Noncardiac steroids and triterpenoid glycosides (saponins) are also present in significant quantities, but their role in pokeweed toxicity is not known.[7] Saponins may potentiate gastrointestinal toxicity and produce vasodilation when given parenterally.

20.1.1.4.3

Diagnosis and Treatment

No specific diagnostic test is available. One must treat horses suspected of having toxicosis symptomatically. One should attempt to evacuate the gastrointestinal tract using laxatives. Adsorbents such as charcoal and protectants may be useful. If animals develop a hemolytic crisis, ancillary therapy such as whole-blood transfusions may be lifesaving. One must maintain fluid and electrolyte balance in such animals to attempt to prevent or minimize hemoglobin nephrosis.[11] 1443

20.1.1.5 ## Nightshade (*Solanum* Species)

20.1.1.5.1 ### Clinical Signs

A number of species of *Solanum* have been incriminated in causing toxicity in horses. However, these plants are rarely a source of natural intoxication to horses. Reported clinical signs are referable to the gastrointestinal and central nervous systems. The primary gastrointestinal signs noted are salivation, abdominal pain, increased borborygmi, and diarrhea. Signs of central nervous system (CNS) dysfunction include mydriasis, dullness, depression, weakness, and progressive paralysis, which can lead to prostration and death.[16,17]

20.1.1.5.2 ### Pathophysiology

Solanine is the toxic substance found in *Solanum* species, is a water-soluble glycoalkaloid capable of producing local irritation,[5,7,11] and is absorbed poorly from the gastrointestinal tract. Intravenous doses caused ventricular fibrillation in rabbits, and intraperitoneal doses caused mild to moderate inhibition of specific and nonspecific cholinesterases.[7]

20.1.1.5.3 ### Diagnosis and Treatment

No specific diagnostic test is available. One should treat animals suspected of having toxicosis symptomatically. Evacuation of the gastrointestinal tract using laxatives and protectants is indicated. Charcoal also has been recommended for treatment of toxicosis in human beings.[7] One should monitor fluid, electrolyte, and acid-base status of affected animals and make corrections as needed.

20.1.1.6 ## Jimson Weed (*Datura* Species)

20.1.1.6.1 ### Clinical Signs

Several species of *Datura* grow throughout North America, all of which can produce signs of toxicosis in livestock. However, these plants are rarely a source of natural intoxication to horses, probably because of the unpalatability of the plant.[12,13] One report of equine acute toxicosis resulted when ingested feed was contaminated heavily with jimson weed seeds. In this report, one horse was affected acutely and died because of gastric rupture and gas-filled intestinal loops. A second horse was treated for several days before being euthanized. Clinical signs noted in the treated horse included abdominal distention with gas-filled intestinal loops, prolonged ileus, mydriasis, tachycardia, hyperpnea, and dry mucous membranes.[14]

20.1.1.6.2 ### Pathophysiology

The toxic substances found in *Datura* species are the tropane alkaloids atropine (a racemic mixture of d- and l-hyoscyamine) and scopolamine (l-hyoscine).[12–14] These substances exert an antimuscarinic effect by competitive inhibition with acetylcholine for receptor sites, resulting in attenuation of the physiologic

response of neuroeffector junctions to parasympathetic nerve impulses. Blockade of the muscarinic receptors of different tissues accounts for the various clinical signs observed.

20.1.1.6.3 Diagnosis and Treatment

One may suspect toxicity when animals exhibit signs compatible with atropine overdose. Identification of seeds in ingesta, gastric lavage contents, or feedstuffs may support diagnosis. Treatment is largely symptomatic, including immediate removal of the offending feed or plants, evacuation of the gastrointestinal tract, and supportive care. The use of pilocarpine and physostigmine to counteract the atropine-like effects of these alkaloids is controversial.[12,13]

20.1.1.7 Blue-Green Algae

20.1.1.7.1 Clinical Signs

The occurrence of algal poisoning in livestock is sporadic, and reports of intoxication in horses are rare. Most cases of toxicity involve other grazing animals, principally cattle and sheep, but horses are reported to be affected.[15] Toxicity may occur during times that favor algal growth in surface water. The two most important factors that favor algal growth are a nutrient source (such as nitrogen or phosphate) and warm temperature. Therefore toxicosis is most likely to occur during periods of warm weather (late spring through fall) when surface water may be contaminated with fertilizer runoff or organic waste high in nitrogen, such as that from feedlots.[15,16]

At least eight genera of blue-green algae are known to be toxic; these are *Anabaena, Aphanizomenon, Microcystis,* Coelosphaerium, Gloeotrichia, Lyngbya, Nodularia, and *Nostoc,* but the first three are of most concern in veterinary medicine. Common signs of algal poisoning are rapid appearance of abdominal pain, diarrhea, muscle tremors, dyspnea and cyanosis, prostration, and death. These signs frequently develop within a few minutes (<1 hour) of ingestion. Some cases may show rapid onset of CNS dysfunction with seizures, prostration, and death. Animals surviving several days may exhibit hemorrhagic diarrhea, muscle tremors, signs of liver damage, and photosensitization.[15,16]

20.1.1.7.2 Pathophysiology

Anabaena flos-aquae contains a low-molecular-weight alkaloid named anatoxin A, or very-fast-death factor. This toxin produces a potent postsynaptic depolarizing neuromuscular blocking agent that has a curare-like action. Death results from respiratory arrest. Blooms of *Aphanizomenon flos-aquae* produce a neurotoxin (saxitoxin) and at least three related toxic compounds of unknown structure.[7,15] Saxitoxin blocks sodium conductance through excitable membranes, which subsequently stops nerve action potential. Death resulting from saxitoxin is from respiratory neuromuscular paralysis.[15]

Microcystis aeruginosa produces at least two toxic substances. Microcystin (fast-death factor) is a lethal, low-molecular-weight cyclic peptide endotoxin that is released by *M. aeruginosa* on cellular decomposition.[7,15] This algal species also contains a cyclic peptide hepatotoxin that is potent, rapid acting, 1444
and causes hepatocellular necrosis and collapse of the hepatic parenchyma. The immediate cause of death 1445
is hemorrhagic shock resulting from the liver lesions.[17]

20.1.1.7.3
Diagnosis and Treatment

No practical means of isolating and identifying toxins from affected animals or suspect water is available. Because the concentrations of algae and toxins can vary tremendously over a short time, recovery of the toxin from water may not be possible when clinical signs are noted. Likewise, no specific assay for toxin has been developed for use on animal tissues. At best one can make a presumptive diagnosis when conditions favoring algal overgrowth are present, coupled with exposure of animals to the algal source and the rapid onset of clinical signs. One can analyze water samples for identification of specific blue-green algae, and their presence may support a diagnosis of algal toxicity.[15]

No specific antidote is available, and animals often are found dead before treatment can be initiated.[15] Algal growth in surface water can be controlled by a variety of herbicides and copper sulfate. When water is treated with these compounds, one must take care to ascertain the compounds are being used in a safe manner for all susceptible livestock.

20.1.2
MEDICATIONS

20.1.2.1
Lincomycin and Clindamycin

20.1.2.1.1
Clinical Signs

Signs of illness in horses generally are not apparent until 2 to 4 days following exposure. Subsequently, affected horses show signs of colitis and endotoxemia, with development of moderate to severe abdominal pain, tachycardia, congested mucous membranes, loose to watery feces that may contain blood, and elevated rectal temperature. Leukopenia may be present initially, and dehydration can develop rapidly. Some horses also develop laminitis during the episode or following acute illness.[15,18]

20.1.2.1.2
Pathophysiology

Lincomycin is elaborated by an actinomycete, *Streptomyces lincolnensis,* and largely has been replaced in human medicine by its derivative, clindamycin.[19] Although no reports of equine toxicosis involving clindamycin were found, one should consider the antibiotic potentially toxic to horses because of its similarity to its parent compound, lincomycin. The oral minimal toxic dose of lincomycin in horses has not been established, but in one report, a dosage of less than 0.5 mg/kg body mass daily for 2 days caused clinical illness.[18]

Several mechanisms have been suggested to cause the necrotic and pseudomembranous lesions in the colon of affected horses. These mechanisms include bacterial overgrowth of certain pathogens, particularly *Salmonella,* Clostridium perfringens, and *Clostridium difficile;* direct toxic action of the antibiotic; and a hypersensitivity reaction to the drug.[15,18,20] Overgrowth of *C. difficile* now is thought to be the primary reason for the toxic signs in human beings.[19] Because these drugs have the potential to disrupt the normal cecal and colonic microflora, one suggestion is that their toxicity may involve colonization and invasion of the colon by pathogenic bacteria and release of bacterial toxins.[20]

20.1.2.1.3 Diagnosis and Treatment

A history of exposure to lincomycin, accompanied by a delayed onset of acute diarrhea and signs of endotoxic shock, suggest possible toxicosis.[15] Signs of colitis and endotoxemia from other causes are similar to those of lincomycin and clindamycin. Feed can be analyzed for lincomycin content.

Specific antidotes are not available. One should remove the suspect feed immediately and should treat affected horses symptomatically. Evacuation of the bowel using laxatives and orally administered gastrointestinal protectants may be useful to prevent further colonization and invasion of pathogenic bacteria. One should maintain fluid volume and normal electrolyte and acid-base concentrations by appropriate means. Other specific therapies for acute colitis, endotoxemia, and laminitis may be found elsewhere in this book.

20.1.2.2 Other Antibiotics

The oral administration of certain other antibiotics also is reported to induce colitis in horses. The use of trimethoprim-sulfonamides and erythromycin base has been associated with diarrhea in a small number of horses.[20] The mechanism whereby they induce colitis also may be related to their ability to alter normal intestinal microflora and allow overgrowth of pathogenic bacteria.

20.1.2.3 Imidocarb

Imidocarb diproprionate has been used to treat *Babesia* infections in horses. The recommended dose for *Babesia caballi* is 2 mg/kg intramuscularly for each of 2 consecutive days. *B. equi* is more difficult to eliminate from the horse and a dosage of 4 mg/kg intramuscularly at 72-hour intervals for a total of five injections is recommended. However, one cannot rely even on this high dosage to eradicate *B. equi*. Higher doses than this are usually toxic to the animal.[21,22]

20.1.2.3.1 Clinical Signs

The clinical signs of toxicity are dose-dependent. All horses given an intramuscular dose of 4 mg/kg exhibited signs of parasympathetic stimulation characterized by lacrimation, excessive sweating, and serous nasal discharge for 30 minutes after treatment.[21] Larger doses, including 16 mg/kg and 32 mg/kg, resulted in horses showing pronounced systemic signs of violent peristalsis and projectile diarrhea, colic, dyspnea, miosis, and depression leading to recumbency for 1 to 4 hours following treatment. Subsequently these horses became anorectic 1 to 2 days following injection. Local swelling at the injection site is almost 1445
always present. Fatal intoxications induced profound depression before coma and death. The median lethal 1446
dose (LD_{50}) was determined to be approximately 16 mg/kg, with mortalities occurring within 6 days of the first injection.[21]

20.1.2.3.2 Pathophysiology

Imidocarb has been proposed to exert its parasympathomimetic activity by inhibiting cholinesterase. Imidocarb is absorbed rapidly from muscle, is disseminated widely in tissues, but selectively concentrates

in the CNS, kidney, and liver. Imidocarb causes diffuse, acute necrosis of the proximal convoluted tubules in the kidney and hepatocellular necrosis and biliary stasis in the liver. The degree of hepatic and renal damage is dose-dependent.[21] The toxic mechanism at the cellular level has not been elucidated.

20.1.2.3.3 Diagnosis and Treatment

Diagnosis of toxicity must be consistent with exposure to the drug. Certain clinicopathologic abnormalities are common in affected horses. Laboratory evidence of hepatic and renal damage is evidenced by increased serum concentrations of aspartate aminotransferase (AST), sorbitol dehydrogenase, and urea nitrogen. Elevated creatine kinase (CK) concentration in serum probably reflects the myositis that develops at the injection site.[21]

No specific antidote for imidocarb toxicity is available. Therapy is largely symptomatic and must aim at supportive therapy for the liver and kidney. Because the toxicity is dose-dependent, no therapy is effective for horses receiving a lethal dose of greater than 16 mg/kg body mass.

20.1.2.4 Atropine

Atropine is a commonly used medication in equine practice in diagnosis and treatment of a variety of disorders. Toxicity from atropine administration most likely results from its physiologic effects on the different body organs and systems rather than from any toxic effect of atropine itself. Complications of excessive atropine administration principally involve abnormal function of the gastrointestinal system.

20.1.2.4.1 Clinical Signs

Clinical signs of atropine overdose in the horse essentially are those of colic.[23] Reduced gastrointestinal motility and ileus may lead to signs of severe abdominal pain characterized by sweating, pawing or kicking at the abdomen, attempts to lie down and roll, and so forth. The severity of signs depends on the amount and degree of gaseous distention of the bowel, the tolerance of the horse to painful stimuli, and any complication associated with severely distended bowel impinging on or affecting another organ or tissue. Reduced motility may be present for several hours. If secondary problems are avoided, the horse usually returns to normal within 12 to 24 hours.

20.1.2.4.2 Pathophysiology

Atropine interacts with muscarinic receptors of effector cells and by competitive antagonism prevents acetylcholine from affixing to the receptor area. The net effect is that physiologic responses to parasympathetic nerve stimulation are attenuated or abolished. Although atropine acts immediately distal to all postganglionic cholinergic nerve endings, the net pharmacologic effect in a particular organ is influenced by the relative dominance of sympathetic or parasympathetic tone in that organ. Salivary and sweat glands are susceptible to small doses of atropine, and increasing dosages are required for a vagolytic effect on the heart and for relaxation of gastrointestinal and urinary tract smooth muscle.

20.1.2.4.3

Diagnosis and Treatment

Most cases of atropine toxicity occur when the drug is used therapeutically. Therefore the primary treatment should be withdrawal or reduced administration of the drug and treatment of any secondary complications of sympathetic nervous stimulation, if necessary. Unless horses become violently painful because of gastrointestinal gas distention, withdrawal of the drug until symptoms subside is usually all that is necessary. Because atropine blockade of muscarinic sites of cardiac muscle, smooth muscle, and glands is caused by competitive antagonism, large doses of acetylcholine or other cholinomimetic drugs may overcome the inhibitory effects of atropine at these sites.

20.1.2.5

Dioctyl Sodium Sulfosuccinate

Dioctyl sodium sulfosuccinate (DSS) is an anionic surface active agent commonly used to treat constipation and intestinal impaction in horses. The recommended dose of DSS is 17 to 66 mg/kg with a maximum dose of 200 mg/kg.[24,25]

20.1.2.5.1

Clinical Signs

Signs of overdose commence within 60 to 120 minutes in affected horses. Initial signs are restlessness and increased intestinal sounds accompanied by steadily increasing heart and respiratory rates. Abdominal pain, watery diarrhea, and dehydration become apparent soon afterward, and horses gradually deteriorate to lateral recumbency and death within 14 to 72 hours.

20.1.2.5.2

Pathophysiology

Much information about the pharmacologic action of DSS remains uncertain.[10] The primary organ of involvement is the small intestine, where epithelial denudation, villous atrophy, and submucosal edema and congestion occur. Dioctyl sodium sulfosuccinate has been suggested possibly to cause epithelial detachment by lowering the surface tension on the basement membranes of intestinal epithelial cells.[25] Once detachment occurs, loss of fluid and electrolytes into the intestinal lumen is extreme. The absorptive capacity of the epithelium is lost, and the osmotic effect of intestinal content causes further loss of fluid into the lumen. With extensive mucosal damage, the horse also becomes much more susceptible to endotoxemia. The rapid death in affected animals is caused by hypovolemic shock, endotoxemia, and circulatory collapse resulting from the loss of fluids and electrolytes into the intestinal lumen. 1446

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20.1.2.5.3

Diagnosis and Treatment

Diagnosis of DSS toxicity depends on observation of the aforementioned clinical signs along with oral DSS administration. No specific antidote for DSS exists, and once clinical signs commence, treatment aims at circulatory support and combating the effects of systemic endotoxin. Specific treatment for circulatory collapse and endotoxemia is covered elsewhere, but medications that might be useful include polyionic electrolyte solutions, electrolyte supplementation, corticosteroids, nonsteroidal antiinflammatory drugs (NSAIDs), bicarbonate, and orally administered gastrointestinal protectants.

20.1.2.6 Nonsteroidal Antiinflammatory Agents

The NSAIDs used extensively in equine practice and until recently were thought to be relatively nontoxic. However, in recent years, excessive intake of these agents has been shown to produce signs of toxicity and death. Phenylbutazone has been studied most widely, but flunixin meglumine (Banamine, Schering Corp., Kenilworth, New Jersey) also has been demonstrated to have toxic effects.

The toxic dose of phenylbutazone appears to be 8 to 10 mg/kg body mass, which must be administered for several days to cause signs of toxicosis.[26–29] Dosages of 15 mg/kg or greater, when given on multiple days, can be lethal to horses, with death occurring as early as day 4 of treatment.[27] Ponies may be at greater risk of developing toxicosis, for 10 mg/kg given once daily to ponies resulted in death of several of the animals by day 7 of administration.[28] Foals have been suggested to be more susceptible than adult horses to developing toxicosis when phenylbutazone is given in therapeutic dosages.[30]

Flunixin meglumine appears to be less toxic than phenylbutazone, but foals given 1.1 mg/kg for 30 days developed signs of toxicosis.[31] In another study, administration of flunixin meglumine at a dosage of 6.6 mg/kg/day intravenously for 5 days was necessary to produce signs of toxicosis in a group of foals.[32]

Excessive intake of NSAIDs has been associated with three syndromes: (1) gastrointestinal ulceration,[26–32] (2) renal medullary crest necrosis,[27,28,33] and (3) ulceration of only the right dorsal colon.[30,34,35] Phenylbutazone toxicosis has caused all three syndromes, but flunixin meglumine has been associated only with gastrointestinal lesions. Ulceration and erosion of the digestive tract almost always affect the glandular stomach, large colon, and cecum. The small intestine can be affected, but the predominant lesions tend to involve the large colon.[26–32] Extensive edema of the colonic mucosa often occurs and may precede the development of erosions and ulcers in the large bowel. In isolated cases, only the right dorsal colon has been affected. Renal medullary crest necrosis occurs along with renal tubular epithelial cell necrosis. Renal dysfunction occurs in horses with toxicosis and may progress to acute or chronic renal failure. In clinical situations, renal dysfunction also may be a manifestation of more prolonged use of NSAIDs or the concurrent use of these products in horses with already compromised renal function.

One should use NSAIDs with caution in horses that are dehydrated or hypovolemic or that have preexisting renal disease because these animals are at greater risk of developing toxicosis. Additional factors that may increase the risk of toxicosis include concomitant use of other NSAIDs or other medications with nephrotoxic potential and administration of phenylbutazone in amounts greater than 8.8 mg/kg/day for periods longer than 4 days.

20.1.2.6.1 Clinical Signs

Signs of NSAID toxicosis include anorexia, depression, colic, diarrhea, melena, weight loss, ventral and peripheral edema, petechial hemorrhages and cyanosis of mucous membranes, and ulcerations of the oral cavity and digestive tract.[26–32,34,35] Oral ulcerations can be extensive and can involve the tongue, hard palate, and mucocutaneous junction at the commissure of the lips. Other signs of endotoxemia—that is, fever, impaired cardiovascular function, hemoconcentration, and neutropenia—may be present in some horses. Renal medullary crest necrosis rarely is considered a primary cause of any clinical sign, but signs

of renal dysfunction can accompany some horses with NSAID toxicity. Signs of toxicity can occur as early as the second to fourth day of treatment when excessive quantities are given.[27]

20.1.2.6.2 Pathophysiology

The mechanisms whereby NSAIDs cause gastrointestinal damage and ulceration still are being elucidated. These agents may exert a local irritative effect on the oral and gastrointestinal tract mucosa.[26,30] However, the major mechanism whereby damage occurs may be by inhibition of prostaglandin synthesis in the bowel, which is necessary for normal mucosal integrity and function.

Prostaglandins are formed in various body tissues by the action of cyclooxygenase on arachidonic acid. The intermediate endoperoxides formed then are converted by specific enzymes to metabolically active prostaglandins. Within the gastrointestinal tract, prostaglandins E_1 and E_2 (PGE_1, PGE_2) are of primary importance and exert a number of beneficial effects; they enhance mucosal microcirculatory blood flow, promote reepithelialization following mucosal injury, stimulate bicarbonate and mucous secretion at the mucosal surface, and decrease hydrochloric acid and pepsin secretions in the stomach. These prostaglandins also appear to have other cytoprotective properties that are effected by unknown mechanisms.[29,32]

The NSAIDs inhibit prostaglandin synthesis by inhibition of cyclooxygenase. Within the gastrointestinal tract mucosa, depletion of endogenous PGE_1 and PGE_2 is suggested to result in vasoconstriction of the 1447

1448 microvasculature, leading to mucosal ischemia and subsequent ulcer formation.[29,30] Support for this theory stems from the ability of synthetic PGE_2 to prevent mucosal injury induced by phenylbutazone administration.[29]

Arachidonic acid metabolism may have other effects on gastrointestinal tract function. Several lipoxygenase enzymes act on arachidonic acid to form leukotrienes, which have a variety of effects on the gastrointestinal tract. These substances influence inflammatory cell chemotaxis, fluid and electrolyte secretion, vasoconstriction, and vasodilation. Therefore the use of NSAIDs may shunt arachidonic acid metabolism toward production of these endogenous eicosanoids and may modify the synthesis of prostaglandins within the gastrointestinal tract and other organ systems.

A recent study proposes that phenylbutazone-induced gastrointestinal ulceration results from direct toxic injury to the mucosal microvasculature.[36] This study suggests that the initial toxic injury induced by phenylbutazone in the intestinal tract is damage to the endothelial cells of the microvasculature. As a result, vascular swelling, stagnation and occlusion of blood flow, fibrin formation, and perivascular leakage occur, with subsequent formation of edema, thrombosis, and necrosis of the mucosa. Eventually the mucosal epithelium is sloughed. This study concluded that vasoconstriction was not the primary cause of the mucosal necrosis, and that once formed, erosions and ulcers may be perpetuated by processes not mediated by prostaglandins, such as bacterial invasion.

The mechanisms involved in production of right dorsal colon ulceration are probably similar to those involved in ulcerogenesis in other regions of the digestive tract. Why the lesion is expressed only in the right dorsal colon in some horses is unknown.

Gastrointestinal edema and ulceration are responsible for most of the clinical signs noted with NSAID toxicity. Protein loss, diarrhea, melena, abdominal pain, and endotoxemia are related directly to abnormal gastrointestinal function induced by toxic levels of NSAIDs.

Renal medullary crest necrosis is thought to be caused by inhibition of prostaglandin synthesis within the kidney by NSAIDs. Prostaglandin E_2, $PGF_{2\alpha}$, prostacyclin, and thromboxane are produced in the kidney, with greater amounts being generated in the medulla than in the cortex. The renal prostaglandins are thought to function as local vasodilators to maintain renal blood flow and glomerular filtration rate under adverse conditions but have little or no control over basal renal blood flow and glomerular filtration rate in healthy animals. As a consequence of prostaglandin inhibition, renal medullary ischemia can occur, leading to medullary crest necrosis.[37] Dehydration and hypovolemia may play a significant role in the development of this lesion, along with concurrent phenylbutazone administration.[33]

20.1.2.6.3

Diagnosis and Treatment

One should suspect NSAID toxicity when compatible clinical signs are evident and the animal has a history of inappropriate drug administration. In addition, certain clinicopathologic abnormalities are fairly characteristic of NSAID toxicosis and aid in diagnosis. Hypoproteinemia and hypoalbuminemia are hallmarks of NSAID toxicity, and serum protein concentration can become dangerously low.[26–30,32,34,35] Other abnormalities that are frequently present include increased concentrations of serum urea nitrogen, creatinine, and phosphorus. Serum bilirubin and AST concentrations occasionally are elevated. Serum calcium concentration invariably is decreased and is another consistent abnormal finding. Decreased serum chloride concentration occurs occasionally, and many horses are neutropenic.[26–30]

Treatment of NSAID toxicity is largely symptomatic and directed toward the specific disorders that are present. In all cases, one should evaluate the use of NSAIDs critically and withdraw them if possible. One should administer plasma to severely hypoproteinemic horses, and broad-spectrum antibiotic therapy may be indicated if signs of sepsis or septicemia are present. One should choose antimicrobials with minimal to no nephrotoxic potential, if necessary. One should correct fluid, electrolyte, and acid-base abnormalities but should use caution when giving fluids to an already hypoproteinemic horse. Treatment of gastric ulceration, endotoxemia, and renal failure are discussed elsewhere.

20.1.3

MISCELLANEOUS AGENTS

20.1.3.1

Arsenic

Arsenicals are found in a number of products, including insecticides, herbicides, defoliants, rodenticides, livestock dips, medications, wood preservatives, paint pigments, detergents, and certain insulation materials.[15,38] Horses are most likely to be exposed to arsenic by eating contaminated forage.[38]

20.1.3.1.1

Clinical Signs

The clinical signs associated with arsenic toxicity are essentially those of a severe gastrointestinal irritant. Most toxicities result from inorganic forms of arsenic, and signs are similar in several animal species.

In peracute cases the animals may be found dead with no premonitory signs. Acute signs of toxicity include severe colic, staggering, weakness, salivation, diarrhea that may contain blood or shreds of mucosa, and signs of shock that indicate cardiovascular collapse. Death usually occurs in 1 to 3 days.[15,38,39] In subacute poisoning, animals may live for several days exhibiting signs of depression, anorexia, colic, diarrhea that may contain blood and mucus, polyuria followed by anuria, and subsequent shock before death. Horses that are poisoned by topical application of arsenic can show signs of blistering and edema of the skin.[15] Chronic arsenic poisoning rarely occurs in domestic animals. 1448

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20.1.3.1.2 Pathophysiology

Many factors play a role in the development of arsenic toxicosis in horses. In general, horses that are debilitated, weak, or dehydrated are more susceptible to toxicosis than normal animals. The formulation of the compound (trivalent arsenicals are more toxic than pentavalent forms), the solubility of the compound, the route of exposure, the rate of absorption from the gastrointestinal tract, and the rate of metabolism and excretion by the individual animal are factors that can influence the toxicity of the various arsenic formulations.[15] The most hazardous preparations are products in which the arsenical is in a highly soluble trivalent form, usually trioxide or arsenite. Sodium arsenite is 3 to 10 times more toxic than arsenic trioxide. The average total oral lethal doses of these compounds for the horse are 10 to 45 g arsenic trioxide and 1 to 3 g sodium arsenite.[15,38]

Soluble forms of arsenic are absorbed from all body surfaces. Less soluble arsenicals such as arsenic trioxide are absorbed poorly from the gastrointestinal tract and essentially are excreted unchanged in the feces. Following absorption, trivalent arsenic is excreted readily via the bile into the intestine, and pentavalent arsenic is excreted via the kidney. Regardless of whether an introduced arsenical is in the trivalent or pentavalent form, all the major actions can be attributed to the trivalent form.[15]

All arsenicals are thought to exert their effects by reacting with sulfhydryl groups in cells. Trivalent arsenic acts primarily by combining with the two sulfhydryl groups of lipoic acid, thereby inactivating this essential cofactor necessary for the enzymatic decarboxylation of the keto acids pyruvate, ketoglutarate, and ketobutyrate. By inactivating lipoic acid, arsenic inhibits the formation of acetyl, succinyl, and propionyl coenzymes A. The net effect is the blocking of fat and carbohydrate metabolism and cellular respiration.[15,38] Trivalent arsenic also may inactivate sulfhydryl groups of oxidative enzymes and the sulfhydryl group of glutathione and other essential monothiols and dithiols. Arsenic also causes a local corrosive action on the intestine.[38]

Arsenic seems to prefer tissues rich in oxidative enzymes such as the liver, kidney, and intestine. The capillary endothelial cells in these organs appear sensitive to arsenic because it relaxes capillaries and increases capillary permeability. Blood vessels with smooth muscle in their walls also dilate. In the intestinal tract, the mucosa easily sloughs away because of the accumulation of fluid in the submucosa. In the kidney, renal tubular degeneration occurs.[38]

20.1.3.1.3 Diagnosis

The clinical signs described previously should cause one to consider inorganic arsenic poisoning. Antemortem laboratory findings are consistent with gastrointestinal, hepatic, and renal damage. Feces may

contain blood, mucus, and increased numbers of white blood cells. The liver enzymes sorbitol dehydrogenase, lactate dehydrogenase (LDH), AST, and γ-glutamyltransferase (GGT) may be increased in serum, and urine might contain protein, red blood cells, and casts. Urine arsenic concentration in affected animals often exceeds 2 ppm.[15]

Postmortem findings are characteristic of a severe gastroenteritis and may include hepatic lipidosis and necrosis. In suspect horses, one should evaluate the liver, kidney, stomach and intestinal contents, and urine for arsenic content. One also can test blood and milk. Levels greater than 10 ppm confirm arsenic toxicosis.[15]

20.1.3.1.4 Treatment

Specific therapy for arsenic toxicity is dimercaprol (BAL). This chelating agent forms a nontoxic and easily excretable complex with arsenic. However, dimercaprol may mobilize stored arsenic in tissues and cause an initial exacerbation of clinical signs by allowing more arsenic to circulate to the intestine and liver. Dimercaprol also can be toxic if overdosed. Signs of overdosage include tremors, convulsions, coma, and death. In horses the recommended dose is 3 mg/kg intramuscularly as a 5% solution in a 10% solution of benzyl benzoate in peanut oil. One should give this dose every 4 hours for the first 2 days, every 6 hours on the third day, and twice daily for the next 10 days until recovery.[38]

Sodium thiosulfate also has been advocated for treatment of arsenic toxicosis, but its efficacy is questionable. The recommended dose for horses is 20 to 30 g orally in 300 ml of water, plus 8 to 10 g intravenously in a 10% to 20% solution.[38]

Symptomatic care of affected animals includes evacuation of the gastrointestinal tract with laxatives and oral demulcents to coat the intestinal tract. One should evaluate fluid, acid-base, and electrolyte indexes and provide support if necessary. Because endotoxemia may develop as a result of the intestinal and liver lesions, prophylactic use of flunixin meglumine at a dosage of 0.25 mg/kg t.i.d. may be beneficial. Other therapy to prevent shock and cardiovascular collapse also may be indicated.

20.1.3.2 Aluminum

One report describes an unexpectedly high incidence of horses on a single premise showing clinical signs compatible with granulomatous enteritis and the presence of abnormally high levels of aluminum in various body organs and tissues of the affected horses.[40] Clinical signs included weight loss with or without diarrhea, hyperkeratosis, ulcerative coronitis, and neurologic deficits compatible with cervical stenotic myelopathy. Laboratory abnormalities included hypoalbuminemia and elevated serum concentration of alkaline phosphatase in some horses. All horses had histologic evidence of granulomatous inflammation of the gastrointestinal tract in varying degrees of severity and distribution. Granulomas occurred in the mucosa, 1449
submucosa, and serosa of the small and large intestine and in the abdominal lymph nodes, portal areas of the 1450
liver, and pancreas. Aluminum was found in granulomas, and elevated aluminum levels were present in kidney and liver tissue.

Chronic environmental exposure to aluminum was postulated as a cause for the condition. Environmental factors (particularly acidic pH of soil, water, plants, etc.) may have an effect on the bioavailability of aluminum, and repeated exposure was suggested as possibly inducing hypersensitivity to aluminum in these horses. In human beings, aluminum is known to induce nonimmunologic (foreign body) and immunologic

granulomas following administration of aluminum-containing vaccines and hyposensitization products.[41] The association between high environmental concentrations of aluminum and increased incidence of generalized granulomatous inflammation in horses warrants further investigation.

20.1.3.3 Petroleum Distillates

Horses are exposed to excessive amounts of crude oil or petroleum distillates primarily by contamination of rangeland with by-products of the oil industry or by iatrogenic application, for petroleum distillates are used commonly as carrier agents for many insecticides.

20.1.3.3.1 Clinical Signs

The most noted signs associated with ingestion of petroleum products are essentially those of gastrointestinal and respiratory dysfunction. Petroleum products are irritating to mucous membranes, and hence prominent signs of gastrointestinal dysfunction might include salivation and fluid feces. The feces actually may contain oil or oily substances. Chronically affected animals also may exhibit anorexia and weight loss over several days to weeks.

Signs of respiratory dysfunction are a common manifestation of petroleum toxicity. Aspiration of the oil or fumes is irritating to pulmonary tissue, and aspiration pneumonia is probably the most serious consequence of petroleum toxicity.[15] Signs of toxicity include increased respirations, anorexia, depression, weight loss, a variable degree of fever, and possibly increased nasal discharge.

Products that are applied inadvertently to the skin might cause some degree of respiratory embarrassment, but they are more likely to cause signs related to the absorption of excessive lead or toxic hydrocarbons.[42] Topically applied agents also may cause signs associated with a contact irritant.

20.1.3.3.2 Pathophysiology

The toxicity of crude oil is correlated with the amounts of gasoline, naphtha, and kerosene content in the oil. Crude oil rich in these low-temperature distillates is more toxic than petroleum containing a lot of sulfur but less of the low-temperature distillates.[42] Petroleum products are irritating to mucous membranes, and their oily nature makes them difficult to remove from skin and mucous membranes and virtually impossible to remove from the respiratory epithelium. Once aspirated, they serve as a focus for foreign body pneumonia that may progress to abscessing pneumonia, pleuritis and pleural effusion, and death.

20.1.3.3.3 Diagnosis

History of possible exposure, clinical signs, and pathophysiologic signs are important in establishing a diagnosis. Suspect contents, that is, gastrointestinal content, may be mixed with water, and if oil is present, it will float to the surface and be readily visible. One sometimes can use infrared spectrophotometry to establish the identity of an oil.[15]

20.1.3.3.4

Treatment

Treatment is supportive. One should evacuate and protect the gastrointestinal tract using laxatives and demulcents. One can remove products applied to the skin using soap and water. Aspiration pneumonia may be frustrating to treat, and supportive care such as fluids and electrolytes, NSAIDs, and broad-spectrum antimicrobial therapy is potentially helpful.

20.1.3.4

Slaframine

Slaframine is an indole alkaloid produced by *Rhizoctonia leguminicola,* a mold that infects red clover and other legumes.[15,43,44] *R. leguminicola* is a ubiquitous soil fungus that infects certain legumes during conditions of high rainfall or humidity.[44] The toxin can survive and persist in dried and baled hay.[15]

20.1.3.4.1

Clinical Signs

The most consistently reported clinical sign is excessive salivation characterized by profuse, viscous, clear saliva.[44] Salivation may begin within 30 to 60 minutes after eating the affected plants, and response from one feeding may persist for up to 24 hours. Other clinical signs are anorexia, polyuria, and sometimes watery diarrhea.[15] One case of abortion in an affected mare has been reported.[44] Clinical signs generally abate within 48 to 96 hours once infected hay is removed from the diet.[15]

20.1.3.4.2

Pathophysiology

Slaframine apparently is activated by liver microsomes following absorption. The active compound seems to have direct histaminergic effects or possibly a histamine-releasing effect, which is borne out in laboratory animal studies in which clinical signs responded better to antihistamines than to atropine.[43]

20.1.3.4.3

Diagnosis

The combination of acute clinical signs of excessive salivation coupled with digestive disturbances and identification of *R. leguminicola* in forage is generally adequate to establish a diagnosis. One can identify slaframine by chemical means, but usually such procedures are unnecessary.[15]

20.1.3.4.4

Treatment

No specific treatment is usually necessary. Animals generally recover uneventfully in 48 to 96 hours following withdrawal of the contaminated forage.[15] Atropine and antihistaminic therapies have been suggested to help control clinical signs,[15,43] but their efficacy is questionable. 1450

20.1.3.5 Pentachlorophenol

Documented instances of horses becoming intoxicated with pentachlorophenol are rare. However, because pentachlorophenol is used routinely as a wood preservative and because other domestic animals, including cattle and swine, are affected, some aspects of this toxin require description.

The chlorophenols (which include pentachlorophenol) are generally not water soluble but are soluble in oils and organic solvents.[42,45] Pentachlorophenol is volatile and can give off toxic vapors.[42] The chlorophenols are absorbed readily from the gastrointestinal tract, by inhalation, and from intact skin and are excreted rapidly via the kidney.[42,45]

Several factors affect the toxicity of chlorophenols. High ambient temperature, physical activity, poor body condition, oily or organic solvent vehicles, prior exposure, and hyperthyroid states serve to enhance toxicity in human beings and other animal species. Cold temperatures, antithyroid drugs, and increased amounts of body fat help to diminish the toxicity.[42]

The mechanism whereby the chlorophenols exert their toxicity involves the energy production sites of mitochondria, where they uncouple oxidative phosphorylation. The chlorophenols act at sites of adenosine triphosphate (ATP) production to decrease or block their production without blocking the electron transport chain. Free energy from the electron transport chain then is converted to body heat. As the body temperature is increased, the heat-dissipating mechanisms are overcome and metabolism is increased. The electron transport chain responds by using more oxygen in an effort to produce ATP, but much of the free energy is liberated as more body heat. Eventually the oxygen demand overcomes the oxygen supply and energy reserves become depleted.[42]

Clinical signs, if observed, include fever, tachycardia, dyspnea, sweating, lethargy, incoordination, weakness, cyanosis, collapse, and death. Less severely affected animals may primarily manifest signs of hyperthermia and oxygen deficiency.[42] Pentachlorophenol in high doses to pregnant animals also is reported to cause embryonic and fetal deaths but is not teratogenic.[15]

Diagnosis of pentachlorophenol toxicity is associated with the combination of clinical signs and blood pentachlorophenol levels greater than 40 ppm. No specific treatment is available, but saline diuresis has been suggested to be helpful in certain instances of human toxicosis.[15]

20.1.3.6 Chlorates

Chlorate salts (sodium chlorate or potassium chlorate) commonly are used as herbicides and defoliants. Horses become exposed by grazing areas that have been sprayed recently or by the mistaken substitution of sodium chlorate for sodium chloride as a feed additive.

20.1.3.6.1 Clinical Signs

Initial signs are those of gastrointestinal irritation and include colic and diarrhea. Hematuria and hemoglobinuria are also present early in the disease course. Within hours, the horse shows dyspnea, cyanosis, and increased respiratory effort. Death can occur suddenly without obvious symptoms.[15]

20.1.3.6.2

Pathophysiology

Chlorates are absorbed readily from the intestine, and once absorbed they continue to exert their damaging effects as long as they are present.[15,42] A dose of 250 g is reported to be lethal to horses.[15]

Chlorates cause toxic changes by three different mechanisms of action:

1. Direct irritation of the gastrointestinal tract
2. Oxidation of hemoglobin to methemoglobin
3. Inducement of severe hemolysis by some undeter-mined action on erythrocyte membranes

The net effect of methemoglobinemia and hemolysis is a severe compromise in the oxygen-carrying capacity of blood, and animals may be affected severely enough to die from anoxia.[42]

20.1.3.6.3

Diagnosis

The prolonged, extensive methemoglobinemia found in affected animals should alert the veterinarian to the possibility of chlorate poisoning. A history of exposure to chlorates also should accompany these clinical signs for one to make a presumptive diagnosis. One can determine chlorate concentration in blood, urine, or tissues analytically, and because chlorates normally are not found in animals, their presence in a suspect sample would confirm poisoning if clinical signs, history, lesions, and response to therapy also suggest this diagnosis.[42]

20.1.3.6.4

Treatment

Once one makes the diagnosis, one should seek out and remove the chlorate source immediately from the environment of the horse. One treats methemoglobinemia with methylene blue at a dose of 4.4 mg/kg given as a 1% solution by intravenous drip. This dose may be repeated in 15 to 30 minutes if clinical response is not obtained.[24,42] Other recommended therapeutic measures include gastric lavage with 1% sodium thiosulfate and the oral administration of intestinal protectants and demulcents.[15,24] Blood transfusion and oxygen supplementation may be beneficial in certain instances.[42]

20.1.3.7

Pyriminil (Vacor)

Pyriminil currently is not commercially available but in previous years was marketed as a rodenticide. Reports of toxicosis in horses are rare,[46] and deaths caused by pyriminil ingestion are not reported.[46,47]

20.1.3.7.1

Clinical Signs

Reported signs in affected horses include severe muscular fasciculation, profuse sweating, dehydration, and mydriasis with a weak pupillary response. Hindlimb weakness, ataxia, persistent inappetence, and abdominal pain also have been reported.[46] Hyperglycemia is a fairly consistent laboratory finding.[15,46] 1451

20.1.3.7.2

Pathophysiology

Pyriminil is absorbed from the gastrointestinal tract and excreted in the urine. Pyriminil acts as a nicotinamide antagonist, but its exact mechanism of action is unknown.[15,24,47] Pyriminil also has been shown to damage the pancreatic β-cells and to depress glucose uptake by erythrocytes.

20.1.3.7.3

Diagnosis

Presumptive diagnosis is based on compatible clinical signs and history of exposure to the rodenticide.

20.1.3.7.4

Treatment

Specific therapy for pyriminil toxicity is reported to be nicotinamide. However, the use of this drug in human beings appears to be effective only when given within 1 hour of ingestion of pyriminil. The reported dosage is 50 to 100 mg nicotinamide intramuscularly every 4 hours for up to eight injections. This dosage is followed by 25 to 50 mg orally 3 to 5 times daily for 7 to 10 days.[15] Other symptomatic therapies that may be beneficial include gastric lavage and the oral administration of mineral oil and activated charcoal.[15,47] Apparently, affected horses recover, for no deaths caused by pyriminil toxicosis are recorded.

20.1.3.8

Tetrachlorodibenzodioxin

The polychlorinated dibenzodioxins include a large number of isomers that differ chemically only in the number and location of chlorine atoms on the dioxin nucleus but that vary greatly in their toxic potential to various animal species. Of the 75 possible isomers of polychlorinated dibenzodioxin, the specific isomer designated 2,3,7,8-tetrachlorodibenzodioxin (TCDD; dioxin) is most toxic and generally is considered the most toxic synthetic molecule known. Tetrachlorodibenzodioxin is a contaminant of certain herbicides and is a by-product of certain chemical manufacturing and combustion processes.[15] The chemical is a highly stable contaminant in the environment, with a half-life in soil of about 1 year.[48]

20.1.3.8.1

Clinical Signs

In one reported outbreak the initial signs began 4 days following exposure and included abdominal pain, polydipsia, anorexia, severe weight loss, alopecia, skin and oral ulcers, conjunctivitis, dependent edema, joint stiffness, and laminitis. A total of 85 horses were exposed, 58 became ill, and 43 subsequently died. The length of illness varied from 4 to 132 weeks in the terminally ill horses, with those having a heavier exposure exhibiting a shorter disease course (average of 32 weeks) than others (average of 74 weeks). In addition, abortions occurred in pregnant mares, and many foals that were exposed only in utero died at birth or shortly thereafter.[48] Other reported signs in animals include gastrointestinal hemorrhage with necrosis and ulceration of the gastrointestinal mucosa, cerebrovascular hemorrhage, hepatotoxicity, and thymic and peripheral lymph node atrophy.[15,48]

20.1.3.8.2
Pathophysiology

Tetrachlorodibenzodioxin is absorbed readily by oral and dermal routes, and following absorption appears to be retained primarily by liver and adipose tissue. The mechanism of action of TCDD in various organs in not well defined. Tetrachlorodibenzodioxin is known to induce microsomal mixed function oxidases in liver and kidney, and hepatic δ-aminolevulinic acid synthetase and aryl hydrocarbon hydroxylase, but the role of these processes in the induction of toxicity of TCDD remains to be elucidated.[15] The mechanism whereby TCDD induces immunosuppression by causing thymic and peripheral lymph node atrophy is undetermined.

20.1.3.8.3
Diagnosis

A combination of the described clinical signs and possible exposure to industrial waste oil products should alert the veterinarian to the possibility of TCDD toxicity. One can confirm dioxin content in tissue by means of gas-liquid chromatography and mass spectroscopy, but few laboratories offer this service, and the analysis is generally expensive.[15]

A characteristic liver lesion seen at necropsy in a number of horses was microscopic evidence of bile stasis, hepatocyte necrosis, bile duct proliferation, and extensive fibrosis that was pronounced around the central veins but minimal in the peripheral liver lobules. Other microscopic changes noted were thickened vascular walls and endothelial proliferation in the smaller blood vessels of several different organs.[48]

20.1.3.8.4
Treatment

No known antidote exists for TCDD toxicity once clinical signs develop. Following their onset, one can offer only symptomatic and supportive care, and one should use every precaution to prevent laminitis. Soil and activated charcoal appear to bind strongly to TCDD and inhibit its absorption, so if known ingestion has occurred, immediate oral administration of activated charcoal may have beneficial effects by reducing the amount absorbed.[15]

20.1.3.9
Monensin

Monensin is one of several biologically active compounds categorized as ionophore antibiotics because they can form lipid-soluble complexes with specific alkali metal cations and transport them across biologic membranes. Monensin is produced by the fungus *Streptomyces cinnamonensis* and is selective in transporting sodium and potassium ions between intracellular and extracellular spaces.[15,49,50] Monensin is used routinely as a poultry coccidiostat and as a feed additive to improve feed efficiency in pasture and feedlot cattle. Horses are the most sensitive domestic animal to monensin toxicosis. The LD_{50} of monensin for the horse is 2 to 3 mg/kg.[15]

20.1.3.9.1
Clinical Signs

Several syndromes of toxicity occur and seem to be dose-related. Peracute toxicity may manifest as a progressive, severe hemoconcentration, hypovolemic shock, and death within a few hours of ingestion.

The acute form of the disorder is characterized by partial to complete anorexia, abdominal pain, occasional watery diarrhea, intermittent profuse sweating, stiffness and progressive muscle weakness most prominent in the hindquarters, progressive ataxia, tachycardia, hypotension, dyspnea, and polyuria. Affected horses may show clinical signs for 1 to 4 days before death.[50–52] Horses surviving sublethal doses of monensin exhibit signs of reduced athletic performance, unthriftiness, and cardiac failure. Cardiac arrhythmias, including atrial fibrillation and tachycardia, a prominent jugular pulse, and pleural and pericardial effusion are apparent.[15] Intravascular hemolysis also may occur to a limited degree.[49] 1452 1453

20.1.3.9.2

Pathophysiology

The primary action of monensin is selective transport of sodium and potassium ions between the intracellular and extracellular spaces. Two mechanisms have been suggested to explain the toxic action.

One theory suggests that monensin interacts with the mechanism regulating potassium entry into cell organelles, especially the mitochondria.[15] Low concentrations of monensin lead to a net accumulation of potassium within the cell, whereas higher doses cause a net loss of potassium from the cell.[49] Because potassium is required for ATP hydrolysis by the mitochondria, the effect of monensin might be to inhibit ATP hydrolysis in mitochondria. As a result, cell energy production is decreased and can result in loss of cell function and death.[15,49]

The second hypothesis suggests that increased intracellular calcium concentration is the mechanism responsible for cell death. When the intracellular calcium concentration is increased, the mitochondria are forced to maintain calcium homeostasis by sequestering the excess calcium. This requires energy, which could take priority over ATP production. When the mitochondria become overloaded with calcium, oxidative phosphorylation is inhibited and less energy is produced to pump calcium out of the cell. When intracellular calcium levels reach a critical level, degradative enzymes are released, swelling of the mitochondria and sarcoplasmic reticulum occur, and cell necrosis and death follow.[15,49]

The heart is the primary target organ of monensin toxicosis, and electron microscopic studies of acute monensin toxicosis in ponies have shown structural changes in myocardial cells consistent with severe mitochondrial damage.[53] In horses ingesting a sublethal dose of monensin, the myocardial sarcolemma is damaged and is replaced by fibrous tissue in the healing process. Myocardial lesions are characterized microscopically by pale myofibers, loss of fiber striation, multifocal vacuolar degeneration, and scattered areas of necrosis. The end result is a structurally weakened heart that can succumb to stress and cause acute collapse of the horse. Other lesions that may be present in affected horses include pericardial, pleural, and peritoneal effusions; hemopericardium; and epicardial hemorrhage. Chronically affected horses also may have hepatic congestion with centrilobular necrosis and hydropic degeneration of the renal tubules.[15,49]

20.1.3.9.3

Diagnosis

One should suspect monensin toxicosis when horses show clinical signs of anorexia, muscle weakness, and heart failure and when a possible exposure to contaminated feed has occurred. One can evaluate suspect feeds for monensin content.

Clinicopathologic abnormalities are nonpathognomonic but include early signs of severe hemoconcentration and dehydration in horses affected peracutely. Serum potassium and calcium may be decreased moderately in the first 12 to 16 hours but then tend to come back to normal levels. Blood urea nitrogen (BUN) and creatinine concentrations are elevated in horses acutely affected but return to normal in surviving animals. Other enzymes that show elevated serum activity include CK, AST, and LDH isoenzyme fractions 1 (cardiac muscle) and 2 (red blood cell origin). Total serum bilirubin also may be elevated.[15,49]

Abnormal findings in urine can include a progressive decrease in urine osmolality during the initial few hours of the disease course.[15] One also might expect elevations in urinary activity of renal tubular enzymes such as GGT and *N*-acetylglucosaminidase. Urinalysis abnormalities tend to correlate well with the degree of renal insult and are nonspecific indicators of renal damage.

20.1.3.9.4 Treatment

No specific antidote exists for monensin. One should treat horses that have ingested a large amount early and aggressively with polyionic fluids to combat hemoconcentration and hypovolemic shock. One should perform electrolyte and acid-base analysis, if possible, and correct deficiencies. One should attempt to evacuate the bowel of the horse using orally administered laxatives such as mineral oil; activated charcoal may help decrease the absorption of monensin. One should keep affected horses as quiet and nonstressed as possible for weeks following exposure to allow the damaged myocardium to heal.[15,49]

Digitalis glycosides should never be used in acutely affected horses because they and monensin have been shown to be synergistic and immediately fatal to cardiac muscle cells. One should use digitalis glycosides only with great caution in the weeks following recovery from the toxic episode. Likewise, one should not give calcium to acutely affected horses for two reasons. First, serum hypocalcemia is transitory, and serum calcium usually recovers to normal by 24 hours. Second, calcium can be dangerously irritating to an already injured myocardium.[49]

One should note that affected horses are susceptible to cardiac damage, which is often permanent. A critical evaluation of cardiac function and the integrity of any previously intoxicated horse that is destined to return to some form of athletic endeavor is judicious. 1453

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20.1.3.10 Lasalocid

Lasalocid is another carboxylic ionophore antibiotic, is a fermentation product of the mold *Streptomyces lasaliensis,* and is used commercially as a poultry coccidiostat and as a feed additive to improve feed efficiency in ruminants.

20.1.3.10.1 Clinical Signs

The signs observed in horses given toxic amounts of lasalocid are similar to those of monensin toxicosis. Affected horses exhibited depression, ataxia, paresis, and paralysis with partial anorexia. Once recumbent, some horses would rise when given assistance. Most horses that survived appeared normal 2 to 3 days following exposure. The lowest dose that caused fatality was 15 mg/kg body mass, but the LD_{50} for a

single oral dose of lasalocid was estimated to be 21.5 mg/kg. Death occurred between 31 and 96 hours following oral dosing in the nonsurvivors.[54]

Results of a toxic feeding study indicated that poultry rations containing approved concentrations of lasalocid (75 to 125 g per metric ton) are not toxic or lethal to horses. This study revealed that horses voluntarily reduced their feed intake with increasing amounts of lasalocid in the ration and refused to eat the commercial premix when offered it in place of their normal ration.[54]

20.1.3.10.2 Pathophysiology

The mechanism of action of lasalocid is thought to be similar to that of monensin. Lasalocid is the least toxic of the ionophores and differs from monensin in that it accepts divalent and monovalent cations.[22]

20.1.3.10.3 Diagnosis

No signs or laboratory findings are pathognomonic for lasalocid toxicity. In suspect horses, one can analyze feedstuffs for lasalocid content.

Abnormal laboratory findings in affected horses include hypocalcemia, hypophosphatemia, and hypokalemia early in the disease course (within 24 hours of exposure), but these values returned to normal ranges by 120 hours after ingestion. Serum activity of AST frequently is increased, as is total serum bilirubin and glucose concentrations. Occasionally the BUN concentration is increased.[54]

20.1.3.10.4 Treatment

Initial treatment should include removal of all suspected feed sources and the oral administration of laxatives to enhance evacuation of the gastrointestinal tract. Other nonspecific supportive care may be helpful, but horses receiving a sublethal dose probably will recover with minimal assistance. If a lethal dose is forced into an animal inadvertently, oral laxatives and adsorbents such as activated charcoal may help bind lasalocid and reduce the amount absorbed.

20.1.3.11 Salinomycin

Salinomycin is an ionophore, also marketed as a coccidiostat, that is related more closely to monensin than is lasalocid. The ionic affinity of salinomycin is predominantly to sodium and potassium, and its mode of action and cellular effects are similar to those of monensin.[22]

In one report of affected horses, the clinical signs were similar to those of the other ionophore toxicoses. The range of clinical signs included anorexia, depression, occasional sweating, colic, dyspnea, weakness, ataxia, and recumbency. Occasionally, horses showed reduced performance for several weeks following exposure, but many horses that became recumbent were destroyed humanely. The clinicopathologic abnormalities exhibited by affected horses included elevated serum activities of CK, AST, and alkaline phosphatase.[55]

The diagnosis of salinomycin toxicity is hampered by the fact that none of the clinical signs are pathognomonic and tissue samples do not reveal toxic levels. However, one may assay suspect feed and intestinal content for the presence and quantity of salinomycin.

Treatment of affected horses is mostly symptomatic because no specific antidote is available. Evacuation of the bowel by laxatives may be helpful in reducing the amount of toxic material available for absorption. One should maintain fluid balance and electrolyte and acid-base indexes within normal ranges. Affected horses generally require an extended convalescence, and one should consider the possibility of a persistent cardiomyopathy.[55] Affected horses should undergo a rigorous cardiac examination before returning to performance events.

20.1.3.12 Cantharidin Toxicosis (Blister Beetle Toxicosis)

Cantharidin toxicosis results from ingestion of dead blister beetles that become entrapped in hay during harvesting. Essentially all reports are of horses being fed alfalfa hay or alfalfa products, but anecdotal reports of horses intoxicated by ingesting grass hay have been communicated to the author. More than 200 species of blister beetles inhabit the continental United States, but toxicity results primarily from beetles of the genus *Epicauta*.[56]

Cantharidin is the sole toxic principle and is contained in the hemolymph, genitalia, and possibly other tissues of the beetle. Cantharidin is a highly irritating substance that causes acantholysis and vesicle formation when in contact with skin or mucous membranes, and the substance is absorbed from the gastrointestinal tract and rapidly excreted by the kidney. Storage of hay does not reduce the toxicity of cantharidin.[56]

20.1.3.12.1 Clinical Signs

The signs associated with toxicosis are many and varied and are dose-dependent. Horses affected with a minimal dose may show only signs of depression, anorexia, and occasionally polyuria, whereas horses ingesting a lethal dose may show signs of profound shock, gastrointestinal and urinary tract irritation, myocardial dysfunction, and hypocalcemia.[56,57] The onset and duration of clinical signs vary from hours 1454
to days, but horses that succumb to cantharidin generally die within 48 hours of onset of signs. Horses that 1455
live longer than 48 hours have a better prognosis for recovery if no complications arise.

The most commonly observed clinical signs include varying degrees of abdominal pain, anorexia, depression, and repeatedly submerging the muzzle in water or frequently drinking small amounts of water. The respiratory and cardiac rates are elevated, and cardiac contractions are occasionally forceful enough to be observed through the thoracic wall. Mucous membranes are congested and cyanotic, and capillary refill time is prolonged. The feces may be watery in consistency but rarely contain blood or mucus. Profuse sweating is typical of horses more severely affected and may be a sign of severe abdominal pain. Affected horses often make frequent attempts to void urine. The urine is grossly normal early in the disease course but later may become tinged with blood or contain clots of blood. Gross hematuria, if it occurs, is usually in the later stages of the disease process. Less commonly observed signs include synchronous diaphragmatic flutter, erosions of the gingival and oral mucous membranes, and occasionally a stiff, short-strided gait similar to that seen in acute myositis. Sudden death also has been reported.[56]

20.1.3.12.2

Pathophysiology

The mechanism of action of cantharidin at the cellular level has not been elucidated fully. Acantholysis and vesicle formation result from disrupted cell membranes. Cantharidin does not have a direct effect on membranes but is thought to interfere with oxidative enzymes bound to mitochondria. These enzyme systems are involved directly in active transport across the plasma membrane, and their failure results in cell death caused by significant permeability changes in the cell membrane.[56]

Hypovolemic shock and pain develop rapidly in more severely affected horses. The normal transfer of fluid, nutrients, and electrolytes across the intestinal mucosa is disrupted because of the morphologic changes induced by cantharidin. Although renal tubular damage is not severe enough to cause death, changes in the renal tubular epithelium also may be related to the development of fluid, acid-base, and electrolyte abnormalities.[56,57]

Hypoproteinemia develops later in the disease course, probably as a result of protein loss across the damaged intestinal mucosa. Protein also is lost into the peritoneal space, and a minor amount may be lost via the urine.[56]

The profound hypocalcemia and hypomagnesemia that occur in many horses have not been explained fully. Calcium loss or derangement of calcium homeostasis or a combination of both is the most likely explanation because the acute onset of the disease eliminates reduced intake as a possible cause. Calcium can be lost via urine, sweat, and as protein-bound calcium through the damaged intestinal wall. An influx of intracellular calcium also may occur in certain tissues. Whether cantharidin has an effect on calcium-binding sites on proteins or in cells is unknown.[56]

The low urine specific gravity in most horses may be caused by decreased permeability of the collecting ducts to water. Other findings, however, point to a mild pathologic insult as a cause of the low urine specific gravity. These findings include the facts that a low specific gravity occurs suddenly within hours of toxin exposure; specific gravity returns to normal in 2 to 4 days in surviving horses; only mild to moderate changes are noted in other renal function tests; and the histologic renal lesions are mild, and neither acute nor chronic renal failure is associated with cantharidin toxicosis in horses.[56]

Myocardial necrosis is a common finding in affected horses and may be caused by the direct effect of cantharidin on cardiac muscle. Dose-related intracellular changes involving the mitochondria, cristae, nuclear chromatin, sarcoplasmic reticulum, and myofibrils have been observed in the cardiac muscle of rabbits that were given cantharidin. A proposed mechanism for these changes suggests that an excess transport of calcium into the myocardial cells occurs, leading to an intracellular calcium overload. This overload may result in a high-energy phosphate deficiency within the cell, leading to necrosis and cell death.[56]

20.1.3.12.3

Diagnosis

One should consider cantharidin toxicosis when horses exhibit signs of abdominal pain, depression, or polyuria, and their diet contains alfalfa hay or alfalfa products. One can make the diagnosis when horses have clinical signs and laboratory findings compatible with cantharidin toxicosis and when one finds beetles in the hay. The beetles can be difficult to identify in hay, so one should search thoroughly. One can

assay cantharidin using high-pressure liquid chromatography and gas chromatography–mass spectrometry techniques.[56,58] Samples to be tested are urine and stomach content from suspect horses.

Laboratory findings are nonpathognomonic, but several abnormalities typically are noted. Packed cell volume (PCV) and serum protein concentrations are elevated early, but hypoproteinemia frequently develops after about 24 hours. Mild hypokalemia can occur but is not a striking feature of this disease. Blood urea nitrogen concentration may be elevated moderately, and hyperglycemia is almost always present initially.[56]

Serum calcium and magnesium concentrations are significantly decreased in most horses and remain low for longer than 48 hours if untreated. The urine generally contains red blood cells and has a low specific gravity, even in the face of clinical dehydration. Abnormal peritoneal fluid findings include increased protein concentration but relatively normal fibrinogen and white blood cell values. Feces are often positive 1455
for occult blood. Serum CK activity may be elevated in more severely affected horses and augurs a poor 1456
prognosis.[56] Although not diagnostic, laboratory findings of prolonged hypocalcemia and hypomagnesemia and elevated CK concentration may help differentiate cantharidin toxicosis from other causes of acute abdominal crisis.

20.1.3.12.4 Treatment

No specific antidote is available for cantharidin. Once one suspects the diagnosis, one should remove all suspect feed from the environment of the horse and should have hay that is subsequently fed to horses carefully examined for the presence of beetles.

One should give mineral oil early to horses suspected of having cantharidin toxicosis. The oil helps evacuate the bowel and also may help reduce the amount of cantharidin available for absorption because cantharidin is lipid soluble. Activated charcoal given via nasogastric tube also may have beneficial effects.[56]

One should administer polyionic fluids intravenously throughout the disease course to correct dehydration and promote diuresis. One also may give diuretics once the horse is volume loaded. Analgesics usually are required because of the severity of the abdominal pain, and glucocorticoids may be necessary to aid in treating shock. One should give calcium gluconate to elevate the serum calcium concentration and should replace calculated deficits of magnesium by slow intravenous infusion.[56]

20.1.3.13 Phosphorus

Elemental phosphorus is available in red and white forms. Red phosphorus is used in manufacturing fertilizers and safety matches and is considered inert and nontoxic. White phosphorus is used as a rodenticide and is commercially available in pastes containing from 1.5% to 5.0% phosphorus. The reported toxic dose for horses is 0.5 to 2.0 g.[15]

20.1.3.13.1 Clinical Signs

The toxic manifestations of phosphorus poisoning are generally threefold: toxic signs commence within hours of ingestion, are followed by a latent period of 48 hours to several days when the animal may appear recovered, and finally recur with greater severity.

The initial signs are characterized by severe abdominal pain and gastrointestinal irritation, with occasional episodes of diarrhea. Blood may be present in feces. Cardiac arrhythmias may occur during this phase, and if the dose is sufficiently large, cyanosis, shock, incoordination, and coma can develop, and the animal may die before the second and third stages develop.[15]

The latent period may occur from 48 to 96 hours following the onset of clinical signs, and during this time the animal may appear normal. The third stage presents as a recurrence of severe abdominal pain, and signs of liver dysfunction may become evident. One may note icterus and a tendency to bleed from the gingiva, stomach, intestine, or kidney.[15]

20.1.3.13.2 Pathophysiology

Phosphorus is absorbed from the gastrointestinal and respiratory tracts. Although dermal exposure may cause skin irritation or burning, absorption does not occur via this route. The mechanism of action of phosphorus is unknown but is noted for causing irritation and necrosis of affected tissue. Phosphorus is also known to cause peripheral vasodilation.[15]

20.1.3.13.3 Diagnosis and Treatment

Clinicopathologic abnormalities reflect hepatic and renal damage. Hypoglycemia may be pronounced, and liver enzymes such as AST, LDH, and sorbitol dehydrogenase are elevated. Renal damage is reflected by increased BUN and creatinine concentrations. One may find albumin, blood, and increased concentrations of amino acids in the urine. The phosphorus concentration in blood is usually normal. Although elemental phosphorus in tissues can be assayed, in time a large portion may be oxidized to phosphates, thereby making confirmation of poisoning difficult by chemical means.[15]

No specific antidote is available for phosphorus intoxication. Therapy is essentially symptomatic and supportive.

20.1.3.14 Thallium

Thallium is toxic to all animals, including human beings, but no reports of clinical toxicosis in horses could be found in the literature. However, a dose of thallous acetate 27 mg/kg orally has been suggested as potentially lethal to horses.[38] Thallium has been used in recent years as a rodenticide, but its use now is restricted only to government agencies.

20.1.3.14.1 Clinical Signs

Thallium ingestion can result in acute, subacute, or chronic syndromes. In the acute form, clinical signs usually begin within 1 to 4 days of ingestion. Initial signs are those of severe gastrointestinal insult and include vomiting, severe hemorrhagic diarrhea, abdominal pain, and anorexia. Labored breathing is apparent early in the disease course, and motor paralysis and trembling may occur. Signs suggesting renal dysfunction also may occur.[15]

The subacute form generally manifests signs in 3 to 7 days after ingestion. Signs of gastric distress and motor disturbances are less marked than in the acute form, but they persist for a longer time. In this form, reddening of the skin and pustule formation occur. A pronounced reddening of the oral mucous membranes also seems to be unique to this particular toxicosis. Other clinical signs observed include conjunctivitis, hair loss, and crusty skin lesions. Secondary bacterial infections also may develop in affected animals.[15]

The chronic stage requires 7 to 10 days to appear. Signs of gastrointestinal and nervous system dysfunction are mild, but hair loss and dry, scaly skin become pronounced.[15] 1456

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20.1.3.14.2 Pathophysiology

Thallium is absorbed readily from the intestinal tract or through the skin; is distributed in all body tissues, although higher levels accumulate in the kidneys and liver; and is excreted principally in feces, and to a lesser extent in urine, and undergoes an enterohepatic cycle for resorption and excretion.[38]

Thallium is thought to combine with mitochondrial sulfhydryl enzymes at a specific yet unknown place in the scheme of sulfur metabolism. Thallium therefore interferes with oxidative phosphorylation within cells. Recent evidence suggests that thallium exchanges for potassium in muscle and nerve cells primarily and also has a necrotizing effect on the intestinal tract, kidney, and occasionally the brain.[15,38]

20.1.3.14.3 Diagnosis

One may suspect thallium toxicosis based on clinical signs, and one can assay urine for thallium content. The finding of thallium in tissue in any amount is diagnostic, but liver and kidney levels tend to be higher than in other tissues.

20.1.3.14.4 Treatment

Chelation therapy using diphenylthiocarbazone (dithizon) at a dosage of 70 mg/kg orally t.i.d. has been recommended for use in dogs.[15] However, cats react adversely to this agent. Its effect in horses is unknown.

Potassium chloride may aid in the elimination of thallium and can be given intravenously or orally, but the oral route is contraindicated in animals that also are being treated with an ion exchange agent.

One can attempt to trap thallium in the intestine with the ion exchange agent potassium ferric cyanoferrate-II (potassium–Prussian blue). Experimentally, potassium– Prussian blue is not absorbed from the intestinal tract and acts to immobilize the thallus ion by exchanging with the potassium of potassium–Prussian blue. Once trapped, thallium is not released readily from potassium–Prussian blue, and fecal excretion of thallium increases.[15,38]

Other forms of symptomatic therapy include intestinal protectants and demulcents, fluid and electrolyte support, analgesics, oral activated charcoal, and general nursing care.[38]

20.2 Toxicoses Causing Signs of Central Nervous System Stimulation

Dealing with horses showing signs of central nervous stimulation can be one of the most difficult and challenging situations the equine internist faces. Symptoms can be hard to control and clients' emotions can run high at the sight of a horse showing severe or uncontrollable signs like hyperexcitability, exaggerated gait abnormalities, and seizures. The immediate clinical emphasis is usually on minimizing the chances and/or severity of self-induced trauma. Etiologic diagnosis can be difficult and protracted.

20.2.1 PLANTS

20.2.1.1 Locoweed

A nervous syndrome in horses, cattle, and sheep has long been associated with eating plants of the genera *Astragalus* and *Oxytropis*. This group of plants is large. The *Astragalus* species that grow in North America number more than 300. Not all species of *Astragalus* and *Oxytropis* are toxic, however, and some species make nutritious forage for livestock.[59] Some debate still exists over the taxonomy of these species, but their clinical signs are essentially the same, and they are discussed together.

The toxic species of *Astragalus* produce three different syndromes in livestock. Some species contain nitroglycosides, which cause methemoglobinemia and competitive inhibition of certain cellular enzymes; others accumulate toxic levels of selenium; and a third group contains alkaloids that cause locoism.[59,60] The first group of plants is of minor importance to the horse, and the selenium concentrators are discussed in the section on selenium toxicosis. A discussion of the clinical syndrome called locoism follows.

The species of *Astragalus* and *Oxytropis* that induce locoism include *A. lentiginosus* (36 varieties); *A. mollissimus* (11 varieties); *A. wootonii* (2 varieties); *A. thurberi; A. nothoxys; O. sericea; O. lambertii;* and *O. saximontana*.[59] Additional *Astragalus* species incriminated in causing disease include *A. argillophilus, A. bisulcatus,* and *A. earlei*.[60] Geographically, locoweeds are found from western Canada southward to include the western United States and northern Mexico.[59]

20.2.1.1.1 Clinical Signs

Locoweeds cause a number of problems in livestock, including neurologic and reproductive dysfunction, emaciation, and habituation.[59] Typical signs exhibited by affected horses include a slow staggering gait, depression, an unthrifty appearance, emaciation, muscular incoordination, and nervousness, especially when the animal is stressed. The affected horse may become solitary and hard to manage and may have difficulty in eating and drinking. In some animals, sexual activity may become suppressed. Visual impairment also occurs in some horses, and mares ingesting locoweed during pregnancy have been known to abort or produce foals with various limb deformities.[60,61] Horses that are affected chronically are generally useless for riding or draft purposes because their behavior is so unpredictable.[59]

The onset of clinical signs varies from as short as 2 weeks to as long as 2 months after the horse starts to graze the plant. The plants generally are considered nonpalatable to horses, but once they start to ingest the plant, they seem to become addicted to it and will search it out.[59] The addiction can extend to subsequent

growing seasons, so clinical signs can worsen progressively in successive years if animals continue to graze the plant.[60] Affected horses can recover if feeding or grazing is discontinued before they become too emaciated and if nutritious forage is given.[59] However, the syndrome eventually may lead to death in chronically affected horses.[60] 1457 1458

20.2.1.1.2 Pathophysiology

The indolizidine alkaloids swainsonine and swainsonine *N*-oxide have been suggested as the toxic principles in locoweeds.[60] These alkaloids were first recovered from *Swainsona* species in Australia.[59,60] Swainsonine inhibits α-mannosidase, a lysosomal enzyme essential in the cellular metabolism of oligosaccharides. As a result, mannose-rich oligosaccharides accumulate in lysosomes and disrupt cellular function. These accumulations are visible microscopically as intracytoplasmic vacuoles. Vacuolization of the renal cortical tubular cells can occur as early as 4 days after feeding of locoweed is started, and neurons of the CNS, including Purkinje cells, may show vacuolization by 8 days. The vacuoles disappear shortly after consumption ceases in the early stages of disease, but if grazing is prolonged, permanent cellular damage occurs. Continuous feeding of the plant for 30 days or longer results in vacuolization of almost all tissues of the body except skeletal and cardiac muscle.[60]

Neurologic signs result from vacuolization of the axons, glial cells, and Purkinje cells of the cerebellum and cerebral cortex. The weight loss and emaciation result from impairment of the liver, pancreas, thyroid, and parathyroid glands. Vacuolization of cells of the retina and decreased lacrimation are responsible for impaired vision in some animals. Vacuoles also occur in lymph nodes, placenta, testicles, and lymphocytes.[59,60] The pathogenesis and lesions of locoism are similar to mannosidosis, a heritable lysosomal storage disease of Angus and Murray Grey cattle.[60]

20.2.1.1.3 Diagnosis and Treatment

No pathognomonic test is available for diagnosis of locoism. One should suspect the diagnosis when horses show clinical signs compatible with locoism and have a history of exposure to the plant. Laboratory testing is nonspecific, and one can expect test results consistent with multiple organ dysfunction. Microscopic lesions of intracytoplasmic vacuolization in various organs, including the CNS, are compatible with a diagnosis of locoism.[59,60] Peripheral lymphocytes that contain intracytoplasmic vacuoles also are considered indicative of locoism, if clinical signs are present.[60]

No effective cure exists for horses with chronic locoism that have had clinical signs for some time. Mild cases usually resolve in 1 to 2 weeks once ingestion ceases, so successful recovery from locoism depends on early recognition of the disease syndrome and preventing horses from further consumption of the plants. Reserpine has been suggested to be helpful in relieving some of the clinical signs of locoism in horses.[60]

20.2.1.2 Nervous Ergotism

Two forms of ergotism are observed in domestic animals, a nervous or convulsive form and a gangrenous form. Horses appear rarely to be affected with ergotism, but the nervous form is reported to occur much more frequently in horses than the gangrenous form.[62,63] Ergotism is caused by a number of alkaloids contained in

Claviceps purpurea, a fungus infecting many grains such as wheat, barley, rye, and oats and wild grasses such as quackgrass, smooth bromegrass, wheatgrass, bluegrasses, and wild rye. The fungal mass, or sclerotium, replaces the seed or kernel of the plant and may have the same general configuration of the seed but is usually larger, dark colored, and hard. Ergotism is rarely of concern in dry seasons, but abundant fungal growth can occur during wet periods.[62] Although well-documented cases of ergotism caused by *C. purpurea* in horses are absent in the literature, a nervous syndrome typical of *C. paspali* poisoning has been observed in horses in Australia. However, the cause of the clinical signs in these horses was suggested to have been the tremorogenic mycotoxins in *C. paspali* rather than the alkaloids found in ergot.[64]

20.2.1.2.1 Clinical Signs

The first sign of nervous ergotism is reported to be dizziness or an unsteady gait. This phase may be interrupted by convulsions and temporary posterior paralysis and drowsiness.[62] Other behavioral effects that have been described include incoordination, lameness, difficulty in breathing, excessive salivation, and diarrhea.[63]

20.2.1.2.2 Pathophysiology

Approximately 40 different alkaloids have been isolated from *C. purpurea*. All of these alkaloids are derivatives of the tetracyclic compound 6-methylergoline, a lysergic acid base structurally similar to a number of biogenic amines such as dopamine, serotonin, and norepinephrine.[7,62] The most pharmacologically potent alkaloids in ergot are ergonovine, ergotamine, ergotsine, ergocristine, ergocryptine, and ergocornine.[62] Additionally, tyrosine, tryptophan, tyramine, histamine, histidine, choline, and acetylcholine have been isolated from ergot sclerotia, but their clinical significance is uncertain.[7]

The mechanism whereby ergot alkaloids induce central nervous signs in horses has not been elucidated. Of 1458
the ergot alkaloids known to affect the nervous system in human beings, bromocriptine is the prototype. 1459
Bromocriptine is a long-acting dopamine agonist that has central stimulating effects and may cause hypotension. Another ergot alkaloid, isoergine (lysergic acid amide), has one tenth the mind-altering potency of the structurally related compound lysergic acid diethylamide (LSD). Lysergic acid diethylamide is thought to produce hallucinations by a series of complex agonist and antagonist actions on several central monoamine neurotransmitters, particularly serotonin.[7]

20.2.1.2.3 Diagnosis and Treatment

The diagnosis of nervous ergotism in horses is based primarily on clinical signs and eliminating other causes of CNS stimulation. One can assay the ergot alkaloid content of feed, but detailed analysis is required to determine the quantity of individual alkaloids present and their potency.[62]

No specific antidote exists for ergot alkaloid toxicity. Treatment of affected horses is largely symptomatic. Recommendations for control include the use of ergot-free feed, crop rotation, plowing deeply because shallow cultivation and seeding leave the sclerotia near the soil surface where they can germinate more readily, and mowing surrounding grasses to limit the spread of fungus into the cultivated crop.[63] Pasture

grasses that may be infested with ergot should be mowed before the development of seed heads because *Claviceps* replaces the seed or kernel of the plant.

20.2.2 MEDICATIONS

20.2.2.1 Carbamates

The carbamate pesticides are composed of cyclic or aliphatic derivatives of carbamic acid, and numerous ones are commercially available.[15,65] Carbamates are absorbed readily through the lungs, gastrointestinal tract, and skin. Carbamates do not accumulate in any particular tissue but do cross the rat placenta, depress fetal acetylcholinesterase, and are not metabolized readily in the fetus.[65] In human beings the carbamates poorly penetrate the blood-brain barrier and therefore produce few CNS symptoms.[7] Carbamates do not require activation by liver enzymes to exert their effect. Toxicity data are not complete for several domestic animals, but lethal doses of the different compounds vary from less than 1 mg up to several hundred milligrams per kilogram of body mass. Carbamates are not stable in the environment and are fairly insoluble in water, but organic solvents and oils can carry the compounds across cell barriers.[65]

20.2.2.1.1 Clinical Signs

Clinical signs can commence within a few minutes to several hours following exposure but are short-lived. The clinical episode frequently is less than 36 to 48 hours in length, with the animal succumbing or recovering during this time.[15,65]

Signs of toxicity in horses reflect muscarinic and nicotinic cholinergic overstimulation. The signs suggestive of muscarinic cholinergic overstimulation include profuse salivation, severe gastrointestinal disturbances characterized by hypermotility, severe pain, abdominal cramps and diarrhea, excessive lacrimation, miosis, sweating, dyspnea, cyanosis, and urinary and fecal incontinence. Affected animals also may cough frequently as a sign of excessive accumulation of respiratory tract secretions. The signs reflected by nicotinic overstimulation include excessive stimulation of the skeletal muscles. The muscles of the face, eyelids, tongue, and the general musculature may twitch. Some animals exhibit signs of generalized tetany whereby they walk in a stiff-legged fashion. This hyperactivity may be followed by weakness and paralysis of the skeletal muscles.[15]

Signs of CNS involvement in domestic food-producing animals may include hyperactivity reflecting excessive stimulation of the CNS, but domestic animals rarely exhibit convulsive seizures. Central nervous system depression is reported to occur more commonly than CNS stimulation.[15]

20.2.2.1.2 Pathophysiology

Carbamates induce excessive stimulation of the parasympathetic nervous system by inhibiting acetylcholinesterase and pseudocholinesterase. The carbamate pesticides occupy the anionic and esteratic sites of acetylcholinesterase, with the esteratic site being carbamylated. Acetylcholinesterase can hydrolyze carbamate pesticides but at a slower rate than that for acetylcholine. Therefore carbamates are reversible inhibitors of acetylcholinesterase, but toxicosis occurs when the amount of pesticide is large enough that the rate of carbamylation of acetylcholinesterase exceeds the rate of hydrolysis of pesticide by

the enzyme.[65] As a result, acetylcholine accumulates in neuroeffector and synaptic regions, resulting in the observed clinical signs of parasympathetic overstimulation.

The continuous stimulation of secretory glands leads to excessive salivation and accumulation of fluid within the respiratory tract and within the lumen of the bowel. Extensive pulmonary edema may occur and along with bronchoconstriction can lead to death in affected animals.[15]

Carbamates are removed from the circulation largely by spontaneous hydrolysis of the carbamate-cholinesterase complex. In addition, blood esterases also can inactivate a portion of the circulating carbamate, and certain liver microsomal enzymes break down the compounds within hours of exposure.[7,65] The clinical signs associated with carbamate toxicity are generally rather short-lived, with recovery occurring in less than 36 to 48 hours in most animals.

20.2.2.1.3 Diagnosis and Treatment

One is most likely to make a diagnosis from a history of possible exposure, clinical signs, and response to atropine treatment. Chemical analyses of body tissue for carbamate residue are usually unrewarding, probably because of the rapid metabolism of the compound. However, finding the pesticide in stomach contents or in feed samples in sufficient quantities to cause toxicosis could confirm the diagnosis.[15]

One also can use cholinesterase activity in blood and tissue to confirm a diagnosis. However, one must use discretion when interpreting these results because recommended therapeutic levels of carbamates applied to animals can result in some depression of blood cholinesterase activity. In one author's opinion, clinical signs of acute carbamate toxicity are associated with blood cholinesterase activity of less than 20% of
normal values. Because the inactivation of cholinesterase by carbamates involves a much weaker and less 1459
stable binding than that by organophosphates, one should not dilute blood samples from suspect animals 1460
and should refrigerate them and analyze them as soon as possible.[15]

One should treat affected animals as quickly as possible. Initial therapy should consist of atropine sulfate at 0.2 mg/kg body mass. One should divide this initial dose, giving approximately one fourth of the dose intravenously and the remainder subcutaneously or intramuscularly. Repeated doses of atropine may be required but should be used only to counteract the parasympathetic signs. The skeletal muscle tremors may not respond to atropine therapy.[15]

Orally administered adsorbents such as activated charcoal may be useful in binding ingested pesticide, and aqueous cathartics may further aid in evacuation of the intestinal tract. One probably should not give mineral oil orally in suspect cases because organic solvents and oils can carry the compound across cell barriers. Dermally exposed animals should be washed with soap and water to prevent further exposure. The oximes such as pralidoxime are of no benefit in treating carbamate toxicosis, and their use may or may not worsen the condition of the animals.[15,65]

20.2.2.2 Organophosphates

The organophosphates are being used increasingly in a variety of ways. Some of the typical uses for these compounds include animal insecticides and parasiticides; plant insecticides; soil nematocides, fungicides,

herbicides, and defoliants; rodenticides; insect repellents; and chemosterilants.[65] Horses can become intoxicated in a variety of ways because these products are used so commonly in the environment.

A wide variety of organophosphorous compounds have been developed, and their toxicity varies greatly among compounds and among animal species. Osweiler, Carson, Buck, et al. have tabulated a good list of various organophosphorous compounds and their relative toxicities.[15] In addition to variations in compound toxicity, a number of physicochemical factors also can affect the toxicity of organophosphorous compounds. The toxicity of these compounds decreases as they are degraded by sun, water, microbes, alkali, or metal ions such as iron or copper. An increase in toxicity may occur by storage activation, a process in which highly toxic isomers of certain pesticides are formed spontaneously in polar solvents or water. This reaction is speeded by heat. Parathion, malathion, fenthion, chlorpyrifos, diazinon, and coumaphos are some of the compounds that can undergo this type of storage activation.[65] The storage activation phenomena provide good reasons to use only freshly prepared preparations of organophosphate compounds on horses.

Other factors that can influence the toxicity of a particular organophosphorous compound are ambient temperature (higher temperatures may increase the toxicity of certain compounds); the vehicle in which the pesticide is dispersed; the age and sex of the animal; and the presence of other chemicals that may alter organophosphate toxicity. The combined effects of two organophosphates may be synergistic or antagonistic, and drugs that compete with organophosphates for target esterases, such as succinylcholine, phenothiazine, and procaine, may enhance organophosphate toxicity. In addition, drugs that have neuromuscular blocking properties (inhalant anesthetics, magnesium ions, certain aminoglycoside antibiotics, and the depolarizing and nondepolarizing neuromuscular blocking agents) also may enhance organophosphate toxicity. The organophosphates are poorly soluble in water but are soluble in organic solvents, fats, and oils. Oily vehicles or organic solvents also can facilitate passage of the organophosphates through the skin.[65]

20.2.2.2.1 Clinical Signs

Clinical signs of organophosphate toxicity are similar to those of carbamate toxicity, and essentially are those of overstimulation of the parasympathetic nervous system, skeletal muscles, and the CNS.

Overstimulation of muscarinic cholinergic sites results in profuse salivation and lacrimation; serous or seromucous nasal discharge; increased respiratory sounds resulting from bronchoconstriction and excessive bronchial secretions; profound gastrointestinal disturbances of increased motility, abdominal pain, and diarrhea; bradycardia; miosis; sweating; coughing; and frequent urination. Signs of nicotinic cholinergic overstimulation include muscle fasciculations, tremors, twitching, spasms, and a stiff or rigid gait. Central nervous system signs frequently include anxiety, restlessness, and hyperactivity.[15,65] If the exposure is not severe enough to result in death of the horse, the horse may require several days or weeks to recover completely.[65]

20.2.2.2.2 Pathophysiology

Organophosphates can be absorbed from the gastrointestinal tract and lungs and through the skin. Following absorption, they are distributed throughout the body but do not accumulate in any particular tissue. Most of the organophosphates must be activated by hepatic microsomal oxidative enzymes before they become potent esterase inhibitors. Phosphorothiolate and the phosphate class of organophosphates do not require activation and can inhibit esterases immediately on entry into the bloodstream.[65]

Organophosphates act as irreversible inhibitors of true cholinesterase and pseudocholinesterase in mammals. They irreversibly phosphorylate the esteratic site of cholinesterases throughout the body. As a result, endogenous acetylcholine is not inactivated. Therefore acetylcholine accumulates in neuromuscular junctions; parasympathetic postganglionic sites in smooth muscle, cardiac muscle, and glands; in all autonomic ganglia; and in cholinergic synapses within the CNS. The result is overstimulation of these sites, leading to clinical signs of toxicosis.[15,65] 1460

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Lethal amounts of organophosphates cause death by a combination of effects of nicotinic, muscarinic, and central cholinergic overstimulation or receptor paralysis. These effects include hypotension, bradycardia, bronchoconstriction and excessive bronchial secretion, inability of the respiratory muscles to work properly, cyanosis, and central respiratory depression. The animal actually dies of asphyxia.[65]

Detoxification of organophosphates is accomplished mostly by serum and liver esterases. However, other enzymes in the liver and in other tissues may attack the pesticides at rates dependent on the class of pesticide, the species, and the age of the animal. Water-soluble metabolites may be formed rapidly and the pesticide excreted quickly in the urine.[15,65]

20.2.2.2.3 Diagnosis and Treatment

One should suspect a diagnosis of organophosphate toxicosis when the horse has a history of possible exposure within the past 48 hours along with characteristic signs of parasympathetic overstimulation. One can perform tests for organophosphate content in body tissues or specimens and in other suspect materials, but the process is tedious. Body specimens analyzed for organophosphate content often yield negative results because the compounds do not stay in tissues long after the animal has been exposed.[15,65]

A test for cholinesterase activity in blood or tissue is the most important aid in determining if an animal has been exposed to excessive amounts of a cholinesterase inhibitor. Blood cholinesterase activity values of less than 20% to 25% of normal are compatible with exposure of the animal to excess organophosphates or other cholinesterase inhibitors. One should note that some depression of blood cholinesterase activity occurs when therapeutic levels of organophosphates or carbamates are used, so one should view these results with discretion.[15,65]

Treatment should involve immediate use of atropine sulfate at 0.2 mg/kg body mass. One should give approximately one fourth of this dose intravenously and the remainder subcutaneously or intramuscularly. One usually needs to repeat this dose at 3- to 6-hour intervals for a day or more. Because atropine does not block the nicotinic cholinergic effects, the horse may continue to show signs of muscle fasciculation or tremors.[15,65] One should use atropine with great discretion in the horse so as not to cause further complications such as gut stasis with atropine overdosage (see Atropine).

The oximes, such as pralidoxime and pralidoxime chloride, act specifically on the organophosphorus-enzyme complex to free the enzyme and also react directly with the organophosphate to form a nontoxic complex that is excreted in urine. However, the use of these products in horses may be economically unfeasible. The recommended dose varies between 20 mg/kg[98] and 25 to 50 mg/kg given as a 20% solution intravenously over several minutes. Oximes are reported to work best in the presence of atropine, so they should be given to the animal following atropine administration. One can repeat treatment with the oximes if signs reappear.[65]

Other treatment measures include removal of the source, if possible; use of orally administered activated charcoal; laxatives to aid in evacuation of the bowel; washing with soap and water if dermal exposure has occurred; and supportive therapy such as fluids and electrolyte administration, if necessary. *Drugs one should avoid when treating organophosphate toxicosis include phenothiazine tranquilizers, succinylcholine, and morphine*.[15,65]

20.2.2.3 Chlorinated Hydrocarbons

The use of chlorinated hydrocarbon pesticides is being discontinued or severely restricted because of their persistence in the environment and their incorporation into the food chain. However, certain agents still are being used, primarily as contact insecticides and as ectoparasiticides.[15,24,65]

The chlorinated hydrocarbon insecticides are poorly soluble in water but are soluble in organic solvents and oils. Oily vehicles or organic solvents also can facilitate penetration of the insecticide through intact skin. This group of compounds also is characterized by volatility, so exposure to the pesticide can occur via inhalation of the vaporized compound.[65] Because these compounds accumulate in body tissues, primarily adipose tissue, signs of toxicosis can occur following repeated exposure to lesser amounts or following a single excessive dose.[24] Toxicity varies greatly among the different compounds, and Osweiler, Carson, Buck, et al.[15] have tabulated the toxicity of a number of these compounds for various animal species.

20.2.2.3.1 Clinical Signs

The chlorinated hydrocarbons act as diffuse stimulants or depressants of the CNS, with the onset of signs ranging from several minutes to several days following exposure. The signs displayed may be progressively severe or explosive and fulminating.[15] Initially the animal may be hypersensitive, apprehensive, or belligerent. These behavioral aberrations may progress to abnormal posturing or frenzied or maniacal behavior. Nervous signs can begin with hypersensitivity and muscle fasciculations beginning around the head and facial area and proceeding caudally eventually to include the hindquarters. These muscle spasms can occur intermittently or continuously. Clonic-tonic seizures often follow and may result in death or may be followed by intermittent periods of CNS depression. Autonomic manifestations of profuse salivation, mydriasis, diarrhea, urination, and bradycardia or tachycardia with arrhythmias may
occur. Some animals may lose coordination and stumble while walking, may walk aimlessly, or may move 1461
in circles. Other notable signs may include increased rate and depth of respiration and fluid sounds in the 1462
lungs. Death may occur within minutes, hours, or days, or not at all.[15,24,65]

20.2.2.3.2 Pathophysiology

Chlorinated hydrocarbon pesticides gain entry into the body via the gastrointestinal and respiratory tracts and absorption through the skin. Once they gain entry into the bloodstream, they are thought to bind to serum lipoproteins and are distributed throughout the body. Eventually an equilibrium is reached in which the pesticide concentration varies among different body compartments. Most of the absorbed pesticide is stored in fat, but brain and fetus also can accumulate significant amounts.[15,65]

Adipose tissue is the main storage tissue for chlorinated hydrocarbons and as such can retain some of these compounds for an extended period of time. The pesticide also is mobilized slowly from fat, which can account for the presence of the pesticide in blood and milk for weeks to months.[65]

The chlorinated hydrocarbons are broken down by liver microsomal enzymes, and the pesticide and its metabolites are excreted in urine, bile, milk, and feces. This first stage of elimination is fairly rapid and may account for 40% to 50% of the compound being eliminated during the first 3 to 4 days after exposure.[65]

The exact mechanism of action of the chlorinated hydrocarbons is unknown, but they act as nonspecific stimulants of the CNS. A suggested mechanism is that the compounds easily enter neural membranes and prolong the time during which some of the sodium channels in the membrane are open during depolarization. In addition, potassium efflux from the cell is hindered. The net effect of these ion imbalances is a decreased transmembrane resting potential that causes a decreased firing threshold and an increased neuronal excitability.[65]

An increase in whole-brain free ammonia concentration and brain glutamine also occurs, but whether these changes are a cause or an effect of the sodium-potassium flux defect is unknown. However, the onset and disappearance of convulsions in animals is correlated with an increase and decrease of brain ammonia concentration.[65]

The depression produced by some chlorinated hydrocarbons may result from rapid depolarizing blockade of neurons of the reticular activating system. Excessive depolarization of the medullary neurons may be responsible for respiratory failure, which is the usual cause of death in chlorinated hydrocarbon pesticide toxicosis. The muscle tremors in chlorinated hydrocarbon toxicosis are thought to be partly central in origin and partly caused by direct depolarizing effects on peripheral motor nerves.[65]

20.2.2.3.3

Diagnosis and Treatment

One can make a tentative diagnosis when animals are known to have been exposed to an insecticide and they are exhibiting signs of convulsive seizures and neuromuscular dysfunction. One may assay tissue samples for residues of the specific compound, but one must interpret results with caution because some of the compounds may be found in fat of normal animals as a result of exposure to small concentrations in the environment. However, parts per million concentrations may have diagnostic significance if history and clinical signs are consistent with chlorinated hydrocarbon poisoning. Brain concentrations of pesticide are reported to be better correlated with toxicosis than are concentrations in body fat.[65] Other suitable tissue specimens include blood, milk, liver, kidney, and gastrointestinal contents.[15]

No specific antidote is available for the chlorinated hydrocarbons, so treatment is symptomatic. For animals exhibiting convulsive seizures or neuromuscular hyperactivity one can give chloral hydrate or pentobarbital intravenously carefully to effect. Sedative doses of these two agents should control most of the behavioral, nervous, and locomotor signs. One usually can discontinue sedation usually after 24 to 48 hours.

One should treat oral exposure with saline cathartics and an adsorbent such as activated charcoal. If exposure was via the dermal route, the animal requires a thoroughly bathing with soap and water. As in all cases of pesticide toxicity, one should eliminate the source of contamination if possible.[15,65]

20.2.2.4 Strychnine

The present day use of strychnine is primarily as a rodenticide. Although strychnine is available to the public through many retail outlets, instances of horses becoming intoxicated by strychnine are rare.[15,65,66] The approximate oral lethal dose for horses is 0.5 mg/kg.[15]

20.2.2.4.1 Clinical Signs

The clinical manifestation of strychnine toxicosis can appear as rapidly as 10 minutes to 2 hours following ingestion. Initial signs include apprehension, nervousness, and muscle stiffness. These signs are followed by violent tetanic seizures that may appear spontaneously or may be initiated by stimuli such as sound, touch, or light. These tetanic spasms may vary from a few seconds to a minute or more and are characterized by extreme extensor muscle rigidity. Apnea frequently occurs during the seizure. Intermittent periods of relaxation occur between seizures but become less frequent as the clinical episode progresses. In lethal cases the convulsive seizures become more frequent until death eventually occurs during a seizure or from exhaustion and anoxia. The entire clinical episode may last less than 2 hours.[15,65]

Additional signs that are reported in horses include sweating, incoordination, prostration, convulsions, and death within approximately 2 hours.[65]

20.2.2.4.2 Pathophysiology

Strychnine is absorbed rapidly from the intestinal tract but not from the stomach. The alkaloid nature of 1462
the compound promotes its ionization within an acid medium, hence minimal absorption occurs from the 1463
stomach.[15] Once absorbed, strychnine is distributed readily throughout the body. Strychnine does not accumulate in any given tissue, but significant concentrations occur in blood, liver, and kidney.[15,65] Strychnine is metabolized in the liver by hepatic microsomal enzymes, and it and its metabolites are excreted in urine.[15] Excretion of strychnine is rapid, with most of a lethal dose being eliminated within 24 hours.[65]

Glycine is an inhibitory neurotransmitter in the spinal cord and medulla that serves to dampen or modulate efferent motor neuron activity. The purpose of this modulating effect is to provide smooth, coordinated muscle contraction and activity that are appropriate and consistent with the requirements for locomotion and respiration. Strychnine acts competitively to antagonize glycine by blocking its uptake at postsynaptic sites on receptors in the spinal cord and brainstem. The result of this blockade is hyperexcitation of muscle groups from lack of normal inhibition. Muscle reflex activity is allowed to proceed in basically an uncontrolled manner. All striated muscles are affected, but the more powerful extensor muscles tend to predominate and produce generalized rigidity and tonic seizures.[15,65]

20.2.2.4.3 Diagnosis and Treatment

One may make a tentative diagnosis of strychnine poisoning based on history of possible exposure, characteristic clinical signs, and a rapid recovery in animals treated in time. To confirm the diagnosis, one may submit tissue samples for evaluation. Liver, kidney, and stomach contents are the most suitable specimens for analysis, but urine and CNS tissues also are useful.[15,65]

Treatment of strychnine poisoning is symptomatic because no specific antidote is available. The horse should be kept in a quiet environment with minimal stimulation. Of primary importance is maintenance of relaxation and prevention of asphyxia. One should give pentobarbital or chloral hydrate solutions intravenously to produce sedation effectively. Complete anesthetization of the horse usually is not required or desired. Other medications have been recommended for use in dogs and should be effective in horses as well. These medications include the centrally acting muscle relaxants methocarbamol (150 mg/kg intravenously) and guaifenesin (110 mg/kg intravenously) repeated as needed; diazepam and xylazine to control seizures; and inhalation anesthetics if necessary.[15] Oxygen therapy and assisted ventilation may be necessary in some animals.

Additional agents that may prove beneficial are activated charcoal given orally, followed by a laxative to evacuate the bowel. Acidification of urine with oral ammonium chloride at 132 mg/kg also may enhance excretion of strychnine. Although toxic doses of strychnine may be depleted from the body in a short time, one may have to maintain relaxation and sedation in the horse for 24 to 48 hours.[15]

20.2.2.5 Metaldehyde

Metaldehyde is used primarily as a molluscicide in snail and slug baits in coastal and low-lying areas. The baits are generally in the form of meal or pellets and are placed around crops or ornamental plants.[15,65] Horses can become poisoned through inadvertent exposure to these baits. No specific toxicity studies could be found regarding horses, but horses are reported possibly to be more susceptible to toxicosis than dogs, in which the acute oral LD_{50} may be as little as 60 to 100 mg/kg.[65] In two separate reports, horses died following ingestion of as little as 60 mg/kg[67] and 120 mg/kg. Experimentally, a parasitized yearling colt died following exposure to 0.1 mg/kg.[68]

20.2.2.5.1 Clinical Signs

The clinical signs reported in horses include acute onset of signs within 1 hour following exposure, excessive sweating, profuse salivation and restlessness, hyperesthesia, incoordination, and tachycardia. One horse exhibited violent muscle spasms just before death.[67] Other signs include muscle fasciculations, clonic spasms, and rapid and deep respiratory movements.[68] Death occurs rapidly (3 to 5 hours) in horses exposed to a lethal amount of metaldehyde[67,68] and is thought to result from acute respiratory failure.[65] Dogs also are reported to exhibit signs of convulsions and elevated body temperature.[15,65]

20.2.2.5.2
Pathophysiology

Metaldehyde is absorbed readily from the gastrointestinal tract. Gastric hydrochloric acid enhances its decomposition to acetaldehyde, and metaldehyde and acetaldehyde are absorbed and readily cross the blood-brain barrier. The exact mechanism of action of metaldehyde is yet to be elucidated.[65]

20.2.2.5.3
Diagnosis and Treatment

A history of possible exposure to a molluscicide coupled with the appropriate clinical signs can lead to a tentative diagnosis of metaldehyde toxicity. One can analyze stomach contents for acetaldehyde, and a formaldehyde-like odor may be present in the stomach contents.[15,65]

No antidote is available for metaldehyde. Treatment is symptomatic and aimed at sedation, removing the compound from the stomach, and supportive therapy such as maintaining proper fluid, electrolyte, and acid-base indexes. Sedatives such as xylazine, acepromazine, and diazepam may be useful to control convulsive behavior. Methocarbamol may help control muscle spasms and fasciculations, and one can give mineral oil orally to aid evacuation of the gastrointestinal tract.[15,65]

20.2.2.6
Methiocarb

This molluscicide has been reported to cause toxicity in two horses: one died and the other fully recovered.[69,70] In each instance the amount ingested was estimated to be from 100 to 125 g of a 4% weight per volume preparation. 1463

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20.2.2.6.1
Clinical Signs

The onset of signs was rapid, beginning within a few minutes of ingestion. Muscle tremors, which became severe, and profuse sweating and salivation were noted. Both horses had increased heart and respiratory rates, and the surviving horse also exhibited signs of abdominal discomfort. Clinical signs gradually lessened until they became absent approximately 12 hours following onset in the surviving horse.[70] The horse fatally intoxicated died about 12 hours after initially showing signs. Postmortem findings included severe generalized pulmonary congestion with froth accumulation in the airways, and a number of large hemorrhagic areas scattered throughout the intestinal tract.[69]

20.2.2.6.2
Treatment

The specific antidote for methiocarb is atropine sulfate.[69,70] Repeated dosing may be necessary. Other suggested remedies include supportive therapy consisting of sedatives, calcium solutions, and mineral oil.[70]

20.2.2.7
4-Aminopyridine

4-Aminopyridine is used commercially as a bird repellent and often is mixed with grain before its distribution. In the one reported instance of 4-aminopyridine toxicity, affected horses were exposed to corn that contained the substance.[71]

20.2.2.7.1
Clinical Signs

The two affected horses began showing signs of profuse sweating, severe convulsions, behavioral abnormalities, and rapid fluttering of the third eyelid. Both horses died within 2 hours of the onset of clinical signs, and about 8 hours after ingesting the contaminated corn. No specific lesions were noted at necropsy. The estimated lethal dose of 4-aminopyridine for these two horses was 2 to 3 mg/kg body mass.[71]

20.2.2.7.2
Pathophysiology

The mechanism whereby 4-aminopyridine causes death has not been elucidated. However, this substance readily crosses the blood-brain barrier and may enhance the release of acetylcholine and other neurotransmitters from prejunctional nerve endings.[72,73] The result is a stimulatory effect on the CNS.

20.2.2.7.3
Diagnosis and Treatment

One can confirm a diagnosis of suspected toxicity by testing the suspect material and stomach contents of affected horses. One can use high-performance liquid chromatography to identify and quantitate 4-aminopyridine in these samples.[71] No specific antidote for 4-aminopyridine toxicity is suggested, but one can give affected horses supportive therapy and mineral oil orally to enhance evacuation of the intestinal tract. From the known mechanism of action of this compound, CNS depressants such as phenobarbital would seem to have some value in treating the CNS signs of affected horses. However, the efficacy of this treatment regimen has not been proved and cannot be recommended at this time.

20.2.2.8
Levamisole

Levamisole has not gained widespread use as an equine anthelmintic, principally because of its limited efficacy in destroying strongyles and because its toxic dose is close to its therapeutic dose. Levamisole is effective, however, in eliminating lungworms, ascarids, and adult pinworms from horses. The drug also has been used in human beings and other animal species in an attempt to enhance immune system function.

20.2.2.8.1
Clinical Signs

Clinical signs associated with levamisole toxicity occur within 1 hour of administration and include hyperexcitability, muscle tremors, hyperactivity, and excessive sweating, and lacrimation. Recumbency may follow these signs, but animals that recover generally appear normal by 12 hours after exposure.[74–76] Adverse effects are more likely following subcutaneous injection than by oral drenching.[76] The toxic dose of 20 mg/kg is close to the therapeutic dose of 15 mg/kg, and 20 mg/kg may cause death in some horses.[77]

20.2.2.8.2

Diagnosis and Treatment

One should suspect a diagnosis when horses exhibit clinical signs suggesting levamisole toxicosis and exposure is known to have occurred. No specific antidote is available, so therapy includes supportive care. Most animals recover uneventfully if a sublethal dose has been given.

20.2.2.9

Carbon Disulfide

Carbon disulfide is seldom used as an anthelmintic in present times but has enjoyed widespread use in past years for treatment of infestation caused by *Parascaris equorum* and *Gastrophilus* species.[78] Carbon disulfide is a manufactured product used as a solvent for resins, pesticides, and waxes and as an agent to remove greases. The chemical is used widely as a fumigant to control insects in stored grain.[15] Carbon disulfide is an exceptional fat solvent and in the pure state is a clear, colorless volatile liquid with a sweet, aromatic odor resembling that of decaying cabbage. The chemical is well absorbed through the skin and lungs and following ingestion.[7,15]

20.2.2.9.1

Clinical Signs

Reported signs of acute toxicity in animals include dyspnea and cyanosis, spasmodic tremors, vascular collapse, prostration, convulsions, coma, and death.[15] Signs referable to local irritation following inhalation might include salivation and coughing. A combination product of piperazine–carbon disulfide and phenothiazine caused transitory signs of overtranquilization and unsteady gait when dosed 8 oz per 45 kg of body mass.[78] Whether any of these effects were related directly to carbon disulfide was not determined.

Chronic exposure causes neuropsychiatric changes, peripheral neuropathies, and cranial nerve dysfunction in human beings,[7] but chronic exposure in horses is unlikely.

20.2.2.9.2

Pathophysiology

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Because carbon disulfide is a potent fat solvent, local skin contact results in erythema and pain, and prolonged contact produces chemical burns and vesiculation.[7] The chemical is irritating to mucous membranes when inhaled or ingested.[7,78]

At the cellular level, carbon disulfide acts to block enzymatic processes by reacting with nucleophilic compounds including pyridoxamine, monoamine oxidase in the cerebrum, and dopamine decarboxylase. Carbon disulfide binds to microsomal enzymes, thereby reducing their activity, and produces a centrilobular hepatic necrosis. In addition, carbon disulfide chelates copper and zinc and therefore can produce disturbances in tract mineral balance.[7]

20.2.2.9.3

Diagnosis and Treatment

No specific diagnostic test is available for use on animals suspected of suffering from carbon disulfide toxicosis. In one report, affected animals had increased BUN and bilirubin concentrations, increased serum

activities of AST and alanine aminotransferase, and increased serum cholesterol concentration. Affected horses also had depressed serum concentrations of protein-bound iodine and magnesium.[78]

In human beings the iodine-azide test is useful to identify carbon disulfide metabolites in urine,[7] but the efficacy of this procedure for diagnostic use in horses is unknown.

Treatment of toxicosis primarily involves removal of the source and symptomatic care.

20.2.3 MISCELLANEOUS AGENTS

20.2.3.1 Nicotine

Nicotine is an alkaloid contained in tobacco leaves. Although toxicosis might occur from ingestion of excessive amounts of tobacco leaves or cured tobacco in cigarettes or cigars, this route of toxicosis is probably rare in horses. Horses are more likely to be intoxicated by ingestion or exposure to the salt nicotine sulfate.[15,65] A concentrated solution of nicotine sulfate (Blackleaf 40) has been used to control leaf-eating insects and occasionally as a premises spray to control certain ectoparasites.[15,24] Horses may ingest this substance from spills of the solution, from contaminated containers, or from foliage that has been sprayed with the product.[65] Topical exposure may result when horses are housed in stables where this product has been used for mite control.[24] The lethal dose of nicotine in the horse is 100 to 300 mg.[15]

20.2.3.1.1 Clinical Signs

The signs of nicotine toxicity are rapid in onset, often occurring within a few minutes following exposure. Initially, the signs noted are those of cholinergic overstimulation, that is, excitement, increased respiration, and salivation. Increased peristalsis and diarrhea also can occur.[15,24] These signs are transitory and are followed rapidly by depression, muscle weakness and ataxia, slow and shallow respiration, and an increased heart rate. Convulsions also can occur. In fatal cases, these signs progress to collapse, coma, and death within minutes to hours following the onset of clinical signs.[15,24,65]

20.2.3.1.2 Pathophysiology

Nicotine is absorbed readily from the oral mucosa, respiratory tract, gastrointestinal tract (excluding the stomach), and through intact skin.[7,15] If ingested, nicotine also can exert a direct, rapid caustic action on the mucosa of the mouth and throat, esophagus, and stomach.[15] The substance is metabolized primarily in the liver, and nicotine and its metabolites are excreted in urine. In human beings, urinary acidification greatly enhances clearance of nicotine and its metabolites.[7]

Initially, and only for a short time, nicotine stimulates the autonomic nervous system ganglia, neuromuscular junctions, and some synapses in the CNS by depolarization of the postsynaptic membrane.[7,15] With toxicosis, however, the stimulation is followed rapidly by a depolarizing-type blockade of all these nicotinic cholinergic receptors.[7,15,65] Large doses result in a descending paralysis of the CNS, and death results from respiratory failure caused by paralysis of the diaphragm and chest muscles.[15,65] The effects of a sublethal dose should diminish in a few hours.[15]

20.2.3.1.3 Diagnosis and Treatment

No diagnostic lesions are present at necropsy. However, the distinct odor of nicotine may be present in stomach contents.[15,65] One can evaluate urine, blood, liver, kidney, and other tissues for nicotine content in suspect cases.[15]

Treatment often is ineffective because of the rapid course of the toxicosis. However, because no specific antidote exists for nicotine toxicity, one should treat affected horses symptomatically. If exposure has been via the skin, washing with soap and water is indicated.[15] Following oral exposure, one can treat affected horses with oral laxatives, tannic acid, or potassium permanganate in an attempt to reduce absorption of the toxin. Activated charcoal also may be beneficial in adsorbing residual nicotine in the intestinal tract.[15,24] Atropine sulfate is reported to be without value in affected horses because it does not protect vital nicotinic receptors in the respiratory muscles and in the CNS from the effects of nicotine.[65] Artificial respiration becomes the only means of maintaining life once the respiratory depression reaches a critical level. This last-ditch effort usually fails.[65]

20.2.3.2 Ammonia

Ammonia toxicosis in the horse can occur in two ways: by primary exposure to ammonia gas or by secondary metabolism of urea to ammonia within the body (see Urea and Nonprotein Nitrogen Substances).[7,15]

Primary exposure to toxic concentrations of ammonia gas is probably rare in horses, even though ammonia is the air pollutant most frequently found in high concentrations in animal facilities. The concentrations of ammonia found in stables may be irritating to horses but probably will never reach lethal concentrations. 1465
Another source of ammonia toxicosis to horses might be compressed anhydrous ammonia, which is used as 1466
an agricultural fertilizer. An accidental spill or spray of anhydrous ammonia onto a horse could have disastrous results that might be lethal.[15] Human beings can detect the odor of ammonia at a concentration of 30 ppm, eye and nasal irritation occurs near 50 ppm, and severe pulmonary dysfunction results from concentrations greater than 1000 ppm. Immediate death occurs at concentrations nearing 1500 ppm.[7]

20.2.3.2.1 Clinical Signs

Signs associated with low concentrations of aerial ammonia are those of irritation to the eyes and respiratory tract. Excessive tearing, shallow breathing, coughing, and nasal discharge are common findings. Higher concentrations can induce laryngospasm and pulmonary edema.[7] Exposure to anhydrous ammonia can result in permanent or impaired loss of eyesight, respiratory disease, and skin burns.[15]

20.2.3.2.2 Pathophysiology

Ammonia is a highly water-soluble, irritating alkaline gas that causes liquefactive necrosis at high concentrations.[7] Because ammonia is so highly water-soluble, it readily reacts with the mucous membranes of the eye and the respiratory tract.[15]

20.2.3.2.3

Diagnosis and Treatment

A diagnosis of aerial ammonia toxicosis is based primarily on history and physical examination findings. Laboratory evaluation is of little value in establishing a diagnosis of inhalation exposure,[15] but laboratory evaluation can be useful to assess the degree of damage to the respiratory tract and to evaluate the effectiveness of therapy.

Treatment involves removing the horse from the source of exposure. If exposure has been severe enough to result in ophthalmic or respiratory tract disease, one should treat these conditions accordingly.

20.2.3.3

Urea and Nonprotein Nitrogen Substances

Urea and other nonprotein nitrogen substances, including various ammonium salts, are added to ruminant rations as a source of nonprotein nitrogen because ruminants can use these compounds to provide a large percentage of their protein nitrogen requirements. Urea has additional uses as a fertilizer and as a substitute for salt in melting snow and ice in metropolitan areas.[15,38] Horses are only mildly susceptible to urea toxicosis, and for horses to ingest sufficient urea or urea-containing feedstuffs to cause clinical signs is highly unlikely.[15,24] However, horses are more susceptible to toxicosis by ingestion of ammonium salts,[15] which may occur by accidental exposure to these substances. In horses, urea is lethal when ingested at a rate of 4 g/kg of body mass, and ammonium salts are lethal at a dose of 1.5 g/kg body mass.[38] Urea and other nonprotein nitrogen formulations are toxic to animals simply because they are hydrolyzed to ammonia, which is responsible for causing the derangements associated with toxicosis. Therefore urea and nonaerosol ammonia toxicosis are considered together.

20.2.3.3.1

Clinical Signs

The spectrum and intensity of signs in ruminants with urea toxicosis vary, and the same is probably true for horses. The clinical course is usually acute and rapid, often occurring from a few minutes to a few hours following consumption.[15] Occasionally, animals are found dead, or they may die quickly following signs of weakness, dyspnea, colic, and terminal tonic convulsions. Other varied signs can be present. Behavioral abnormalities such as restlessness and dullness may be present. Excitement and even belligerency can follow these signs. Nervous signs including hyperesthesia, tremors, and muscle twitching and spasms can occur. Autonomic nervous system derangements can include salivation, bradycardia, hypertension, and severe colic. More terminal signs can include increased and labored respirations, cardiac arrhythmias, frothing at the mouth, and cyanosis. Intermittent tonic-opisthotonic seizures also can be elicited near death. The onset of signs may range from 10 minutes to 4 hours, and death may occur in a few hours to 3 to 4 days.[38]

20.2.3.3.2

Pathophysiology

Urea is hydrolyzed to ammonia by the action of the enzyme urease. This reaction is speeded by an alkaline pH, and in the horse these requirements are found in the cecum. In horses, urea is absorbed from the small intestine and excreted via the urine. The only urea that might contribute to toxicity in the horse would be that excessive amount that reaches the cecum and is available for hydrolysis.[24]

In normal animals, ammonia liberated from nonprotein nitrogen sources can be in the form of the ammonium ion. This ion is soluble, but its charge prevents it from being absorbed across membranes. Ammonia is also soluble, however, but because it lacks an ionic charge, it can be absorbed readily across membranes to enter the bloodstream.[38]

Ammonia is a normal by-product of tissue metabolism, and in the hepatocytes it is converted to urea by the urea cycle or is incorporated into glutamic acid in the synthesis of glutamine. Toxicosis occurs when the amount of ammonia absorbed into the bloodstream exceeds the ability of the horse to detoxify it.[38]

The primary mechanism of ammonia toxicosis is thought to be inhibition of the citric acid cycle, but the exact mechanism by which this occurs is not known.[38] Ammonia saturation of the glutamine-synthesizing system has been suggested to have an inhibitory effect on the citrate cycle, creating a decrease in its intermediates and a subsequent decrease in cellular energy production and respiration. As the citrate cycle fails, cells begin to malfunction. Cellular energy and respiration deficits may cause ultrastructural damage 1466
leading to degenerative changes and eventual cell death. The role of ammonia in causing signs of 1467
encephalopathy is controversial and not well understood.[79]

Laboratory abnormalities associated with ammonia toxicosis include elevated serum potassium, phosphorus, lactic acid, glucose, and concentration of the liver enzyme AST. Urine output decreases, and PCV increases as impending cardiac failure and shock ensue.[38]

The ultimate cause of death is inconsistent in urea toxicosis and poisoning by ammonium compounds. Cardiac failure may be induced by hyperkalemia, or ventricular fibrillation may result from the myocardial effects of ammonia itself. Convulsions may be prolonged and responsible for fatal anoxia. Pulmonary edema may be a complicating factor in some cases. Death also has been postulated to result from asphyxiation.[38]

20.2.3.3.3 Diagnosis and Treatment

Animals that die of ammonia toxicosis exhibit no characteristic lesions. Generalized venous stasis and congestion of organs may be present, along with pulmonary edema and scattered petechiation and ecchymoses. A strong odor of ammonia may be present, but this is probably much more characteristic of ruminants than of monogastric animals.[38]

Clinical signs and history can be helpful in establishing a diagnosis. One can perform laboratory evaluation for blood ammonia, but one must interpret the results with caution.[38] Storage of the sample, length of time between death and time of sampling, and length of time between sampling and analysis can influence the blood ammonia concentration. One should freeze tissue specimens immediately if they are to be analyzed.[15] One also can analyze suspect feeds for urea or nonprotein nitrogen content.

Treatment is often unrewarding because of the rapidity of onset. No specific antidote is available for ammonia, so therapy is mostly symptomatic. Orally administered laxatives such as mineral oil may be beneficial. One should correct any deficits in fluid volume or abnormalities in acid-base or electrolyte concentrations. One can control horses that are convulsing with pentobarbital, and one should maintain a patent airway. Assisted ventilation, if necessary, is usually futile because of the poor survival of animals affected so severely.[38]

20.3 Toxicoses Causing Signs Relating to Central Nervous System Depression

As with toxic problems associated with other primary clinical signs, it can be difficult to determine the source of central nervous depression. The principal task is to rule out the existence of primary cranial disease. Even with the availability of advanced imaging equipment, this can be difficult. Unless the clinical signs and/or history are definitive, the diagnosis of a toxic problem is often presumptive.

20.3.1 PLANTS

20.3.1.1 Black Locust *(Robinia pseudoacacia)*

No clinical reports of black locust toxicity were found in the literature. The tree has been described as being toxic to horses, however.[5,6]

The toxic principle is classified as a lectin, which is present in seeds, sap, roots, wood, leaves, and bark of the plant. Lectin has been used in cytologic research because of its ability to stimulate glycoprotein biosynthesis and cell proliferation in lymphocytes of various animal species.[80] Horses may become intoxicated by ingesting the bark. Only small amounts of lectin are reported to precipitate clinical disease.[6]

The clinical signs reported are mental depression, weakness, posterior paralysis, an irregular heart rate, pale mucous membranes, and anorexia. One also may note abdominal discomfort and diarrhea of varying degrees.[5,6]

No definitive diagnostic test is available. Treatment of suspected toxicosis is mostly symptomatic and should include removal of the source, evacuation of the intestinal tract, and maintenance of normal fluid, electrolyte, and acid-base indexes.

20.3.1.2 Bracken Fern *(Pteridium aquilinum)*

Bracken fern is found most commonly in forested areas, burns, or in abandoned fields in the northern and western United States.[6] Toxicity can occur at any time of year, but horses are more likely to consume the plant in late summer and fall when other forage is scarce. However, horses also might acquire a taste for the plant in pastures or when it is incorporated in bedding. Horses also can become intoxicated from hay contaminated with large amounts of bracken fern. The entire plant is considered to be toxic.[5]

20.3.1.2.1 Clinical Signs

Signs of toxicosis occur after the horse has been consuming the plant for 30 to 60 days. Horses also can exhibit signs even if they have not ingested bracken fern for 2 to 3 weeks.[5] The signs most frequently reported are incoordination, which may progress to severe ataxia; postural abnormalities, including arching of the back, crouching, and a base-wide stance; muscle fasciculations, which can progress to severe tremors; and bradycardia with cardiac arrhythmias early in the disease course, but tachycardia is most prevalent terminally. Terminal stages of the disease are characterized by signs of opisthotonus and clonic

convulsions.[5,6] One report also describes an affected horse showing signs of colic and acute hemolytic anemia.[81]

20.3.1.2.2 Pathophysiology

The agent in bracken fern that is toxic to horses is thiaminase.[5,6] Bracken fern also is reported to contain a heat-stabile antithiamine factor,[82] a radiomimetic factor capable of inducing bone marrow suppression, 1467
and a β-glucopyranoside which may enhance the release of endogenous histamine.[6] The significance of 1468
these last two compounds in the development of toxicity in horses is unknown. Thiamine plays an integral role in carbohydrate, fat, and protein metabolism and acts as a cofactor in enzymatic pathways responsible for energy production. Thiamine is an important cofactor in the decarboxylation of pyruvate to acetyl coenzyme A, which subsequently enters the tricarboxylic acid cycle.

Thiamine deficiency acts to interrupt these cellular energy processes and also limits certain metabolic pathways available for pyruvate metabolism, resulting in the systemic accumulation of a variety of metabolites, including pyruvate and lactate.

20.3.1.2.3 Diagnosis and Treatment

History and clinical signs are helpful in arriving at a diagnosis. No pathognomonic lesions or laboratory abnormalities occur, but expected laboratory findings include elevated blood pyruvate concentration and decreased plasma thiamine and red blood cell transketolase concentrations.[6]

One should administer thiamine to affected horses at a dose of 0.25 to 0.5 mg/kg body mass daily intravenously, subcutaneously, or intramuscularly for several days. Initially, one can give thiamine at a dose of 5 to 10 mg/kg intravenously, but one should dilute this dose in fluids and give it slowly because of the frequency of adverse reactions when thiamine is given intravenously. One usually can prevent thiamine deficiency by dietary supplementation of yeast or cereal grains.[6]

20.3.1.3 Equisetum *(Equisetum arvense)*

Equisetum, commonly called horsetail, marestail, or scouring rush, has geographic distribution similar to that of bracken fern. Like bracken fern, equisetum is unpalatable to horses, and toxicosis usually results from hay contaminated with the plant.[5,6]

Thiaminase is the toxic principle found in equisetum, and the clinical signs, pathogenesis, and treatment are virtually identical to those of bracken fern.[5,6]

20.3.1.4 Milkweed *(Asclepias* Species*)*

Several species of *Asclepias* have been reported to be toxic to large animals,[6,83] but no specific reports of horses intoxicated by this plant were found. The plants are reported to be distasteful to animals and not commonly grazed, but the plant may become incorporated into hay.

Affected animals are reported to have a weak, rapid pulse; dyspnea; loss of muscular control; and muscular spasms. Salivation, bloating, and convulsions also may occur.[6,83] Most animals that reach the convulsive stage die.[83]

Numerous compounds have been isolated from *Asclepias* species, including resinoids and cardioactive glycosides. One resinoid produces smooth muscle spasms of the gastrointestinal tract.[6] The mechanism of action of the other toxins has not been elucidated.

No specific antidote is available,[6] but supportive care, including evacuation of the gastrointestinal tract, is indicated.

20.3.1.5 White Snakeroot *(Eupatorium rugosum)* and Rayless Goldenrod *(Isocoma wrightii)*

Cases of white snakeroot intoxication have been reported primarily in the eastern half of the United States, from Michigan south to Alabama and eastward.[84] The toxic principle is tremetol, which has been described as a fat-soluble, high-molecular-weight alcohol.[43,85] Tremetol poisoning is reported to be most prevalent in dry years or in circumstances in which animals are subjected to inadequate pasturage. The toxin is excreted slowly and therefore tends to accumulate in animals grazing the plant. Because of this cumulative effect, repeated small doses can result in toxicosis as well as a single, larger exposure to the plant. A total amount of green plant varying from 1% to 10% of body weight may be lethal to horses.[84] The toxic principle remains in the dried plant after freezing.[85] In the southwestern United States, rayless goldenrod is the source of tremetol, and ingestion of this plant produces the same clinical syndrome as that of white snakeroot intoxication.

20.3.1.5.1 Clinical Signs

Depression, a stiff gait with frequent crossing of the hindlimbs, and patchy, profuse sweating seem to be the most profound signs of tremetol toxicity. Other, less frequently noted findings include muscle tremors, particularly of the shoulders and limbs; labored or shallow respirations; normal to subnormal body temperature; pupillary dilation; cardiac arrhythmias; and darkly discolored urine.[84,85]

The time of onset of signs can vary considerably from less than 2 days to as long as 3 weeks after the last exposure to the plant. Most horses showing clinical signs die. Recovery is reported to be rare and usually is prolonged and often incomplete. However, a recent report describes two horses that apparently fully recovered from suspected tremetol toxicity.[85] Death often follows the appearance of clinical signs within 1 to 3 days.[84]

Laboratory abnormalities routinely noted include hematuria, hemoglobinuria and proteinuria, mild elevations in serum alkaline phosphatase activity, elevated AST concentration, and significant elevation of serum CK activity. Acidosis, hyperglycemia, and glucosuria also have been documented.[84,85]

Postmortem findings primarily consist of mild renal tubular degeneration and necrosis; nonsuppurative colitis; pulmonary congestion; increased amounts of pleural and peritoneal fluid; and moderate to severe centrolobular vacuolar changes in the liver. Other significant findings can include pericarditis and extensive, patchy myocardial degeneration and necrosis. Extensive, minute epicardial hemorrhage also has 1468

been reported.[84,85] One reported case also exhibited moderate, multifocal degeneration of skeletal muscle.[84] 1469

20.3.1.5.2

Pathophysiology

The mechanism whereby tremetol causes the aforementioned lesions and signs remains unknown.

20.3.1.5.3

Diagnosis and Treatment

Diagnosis of tremetol toxicity is based on observation of the described clinical signs and concurrent clinicopathologic abnormalities. Additionally, evidence that the affected horses have been exposed to white snakeroot should be present, and when available, necropsy findings should be compatible with those described for tremetol toxicity. Isolation of tremetol from suspect samples has not been reported, and attempts at recovering the substance from blood, urine, liver, kidney, and stomach and cecal contents from affected horses have been unsuccessful.[84]

Treatment is symptomatic. The primary goal of therapy is promptly removing the horse from exposure to the plant and providing supportive care. One should attempt to evacuate the gastrointestinal tract of the horse with laxatives such as mineral oil. Activated charcoal also has been suggested as being beneficial in removing the toxin.[85] Based on observed histopathologic abnormalities, volume diuresis of affected animals seems appropriate, and one should attempt to maintain normal acid-base and serum electrolyte concentrations. One should provide all affected horses and herdmates with adequate and suitable forage and ample fresh water.

20.3.1.6

Yellow Star Thistle *(Centaurea solstitialis)* and Russian Knapweed *(Centaurea repens)*

Both yellow star thistle and Russian knapweed cause nigropallidal encephalomalacia in horses. These plants are found scattered over much of the western United States and are most abundant in nonirrigated pastures during the dry seasons of summer and fall. Both plants have a minimal moisture requirement and so may be the only green plants remaining in a dry season. Consequently, most poisonings occur during the summer or fall months.[86–88]

Horses apparently reject the plants when more suitable vegetation is available, for the disease does not occur in horses grazing improved pastures or grassland range.[88] However, some horses are reported to develop a craving for the plant and selectively seek it out.[86] Horses may eat the weeds, occasionally or frequently, without becoming ill, and continuous and protracted exposure to the plants is necessary for toxicosis to develop under experimental conditions. Feeding trials have shown that horses must consume an amount of weed equivalent to 59% to 200% of their body weight of yellow star thistle and 59% to 63% of their body weight of Russian knapweed for 3 to 11 weeks of continuous feeding before clinical signs develop. The plants retain their toxicity when dried and incorporated into hay. All ages may be affected, but in general, younger horses seem more prone to the disease. One study reported a median age of about 2 years in affected horses.[88] Horses appear to be the only animals that develop nigropallidal encephalomalacia when exposed to these plants.

20.3.1.6.1 Clinical Signs

The onset of signs is always sudden, beginning with variable degrees of impairment of eating and drinking. Coordinated movements of prehension, mastication, and deglutition are often lacking. Affected horses are unable to chew adequately and propel the food to the back of the mouth. Some horses may show only faulty prehension, whereas others are unable to eat at all. Most horses, however, appear to be able to swallow if feed or water gains access to the posterior pharynx. More severely affected horses may attempt to drink by immersing their muzzle deeply into the water in an attempt to force water into the posterior pharynx.[86,88–90]

Hypertonicity of the facial muscles is a characteristic sign, particularly when feed is offered.[86] The horse often holds the mouth partially opened with the lips retracted, resulting in a fixed facial expression. The tongue may protrude from the mouth, and many horses display constant chewing movements.[86,90]

Other characteristic signs include weight loss, mild to moderate depression, and yawning. Most horses can be roused readily from somnolence by mild stimulation. Few animals may show aimless, slow walking or circling early in the disease course. The gait is usually normal, but occasional deficits are apparent, including stiffness, slowness, ataxia, tetraparesis, and conscious proprioceptive deficits.[86,90] In cases of prolonged exposure, a wobbly, shuffling gait may occur because of weakness. Sensation and reflexes appear normal, and the animals are afebrile.[90]

Horses less severely affected may adopt unusual means of eating by scooping feed into the mouth. These animals may survive for months, but complete recovery has not been observed in confirmed cases of the disease. In some instances, however, residual signs may become almost undetectable.[88] Death of affected horses is from starvation and dehydration.

20.3.1.6.2 Pathophysiology

The pathogenesis of the lesions is unknown, but these plants have been postulated possibly to contain a specifically toxic substance or to lack some nutritional component necessary for the health and well-being of the horse.[89,90] Several sesquiterpene lactones and polyacetylenes have been isolated from these plants, but their significance remains undetermined.[91]

20.3.1.6.3 Diagnosis and Treatment

The antemortem diagnosis of nigropallidal encephalomalacia is based largely on observation of clinical signs and prolonged exposure of the horse to the plants by grazing or by severely contaminated hay. If available, magnetic resonance imaging can identify characteristic lesions on T1-weighted, T2-weighted, and proton density images. These lesions do not contrast enhance after gadolinium- 1469
diethylenetriaminopentaacetic acid administration.[92] Characteristic necropsy findings include bilaterally 1470
symmetric softening and necrosis in areas of the globus pallidus and substantia nigra. These areas are usually sharply defined and may be cavitary.[86,90]

No known treatment exists for affected horses. Prevention requires keeping horses from the plant and providing adequate, suitable forage.

20.3.1.7 Milk Vetch and Timber Milk Vetch (*Astragalus* species)

Most of the variants of *Astragalus miser* are referred to as timber milk vetch or milk vetch. These plants are found primarily in the western United States from northern Mexico to Canada, and are essentially a cause of toxicosis to ruminants. Horses are reported to be poisoned by this group of plants, but there are no reports of the lesions.[59]

The disease in ruminants is characterized primarily by general depression, mental dullness, incoordination, and eventual hindlimb paralysis. Respiratory distress, cyanosis, and acute collapse also may occur. Acute and chronic forms of the syndrome are reported.[59]

The toxic principle, referred to as miserotoxin, is a β-d-glycoside of 3-nitro-1-propanol, which is metabolized in the intestinal tract to the highly toxic compound 3-nitro-1-propanol. Miserotoxin is broken down into inorganic nitrite and a three-carbon side chain. The nitrite is responsible for producing methemoglobinemia in animals but is not the primary cause of death.[59]

No specific antidote is recommended. Poisoning is prevented by controlling the plants with herbicides and preventing livestock from grazing the plant.

20.3.1.8 Mushroom *(Amanita verna)*

One report describes fatal intoxication of a horse by ingestion of toadstools *(Amanita verna)*.[93] Mushrooms are apparently unpalatable to livestock species, but the described horse also suffered from meningioangiomatosis, a rare benign tumor of the meninges and brain. The authors speculate that the presence of the tumor may have resulted in abnormal mentation and altered the normal eating behavior of the horse, resulting in mushroom consumption.

20.3.1.8.1 Clinical Signs

The described horse had acute onset of depression, head pressing, ataxia, and repeated recumbency with difficulty in rising. Clinical signs of shock, including hypothermia, poor capillary perfusion, and weak pulse also were apparent. The horse was euthanized because of continued deterioration and lack of response to supportive therapy.

20.3.1.8.2 Pathophysiology

Amanita mushrooms contain a number of biologically active cyclic peptides including amatoxins, phallotoxins, phallolysin, and antaminide. Some of these peptides affect actin polymerization and cause hepatocytes to lose their cytoskeletal organization and cellular attachments, resulting in hepatocellular dissociation and necrosis. Amanitins are peptides that inhibit RNA polymerase II within the nucleus, thereby preventing DNA transcription and subsequent protein synthesis. The end result is acute submassive hepatic necrosis, which was present in the described horse.

20.3.1.8.3 Diagnosis and Treatment

Diagnosis in the described horse was based on histologic findings of submassive hepatic necrosis, acute onset of clinical signs, and presence of partially digested mushrooms in stomach contents. No specific therapy is recommended, although prompt evacuation of the gastrointestinal tract coupled with supportive care would seem prudent. The quantity of mushrooms ingested appears to be the primary prognostic indicator. If sufficient amounts of toxin are absorbed, prognosis is grave and often leads to hepatic coma in other species.

20.3.2 MEDICATIONS

20.3.2.1 Piperazine

Piperazine has been used widely as an equine anthelmintic, particularly because of its efficacy against ascarids. Numerous derivatives, principally salts of piperazine, have been developed, and these compounds have a wide margin of safety in all animals. Chemically, piperazine is a diethylenediamine that is freely soluble in water and glycerol. Piperazine is a strong base and therefore readily absorbs water and carbon dioxide.[77]

Piperazine and its salts are absorbed readily from the anterior gastrointestinal tract. Following absorption, piperazine is metabolized partially in tissues, but approximately 30% to 40% is excreted in urine. Urinary excretion is usually complete within 24 hours. Piperazine is reported to be virtually nontoxic under ordinary circumstances for adult horses and foals.[77]

Reports of toxicity in horses are rare, but the amount of compound necessary to produce clinical signs of toxicity apparently varies among the different piperazine salts. Foals treated with six times the normal dosage of piperazine adipate failed to show any ill effects. However, foals and adult horses treated with 6 times the normal dosage of piperazine citrate[94] and piperazine monohydrochloride[74] showed clinical signs of toxicity. Toxicity apparently results from the inadvertent overdosage of certain piperazine salts.

20.3.2.1.1 Clinical Signs

The clinical signs of piperazine toxicity are primarily those of depression and incoordination. Horses given excessive amounts of piperazine citrate began to show clinical signs over 12 to 48 hours following ingestion. The horses were depressed and incoordinated. Walking produced a moderate incoordination in
all legs, and when standing, affected horses would sway as if their sense of balance were affected 1470
adversely. Hyperesthesia to touch but not to sound was apparent. Other findings included dilated pupils 1471
that responded slowly to light, gross muscle tremors, and constipation. All laboratory values measured were within normal limits. These included a hemogram and serum electrolyte and muscle enzyme concentrations. Appetite initially was depressed in affected horses but returned to normal with recovery.[95]

The one horse experimentally intoxicated with piperazine monohydrochloride began showing signs of CNS depression 6 hours after treatment. By 24 hours after treatment, the horse was unsteady and reluctant to move. By 4 days after treatment, the horse stood quietly in a normal stance. Anorexia was notable during the entire posttreatment period, and the horse died on the eleventh day following treatment.[74]

20.3.2.1.2

Pathophysiology

The mechanism whereby piperazine produces its toxic effects in horses has not been elucidated. Piperazine and its derivatives have an anticholinergic action at the myoneural junction in ascarids. The resulting neuromuscular blockade produces a narcotizing or paralytic effect on the worm.[77] Whether this same mechanism is responsible for the toxic effects observed in horses remains to be proved.

20.3.2.1.3

Diagnosis and Treatment

Diagnosis of piperazine toxicity depends on observation of clinical signs and a history of inadvertent overdosage. No laboratory values appear to be altered in affected horses, but because the drug is excreted in the urine, measurement of urinary piperazine concentrations would seem valuable. One should attempt to measure piperazine concentration in the urine early in the disease course because urinary excretion is practically complete within 24 hours following ingestion.

No treatment other than supportive care is indicated. All horses intoxicated with piperazine citrate made a complete recovery within 2 to 3 days. The one horse that died following piperazine monohydrochloride administration was not treated, and necropsy abnormalities were only related to debilitation from prolonged anorexia.[74]

20.3.2.2

Reserpine

Reserpine is the most widely used and studied pure alkaloid prepared from the plant *Rauwolfia serpentina* and other *Rauwolfia* species.[7] The drug has found little application in clinical veterinary medicine but has been used in horses when a period of prolonged tranquilization lasting several days was desired.[96,97] The therapeutic and toxic dosages of reserpine are close in horses, which increases the likelihood of toxicosis even under the most judicious circumstances. An effective parenteral dose of 1 to 4 mg per 450 kg body mass has been suggested, but 5 to 10 mg per 450 kg has resulted in signs of toxicity.[97]

Reserpine is absorbed readily from the oral and intramuscular routes, and peak blood levels in human beings occur in 1 to 2 hours. The compound is distributed widely into a variety of tissues, including the brain, liver, spleen, kidneys, and adipose tissue. The alkaloid also localizes in the adrenergic neurons. Reserpine is metabolized in the liver and gastrointestinal tract, and approximately 6% of the dose is excreted in the urine within the first 24 hours. Up to 60% of a dose is excreted in the feces within 96 hours.[7]

20.3.2.2.1

Clinical signs

Signs of toxicosis in horses can occur rapidly, within 3 to 6 hours following intravenous administration.[97,98] Initial signs include marked depression, generalized profuse sweating, and flatulence. Horses may exhibit sporadic episodes of violent, coliclike behavior followed by abrupt recumbency and somnolence. Additional signs noted early in the disease course are increased gastrointestinal sounds and diarrhea, muscle trembling, sinus bradycardia, and second-degree atrioventricular blockade. Affected horses also have miosis and ptosis of the upper eyelids, and males develop paraphimosis.[98] Signs of tranquilization

persist in affected horses until norepinephrine stores are replenished.[96] In horses experimentally intoxicated with reserpine, clinical signs had abated within 60 hours of exposure.[98]

20.3.2.2.2

Pathophysiology

Reserpine is a sympatholytic agent that acts at the presynaptic nerve terminal of postganglionic adrenergic neurons to cause a depletion of norepinephrine. Reserpine impairs the magnesium- and ATP-dependent uptake mechanism whereby norepinephrine is accumulated and stored in intraneuronal vesicles within the terminal sympathetic nerve endings. As a result, norepinephrine is released from granular storage sites into the neuronal cytoplasm, where it is metabolized by cytoplasmic monamine oxidase. Reserpine interferes with catecholamine synthesis by blocking dopamine uptake into storage vesicles, where it is enzymatically converted to norepinephrine. Reserpine also crosses the blood-brain barrier, where it causes the brain neurons to become depleted of serotonin, dopamine, and norepinephrine.[7,96]

The end result is that norepinephrine is no longer available to cross the synaptic cleft and excite the postsynaptic membrane receptor. This transient deficiency of an adrenergic neurotransmitter allows a physiologic state to develop whereby profound parasympathetic tone predominates. Reserpine binding at the presynaptic neuron site is reversible. The prolonged effects result from the high lipid solubility of the drug, which allows it to persist in the body.[7,97]

20.3.2.2.3

Diagnosis and Treatment

One can perform qualitative analysis for reserpine on serum and urine samples, but testing is not easily available at most commercial laboratories. Thin-layer or high-performance liquid chromatography is used to identify the drug, which can be detected in serum up to 5 days following a 2- to 4-mg intravenous dose.[96]

No specific antidote is available.[7] Treatment of affected horses is mostly symptomatic, but pressor agents 1471
such as methamphetamine have been recommended.[97] One should use such agents only in horses that are 1472
volume-loaded, so as to maintain fluid, acid-base, and electrolyte indexes in a normal range.

20.3.2.3

Iron

Iron toxicosis in horses usually results from iatrogenic overdose of injected or oral products given to foals or from accidental consumption of iron-containing supplements. Supplemental iron is available in injectable and oral formulations, and presently more than 120 iron preparations are on the market.[15] Iron dextrin, iron polysaccharide, iron sorbitol, and ferric ammonium citrate are available injectable preparations. Oral formulations include iron salts such as ferris sulfate, citrate, or ammonium citrate; ferrous sulfate, chloride, glutamate, lactate, fumarate, or carbonate; or ferric phosphate with sodium citrate. Chelated iron compounds are about one fourth as toxic as other compounds.

The toxicity of iron is least by the oral route, with intramuscular and intravenous routes being increasingly more toxic. Because most animals do not have a mechanism for iron excretion, the toxicity of iron depends on the amount of iron already present in the body.

20.3.2.3.1

Clinical Signs

In animals, two syndromes of iron toxicosis are reported. A peracute syndrome is represented by sudden death within a few minutes to hours after injection. This syndrome may resemble an anaphylactic reaction, but the triggering mechanism is unknown. A subacute reaction characterized by progressive depression, icterus, and disorientation leading to coma and death seems to be the more typical syndrome seen in horses.[15,99,100]

20.3.2.3.2

Pathophysiology

Experimentally, 5% to 10% of oral iron is absorbed in the small intestine, primarily from the duodenum and jejunum, by a rate-limited mucosal transfer system. The ferrous forms are absorbed to a greater extent than the ferric forms, but both can be absorbed if they are in the ionized state. Phosphates reduce the absorption of iron, and a high-sugar diet increases absorption. Once absorbed, iron is bound in serum by transferrin.[15]

Toxic doses of orally administered iron overwhelm the mechanism controlling absorption of iron from the intestine, resulting in massive iron absorption. Toxicity occurs when serum iron levels exceed the iron-binding capacity of transferrin. Free circulating iron then damages blood vessels, may cause erosion and ulceration of the stomach and intestine, causes hepatocellular necrosis and fatty degeneration of the myocardium, and can produce cerebral edema.[7,15] In horses, hepatic failure appears to be the cause of death.[99]

At the cellular level, excessive iron causes extensive peroxidation of lipids in biologic membranes. A resulting decline in the ratio of unsaturated to saturated fatty acids leads to increased membrane rigidity, reduction of membrane potential, and increased permeability to various ions, leading to rupture of the membrane. The intracellular organelles adversely affected include the mitochondria and the lysosomal and sarcoplasmic membranes.[101] Elevated serum iron also inhibits the thrombin-induced conversion of fibrinogen to fibrin, thereby adversely affecting coagulation and enhancing any hemorrhagic process.[100] Histologically, affected livers are characterized by small size and have prominent bile duct proliferation, periportal fibrosis, and hepatocellular necrosis.[7]

20.3.2.3.3

Diagnosis

Measurement of serum iron concentration is the best method of confirming a diagnosis of iron toxicity.[15] In addition, a history of iron administration coupled with clinical signs and laboratory evidence of hepatocellular damage and cardiovascular collapse is highly suggestive of toxicity.

Abnormal laboratory findings include prolonged partial thromboplastin time (PTT) and prothrombin time (PT), high concentrations of aromatic amino acids (tyrosine, phenylalanine, tryptophan, methionine), elevated plasma ammonia concentration, and increased activities of the liver-derived enzymes alkaline phosphatase and GGT. In addition, a high ratio of aromatic to branched-chain amino acids and an elevated total serum bilirubin content often are found.[99]

20.3.2.3.4

Treatment

Treatment of peracute iron toxicosis is usually unrewarding. Once clinical signs become evident, major organ damage is usually present. No specific treatment for horses affected with iron toxicity is known. Treatment of affected dogs has included supportive therapy with glucose and norepinephrine, and magnesium oxide given orally to help complex the ingested iron. Experimentally, a specific chelator of ferric iron, deferoxamine (Desferal, Ciba-Geigy Corp., Summit, New Jersey) has been used at 0.75 mg/kg/min intravenously to chelate the circulating iron in dogs. One should give this drug slowly by intravenous drip because it can cause a sharp decline in blood pressure. Plasma extenders and intravenous fluids also have been used to counteract cardiovascular shock present in some dogs.[15]

In cases of human toxicosis, gastric lavage, chelating agents, and cathartics (sodium sulfate and magnesium sulfate) have been used. The use of oral bicarbonate solutions to decrease iron absorption is controversial, and activated charcoal is given orally to absorb the iron-deferoxamine complex, even though charcoal does not effectively bind free iron.[7]

The chelator of choice in human toxicosis is deferoxamine. The drug is produced by the bacteria *Streptomyces pilosis* and is a specific chelator of ferric iron. One gives deferoxamine intravenously for maximum effect; the drug has a plasma half-life of about 1 hour. Deferoxamine is detoxified by the liver, but the iron-deferoxamine complex is excreted via the kidneys.[7] 1472

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20.3.2.4

Carbon Tetrachloride

No current indication exists for carbon tetrachloride therapy in horses; therefore toxicosis results from accidental exposure to the substance. This product currently is used in the industrial manufacture of aerosol propellants, solvents, and fluorocarbon refrigerants. Carbon tetrachloride is a clear, highly volatile but nonflammable liquid that has an odor similar to that of ether and is well absorbed by the lungs and gastrointestinal tract and is concentrated in fat stores in the body.[7]

20.3.2.4.1

Clinical Signs

In animals, death following exposure to carbon tetrachloride occurs peracutely, that is, within 24 hours, as a result of anesthetic depression and severe pulmonary edema or 3 to 7 days later as a result of hepatic and renal failure. The latter syndrome is most typical of farm animals exposed to carbon tetrachloride. The peracute syndrome is characterized by immediate onset of staggering and falling, with progressive narcosis, collapse, convulsions, and death following. The more typical syndrome is characterized by anorexia, depression, weakness, jaundice, and diarrhea of several days' duration. In sheep, death usually is preceded by coma. Affected animals occasionally exhibit other signs of hepatic failure, such as photosensitization, and they are reported to be more sensitive to various environmental stressors.[102] Because carbon tetrachloride is also a potent renal tubular toxin, affected animals can exhibit signs suggestive of acute renal failure (see Toxicoses Causing Signs Relating to the Urinary System).

20.3.2.4.2

Pathophysiology

Carbon tetrachloride causes acute fatty degeneration of the liver by blocking the formation and release of low-density lipoproteins. Carbon tetrachloride also induces hepatic necrosis, but the exact mechanism whereby this occurs has not been elucidated fully. Cleavage of the carbon-chloride bond, which is thought to proceed via the microsomal cytochrome P-450 reductase and NADPH-dependent reductive pathways, produces trichloromethyl and monoatomic chlorine-free radicals. These radicals lead to lipid peroxidation, and energy processes and protein synthesis within the endoplasmic reticulum subsequently are disrupted. In addition to the lipid peroxidation, binding of these radicals to cell organelles contributes to the hepatic damage.[7]

Carbon tetrachloride also produces acute tubular necrosis primarily of the proximal tubules and the loop of Henle in the kidney.[7] Inhalation of carbon tetrachloride causes immediate, acute CNS depression and diffuse pulmonary edema.[7,102]

20.3.2.4.3

Diagnosis

One should suspect a diagnosis of carbon tetrachloride toxicity when horses show clinical signs and laboratory evidence of acute hepatorenal disease and exposure to the toxin is known to have occurred. Laboratory findings suggesting acute hepatic dysfunction include elevations in serum activities of the liver enzymes sorbitol dehydrogenase, AST, and isoenzyme 5 of LDH.[103] Increases in plasma bile acid concentration and plasma bilirubin also are apparent.[104]

Laboratory findings suggesting renal tubular damage include elevated BUN and creatinine concentrations, along with abnormal urine findings of proteinuria, casts, and occasionally blood cells. Affected horses may be expected to lose their urine concentrating ability, and urinary activity of renal tubular enzymes such as GGT and LDH should be increased early in the course. As a result of renal dysfunction, other homeostatic mechanisms are disturbed, which can lead to changes in fluid balance, acid-base disturbances, and serum electrolyte abnormalities.

20.3.2.4.4

Treatment

No specific antidote is available for carbon tetrachloride toxicity. One should initiate supportive therapy for acute hepatic and renal dysfunction (see Chapter 14 and Chapter 17.4)

20.3.2.5

Propylene Glycol

Propylene glycol is used commercially as a diluent for injectable drugs and as a glucose precursor in the treatment of hypoglycemia in ruminants. Horses may become intoxicated by the inadvertent use of propylene glycol if it is mistaken for a similar appearing liquid paraffin preparation. Reports of toxicosis in horses are rare.[105,106]

Propylene glycol has a low oral toxicity in human beings. Approximately 45% of the absorbed dose is excreted unchanged via the kidney, whereas the remainder is metabolized by hepatic alcohol dehydrogenase to acetate, pyruvate, and lactate.[7]

20.3.2.5.1

Clinical Signs

Adverse signs occur within 10 to 30 minutes following a toxic dose of propylene glycol. Initial findings include salivation and profuse sweating, ataxia, depression, and tachypnea. Additional signs can include cyanosis, seizures, and coma.[105] Diarrhea also has been reported in one horse experimentally given a large dose of propylene glycol.[106] Death has occurred 1 to 3 days following ingestion of excessive amounts of the product.[105,106]

20.3.2.5.2

Pathophysiology

Propylene glycol is metabolized by hepatic alcohol dehydrogenase to form acetate, lactate, and pyruvate. Following a large exposure, excessive amounts of these products accumulate, resulting in severe systemic lactic acidosis.[7] The clinical signs and toxicologic findings result from the effects of this severe acidemia on various body organs and tissues.

The acute oral LD_{50} for horses has not been established, but the doses for rats, rabbits, and dogs are 32, 18, and 9 ml/kg, respectively. One 450-kg horse died following intubation with 3.8 L (7.6 ml/kg) of propylene glycol,[105] but other horses have survived this dose. Toxic signs were reported in horses receiving 1.9 to 7.6 L of propylene glycol, but the only death occurred in the horse getting 7.6 L.[106] 1473

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20.3.2.5.3

Diagnosis and Treatment

One can confirm exposure to propylene glycol by chemical analysis of serum and tissues and by use of gas chromatography with flame ionization. Necropsy findings associated with propylene glycol toxicosis may be minimal.[105] The horse given 7.6 L exhibited sloughing of the gastric mucosa, diffuse enterocolitis, renal congestion, and brain edema.[106] Histopathologic findings typically include hepatic necrosis, renal tubular necrosis and infarcts, myocardial perivascular edema, and pulmonary edema.[105,106]

Because no specific antidote is available, treatment of propylene glycol toxicosis aims at alleviating the severe acidemia that develops and providing supportive care for the other organs and tissues that may become compromised. One should give sodium bicarbonate solutions to treat the acidosis. Where possible, one should monitor blood pH and adjust bicarbonate administration according to need. One should give fluids intravenously to aid diuresis and to maintain normal fluid volume. One should evaluate pulmonary and renal function thoroughly in affected horses and should take precautions to prevent further pulmonary edema from developing. Such precautions may require the use of diuretics and careful monitoring of the fluid administration rate.

One should maintain serum electrolyte concentrations in normal ranges, and oxygen therapy may be beneficial in horses exhibiting tachypnea and cyanosis. Activated charcoal has been recommended in treating human toxicosis.[7]

20.3.3 MISCELLANEOUS AGENTS

20.3.3.1 Triclopyr

Triclopyr is a herbicide used to control hardwood species on road rights-of-way, industrial sites, and forest planting sites. Horses may become exposed to the herbicide by grazing areas that have been treated previously with the product. Spontaneously occurring instances of triclopyr toxicity were not found in the literature.

An experimental study has been conducted to determine the toxic level of triclopyr to ponies. Ponies given 60 mg/kg/day for 4 days did not show any clinical sign of illness. Ponies given 300 mg/kg/day for 4 days did develop clinical signs. This study indicated that the toxic dosage in ponies was 5 times the estimated maximal intake for the highest recommended usage rate as a herbicide.[107] Therefore poisoning from the proper use of this herbicide is unlikely.

20.3.3.1.1 Clinical Signs

Initial signs of depression and decreased gastrointestinal motility were first noticed on the fourth day of the trial. Additional signs that developed were ataxia, weakness, muscle tremors, increased respiratory rate, cyanotic mucous membranes, and normal to slightly elevated body temperature. Some ponies became recumbent as clinical signs progressed. Two ponies died on the fifth and sixth days of the trial, and two other ponies were euthanized on the fifth day. The remaining two ponies were only mildly affected and recovered.

No significant changes were apparent in clinical chemistry values. Gross necropsy lesions consisted of pale livers and pale swollen kidneys, and a few horses had excessive intestinal fluid contents. Microscopic changes were mild and were those of nonspecific hepatosis and nephrosis.[107]

20.3.3.1.2 Pathophysiology

The mechanism whereby triclopyr produces clinical disease in horses has not been elucidated.

20.3.3.1.3 Diagnosis and Treatment

Toxicosis caused by triclopyr appears highly unlikely under natural circumstances.[107,108] Diagnosis of suspect cases is based on clinical signs and a history of exposure to the herbicide. No specific antidote is available. Affected individuals require supportive and symptomatic care.

20.3.3.2 Aflatoxin

Aflatoxicosis is apparently a rarely documented disorder of horses, as evidenced by the paucity of clinical cases reported in the literature.[109–111] In reported instances, toxicosis developed in horses being fed contaminated feedstuffs, but horses also have been affected experimentally by forced feeding of aflatoxin-contaminated material.[112,113]

Aflatoxins are toxic metabolites produced by the fungi *Aspergillus flavus* and *A. parasiticus*. These molds are ubiquitous in nature and normally can be found in stored feeds. The molds are not inherently toxigenic, but under environmental conditions of adequate temperature and humidity, the molds can grow rapidly and produce large amounts of aflatoxin. Many feedstuffs can support the growth of these molds, but cereal grains, cottonseed meal and cake, and peanuts seem to be affected most commonly.[38]

These molds produce five major aflatoxins: B1 and B2, which fluoresce blue under long-wave ultraviolet light; G1 and G2, which fluoresce green; and M1 aflatoxin, which is present in milk. Of these, B1 is the most important because of its toxicity and because it occurs most abundantly under natural conditions.[38]

Aflatoxins are a group of polycyclic, unsaturated compounds that have a coumarin nucleus coupled to a reactive bifuran system and a pentenone or lactone. These toxins are insoluble in water and are heat resistant; they are absorbed rapidly from the gastrointestinal tract and bound to serum albumin. Most of the toxins are removed from the bloodstream in the liver, where aflatoxins bind to macromolecules such as DNA, endoplasmic steroid-binding sites, and certain enzymes within the hepatocytes. In the liver a variety of metabolites are produced at rates that vary among species. The metabolites may be lipid-soluble or water- 1474
soluble conjugates and are excreted in bile. At least some of the metabolites undergo an enterohepatic cycle 1475
of absorption and excretion. The aflatoxins and their metabolites are excreted in urine and feces, and complete elimination may take several days. The aflatoxins are not known to be stored in any particular tissue.[38]

Acute and chronic aflatoxicoses are reported in a variety of animal species, including human beings. However, clinical reports of equine disease are concerned primarily with acute intoxication. The acute oral LD_{50} of aflatoxin B1 in horses is reported to be greater than 2 mg/kg and for foals is 2.0 mg/kg. One author reported signs of toxic hepatopathy and gastrointestinal upset in horses that consumed feed containing 2 to 50 ppb of aflatoxin B1. Toxicity of aflatoxins also is reported to be enhanced by riboflavin, exposure to light, and a diet low in protein, choline, and vitamin B_{12}.[38]

20.3.3.2.1 Clinical Signs

Clinical signs associated with acute aflatoxicosis include anorexia, elevated temperature, increased heart and respiratory rates, ataxia, depression, lethargy, convulsions, icterus, colic and abdominal straining, bloody feces, and death. These signs were exhibited by horses experimentally intoxicated with aflatoxin at dosages of 2 to 5 mg/kg. Onset of signs began as early as 4 hours after dosing, and deaths occurred from 68 hours to 32 days after intoxication.[110,112,113] An additional sign noted in a naturally occurring instance of toxicosis was subcutaneous hemorrhages.[110]

The feed concentration of aflatoxin B1 necessary to cause signs of chronic aflatoxicosis in horses has not been established, and reports of chronic aflatoxicosis were not found in the literature. Signs of chronic aflatoxicosis in other species have included reduced feed efficiency, rough hair coats, anemia, anorexia, depression, mild jaundice, and occasionally abortion.[38] One should note that experimental trials have indicated that some horses refuse mold-contaminated grain and that well-fleshed animals may be at lesser risk of developing aflatoxicosis than unthrifty animals.[110,112]

20.3.3.2.2 Pathophysiology

The hepatic cytotoxicity of aflatoxins is thought to be related to their binding to intracellular macromolecules. Aflatoxin B1 binds to nuclear DNA to inhibit RNA synthesis, which subsequently inhibits synthesis of intracellular enzymes and other proteins. Aflatoxin also binds to endoplasmic steroidal ribosome-binding sites, resulting in ribosomal disaggregation. Metabolites of aflatoxin B1 also can bind to cellular macromolecules, and most of the cytotoxicity of aflatoxin B1 appears result from binding of certain of these metabolites rather than from aflatoxin B1 itself. This impairment of protein synthesis and the related ability to mobilize fats is thought to cause the early lesions of hepatic necrosis and fatty degeneration of the liver in aflatoxicosis.[15]

The possibility of other mechanisms being responsible for the other signs and lesions noted with aflatoxicosis has been suggested. Aflatoxins are known to be carcinogenic, they can be immunosuppressive, and they also can inhibit synthesis of clotting proteins. The mechanisms whereby these changes take place have not been well described.[38]

20.3.3.2.3 Diagnosis and Treatment

Definitive diagnosis of aflatoxicosis can be difficult because of the nonspecificity of many clinical signs and because the disease can mimic many other conditions. Experimentally intoxicated horses have elevations in serum concentrations of AST, alanine aminotransferase, GGT, iditol dehydrogenase, and arginase.[110,112,113] Other laboratory abnormalities have included hypoglycemia, hyperlipidemia, lymphopenia, and elevated PT.[109,110,113]

Gross necropsy lesions typically consist of variable degrees of hepatic degeneration and necrosis, visceral petechial hemorrhages, and hemorrhagic enteritis.[109,110,113] Other lesions noted at necropsy include encephalomalacia of the cerebral hemispheres, myocardial degeneration, fatty infiltration of the kidney, and subcutaneous and intramuscular hemorrhage.[109,110] Histopathologic abnormalities may include fatty degeneration and necrosis of hepatocytes, bile duct hyperplasia, periportal fibrosis, and inflammatory cell infiltration into the liver. Renal lesions have included lipid accumulation in the tubular epithelial cells of the proximal tubules and protein precipitation in the tubular lumen.[109,113]

Although metabolites of *Aspergillus* species fluoresce under ultraviolet light, the presence of fluorescence in suspect feed samples is not pathognomonic for aflatoxin. One can assay aflatoxin definitively in feed samples by a minicolumn technique using thin-layer chromatography.[110] One also can quantitate aflatoxin in animal tissues.[109,113] Mold culture is nondiagnostic.[38]

Treatment is largely symptomatic and must include removal of the offending feed material, if it still is being fed. An easily digested, low-fat diet containing appropriate protein has been recommended. Multiple vitamin supplementation might be beneficial, and one should initiate treatment of specific organ dysfunction. In acutely intoxicated horses, charcoal administered orally has been recommended.[38] Prevention of the condition requires storing feeds in a suitable environment that discourages mold growth and the feeding of noncontaminated feedstuffs.

20.3.3.3

Leukoencephalomalacia

Equine leukoencephalomalacia, a sporadically occurring disease of horses, ponies, donkeys, and mules, has a worldwide distribution. The disease is usually seasonal, with most cases occurring from late fall through early spring, and most outbreaks have been associated with a dry growing period followed by a wet period. [114,115]

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This malady is caused by the mycotoxin fumonisin B1, a metabolite of *Fusarium moniliforme*. Equidae become affected usually by ingesting *F. moniliforme*– infected corn, but the problem also has been associated with the consumption of commercially prepared diets.[114–117] Infected kernels often have a pink to reddish-brown discoloration, and damaged kernels and cob parts have a much greater concentration of fumonisin B1 than do undamaged kernels.[115,117] Feeds containing less than 10 ppm fumonisin B1 have not been associated with disease, but concentrations of greater than 10 ppm can be lethal to horses.[118]

20.3.3.3.1

Clinical Signs

Two clinical syndromes are associated with fumonisin B1 intoxication. The more common is the classic neurotoxic syndrome, but hepatotoxicosis also occurs in some horses. Older animals may be more susceptible than younger animals, and clinical signs become evident approximately 3 to 4 weeks following daily ingestion of contaminated feed. Onset of signs is typically abrupt and death usually occurs within 2 to 3 days.[114,115] Occasionally, horses are found dead with no premonitory signs.[114]

The neurologic syndrome is characterized initially by incoordination, aimless walking, intermittent anorexia, lethargy, depression, blindness, and head pressing. These signs may be followed by hyperexcitability, belligerence, extreme agitation, profuse sweating, and delirium.[114,115] Recumbency and clonic-tetanic convulsions may occur before death. Recovery from acute episodes has been reported, but some horses retain neurologic deficits.[115]

Clinical signs associated with the hepatotoxic syndrome are swelling of the lips and nose, somnolence, severe icterus and petechiae of mucous membranes, abdominal breathing, and cyanosis. Affected horses also had acute onset of clinical signs with death occurring within a few hours to days.[114,115]

20.3.3.3.2

Pathophysiology

The gross lesions typical of equine leukoencephalomalacia include liquefactive necrosis and degeneration of the cerebral hemispheres, but degenerative changes also can occur in the brainstem, cerebellum, and spinal cord.[114–117] Necrotic areas can vary in size, and regions adjacent to the necrosis are often edematous and rarefied.[115,116]

Gross hepatic lesions in affected horses generally are not pronounced. The liver may be slightly swollen, have a yellowish-brown discoloration, and contain irregular foci or nodules scattered throughout the parenchyma. Histologic abnormalities noted in the liver may include centrilobular necrosis and fibrosis, fatty infiltration of hepatocytes, portal fibrosis, biliary stasis, and bile duct proliferation.[114–116]

The mechanism whereby these changes occur has not been elucidated. The following have been suggested: the brain lesions may be induced by smaller quantities of infected corn ingested during a long period, and ingestion of higher quantities may produce fatal hepatotoxicosis in a shorter time.[115,119]

Several metabolites of *F. moniliforme* have been identified in feeds associated with outbreaks of equine leukoencephalomalacia. These include fusarin C, moniliformin, fusaric acid, 2-methoxy-4-ethylphenol, fumonisin B1, and fumonisin B2.[116,117] Toxicity information about fumonisin B2 is unknown, but moniliformin, fusaric acid, and 2-methoxy-4-ethylphenol do not produce leukoencephalomalacia when injected intravenously into donkeys. Their role in the pathogenesis of the liver lesions in equine leukoencephalomalacia is likewise unknown. However, the neurologic and hepatotoxic syndromes can be produced by oral and intravenous administration of fumonisin B1.[116]

20.3.3.3.3 Diagnosis and Treatment

The diagnosis of equine leukoencephalomalacia has been based mostly on observation of clinical signs coupled with a history of exposure to moldy corn. Typical postmortem lesions, when available, confirm the diagnosis.

Clinicopathologic abnormalities are nonspecific and usually indicate some degree of liver dysfunction. Increased serum concentrations of bilirubin, AST, GGT, and LDH have been reported.[114,116,119] Cerebrospinal fluid abnormalities can include increased protein and total nucleated cell counts and an increased concentration of myelin basic protein.[115,119]

With the recent identification of fumonisin B1 as the causative agent, however, analytic methods have been developed to assay this toxic metabolite in feed material. One can analyze suspect feed samples for fumonisin B1 by thin-layer chromatography, high-performance liquid chromatography, or gas chromatography/mass spectroscopy. Feed containing greater than 10 ppm fumonisin B1 is not safe to feed to horses.[118] Because the disease requires a fairly prolonged exposure to infected corn, one should submit an appropriate feed sample for analysis. Feed currently being ingested may not be contaminated with the mold.

Treatment of equine leukoencephalomalacia is largely supportive because no specific antidote is available for fumonisin B1. One should sedate horses that are hyperexcitable to minimize injury to themselves and their handlers. One should initiate supportive therapy for hepatic dysfunction if liver damage is evident, and some horses may require forced feeding and watering if they become unable to eat and drink. One may give mannitol or DMSO to aid resolution of cerebral edema, and one may give laxatives and activated charcoal to eliminate toxins already in the digestive tract, but their usefulness is probably minimal because this disease is not an acute intoxication. Contaminated feed should be removed immediately from all exposed horses, and pastured horses should be moved to pastures without access to corn. Prevention aims 1476
to provide suitable feed material to horses and to store grains, particularly corn, under conditions that 1477
discourage mold growth.

20.3.3.4 Trichothecenes

The trichothecenes are a group of compounds elaborated primarily by *F. tricinctum* and other *Fusarium* species. Only four of approximately 40 trichothecene derivatives have been found to occur naturally in

feedstuffs. These four include T-2 toxin, deoxynivalenol, diacetoxyscirpenol, and nivalenol.[120,121] Of these, only one reported episode of T-2 intoxication in horses was found in the literature.[121]

In the reported outbreak, horses showed clinical signs and laboratory abnormalities similar to those of equine leukoencephalomalacia caused by fumonisin B1. Gross lesions in necropsied horses were also similar to those of equine leukoencephalomalacia. *F. tricinctum* was isolated from all suspect feed samples, and T-2 toxin was detected in varying concentrations in all examined feed samples. Other *Fusarium* metabolites detected in this outbreak included HT-2, verrucarin A and J and roridin A. The toxicity of these metabolites in farm animals is not yet determined.[121]

20.3.3.5

Lead

Lead is reported to be one of the more common toxicants found in veterinary practice. Materials that can serve as a source of lead toxicity to animals include lead-based paints, putty and caulking materials, used crankcase oil, greases, linoleum, leaded gasoline, solid lead solder, roofing materials, asphalt, and industrial effluents contaminating streams or forage. Discarded automobile batteries and water from lead plumbing also might serve as a source of toxicity.[65,86]

Acute and chronic forms of toxicosis can occur depending on the amount of lead ingested and the time frame in which ingestion occurs. Lead toxicosis in horses is usually chronic and is associated with some type of forage contamination.[122,123] Foliage near lead smelters often contains excessive lead, and grasses located near busy highways have been reported to contain high lead concentrations.[86,123] Horses appear to be much more sensitive than cattle to prolonged, low-dose exposure to lead, yet they are much less sensitive than cattle to short-term exposure of large doses.[65,122] The acute oral lethal dose of lead acetate in horses is 500 to 750 g total dose, but chronic toxicosis can arise when 1 to 7 mg/kg/day is ingested over a period of days, weeks, or months.[65]

Numerous factors can influence the toxicity of lead in horses. Young animals and malnourished animals are reported to be more susceptible than older animals; solid lead is not as toxic as the more soluble salts, which are absorbed more readily; and concurrent exposure to lead and cadmium results in increased severity of clinical signs of lead poisoning. In addition, lead may interact with other minerals to affect toxicity. High levels of dietary calcium cause decreased gastrointestinal absorption of lead.[65,86,123]

20.3.3.5.1

Clinical Signs

The clinical signs of lead toxicity in horses are caused primarily by peripheral nerve dysfunction. The motor nerves are at greater risk, with minimal sensory perception loss in affected horses. Initially, affected horses may appear weak or have slight incoordination. Depression and weight loss become apparent and worsen over the disease course. Laryngeal and pharyngeal paralysis, dysphagia, dysphonia, and proprioceptive deficits occur. As the disease progresses, horses may exhibit flaccidity of the rectal sphincter, paresis of the lower lip, and difficulty in prehension, mastication, and deglutition. Aspiration pneumonia resulting from dysphagia and regurgitation of food is common, and fine muscle tremors may occur intermittently. Terminally, horses may show severe incoordination, anorexia, emaciation, and almost complete pharyngeal and esophageal paralysis with inability to swallow food or water. Seizures also may occur terminally. Colic and diarrhea may be apparent, but they are not common signs.[86,122,123] Progression of these disease signs may require weeks.

Dietary lead crosses the placental barrier, and mares chronically exposed to lead in late pregnancy may deliver premature or small, weak foals. These foals are at greater risk of developing secondary disease complications.[122]

20.3.3.5.2 Pathophysiology

Lead enters the body primarily through ingestion. Inorganic lead cannot readily penetrate the skin, but organic forms such as tetraethyl lead and tetramethyl lead are absorbed through the skin. However, exposure of horses to organic lead compounds would seem to be a rare occurrence. Metallic lead shot or bullets lodged in tissues do not dissolve because tissue pH is too high.[65]

Metallic lead and lead sulfide are less absorbed than the acetate, carbonate, hydroxide, oxide, and phosphate salts. Only 1% to 2% of ingested lead is absorbed, but if sufficient quantities of soluble salts are ingested, a significant amount of lead can cross into blood. Even though intestinal absorption is inefficient, increases in blood lead concentrations occur within 3 hours of dosing.[65,86]

Once absorbed, a large portion of lead is carried on erythrocyte membranes where it is bound irreversibly to erythrocyte proteins. Much of the remaining lead becomes bound to albumin, and only a small proportion of the absorbed lead is actually free in serum. Unbound lead is in equilibrium with lead bound to erythrocytes and albumin, and distribution to various tissues takes place from the unbound fraction.

Much of the blood lead is removed in the liver. Within the liver, cellular trapping of lead is thought to occur by lead binding to cytoplasmic proteins called metallothioneins. Lead also accumulates within the 1477
renal cortex, where it becomes trapped as intranuclear lead proteinate inclusions in tubular epithelial cells. 1478
Unbound lead is excreted into milk, and it readily passes membrane barriers such as the placenta and the blood-brain barrier to become distributed in many body tissues.[65]

Unbound lead becomes immobilized and bound to bone substance, particularly in the physeal region, by an unknown mechanism. However, bone is considered to be the “sink” for lead and eventually may contain greater than 90% of the total body burden of lead. Deposition of lead into bone is a slow, gradual process, entailing redistribution of lead from other soft tissues. In this manner, bone serves as a detoxification mechanism under conditions of chronic exposure to small concentrations. Bone cannot hold an infinite amount of lead, however, and when saturation occurs, signs of toxicosis may appear suddenly because of rising blood and soft tissue concentrations following continued exposure.[65]

Bile and feces are thought to be the primary pathways of excretion of lead, and feces may contain unabsorbed lead and lead that has undergone enterohepatic circulation. Gastrointestinal secretions, including pancreatic secretions, also might be involved in elimination of lead from the body.[65,86]

The mechanism of action of lead at the cellular level is still under scrutiny. The known toxic effects of lead include inhibition of sulfhydryl groups of enzymes essential to cellular metabolism and inhibition of heme synthesis. Lead also is known to cause a decrease in local concentrations of the essential trace metals copper, iron, and zinc. These metals have important functions in mitochondrial enzymes, and interference by lead of these may affect cellular respiration, oxidative phosphorylation, and the ATP synthetase complex adversely.[65]

The peripheral neuropathy associated with lead toxicosis in horses is thought to be caused by peripheral nerve segmental demyelination, which impedes nerve impulse conduction and contributes to the clinical signs observed. The metabolic inhibitory effects of lead are speculated to cause the demyelination.[65,122]

Lead is known to damage the blood-brain barrier and capillary endothelial cells, resulting in cerebral edema and hemorrhage. Additionally, the damaged blood-brain barrier may allow cytotoxic solutes normally excluded from the brain to enter the brain substance.[65] Whether these mechanisms have any appreciable effect on the development of clinical signs observed in the horse is unknown.

Inhibition of heme synthesis is an important aspect of lead toxicosis in several animal species. This pathologic mechanism also occurs in the horse, but its significance to the overall disease course is limited. The result of heme metabolism interference and altered function of other erythrocyte proteins is a shortened erythrocyte half-life that can produce a normochromic, normocytic anemia, which is generally marginal in affected horses (PCV of 25% to 30%).[122,123] The anemia also may be accompanied by nucleated red blood cells and Howell-Jolly bodies in peripheral blood.[123]

Two enzymes in the heme synthesis pathway that are particularly susceptible to lead are δ-aminolevulinic acid dehydratase (ALA dehydratase) and ferrochelatase. Inhibition of ALA dehydratase results in reduced levels of porphobilinogen in erythrocytes and an accumulation of ALA dehydratase, which is excreted in urine. Interference with ALA dehydratase also may be partly responsible for brain damage associated with lead toxicity in some species. Inhibition of ferrochelatase limits the formation of heme from protoporphyrin, resulting in an accumulation of unmetabolized porphyrins. These include protoporphyrin I, which is retained in the erythrocyte; uroporphyrins, which are excreted in urine; and coproporphyrins, which are excreted in feces. Lead also interferes with pyrimidine-specific 5′-nucleotidase activity, resulting in basophilic stippling of affected erythrocytes.[86]

Another important implication of lead toxicity is the suggestion that lead may be immunosuppressive via interference with cell-mediated immune responses.[65,86] The mechanism of this effect is unknown, and its significance to equine lead toxicosis in unclear.

20.3.3.5.3 Diagnosis and Treatment

Confirmation of suspected lead poisoning often is based on determination of blood or tissue lead concentrations. Blood levels of 0.35 ppm or greater are diagnostic if horses are showing clinical signs, but blood lead concentrations do not reflect the severity of poisoning.[122,123] Lead concentrations greater than 4 ppm in liver, 5 ppm in kidney, and 30 ppm in bone are considered diagnostic of lead intoxication in horses.[65]

In instances of chronic toxicity, however, blood lead values may be within a normal range. In such instances, diagnosis of toxicity may be aided by administration of calcium disodium EDTA, which chelates lead in bone stores and increases the lead concentration in plasma. The soluble lead complexes then are excreted in urine, with a resultant manyfold increase in urinary lead concentration within a few hours of EDTA administration.[65,86,121] The recommended dose of calcium disodium EDTA is 75 mg/kg intravenously.[86]

Clinicopathologic aberrations include increased concentrations of erythrocyte aminolevulinic acid and erythrocyte porphyrins and decreased activity of erythrocyte ALA dehydratase. Increased amounts of coproporphyrins, uroporphyrins, and aminolevulinic acid are found in urine, but measurement of erythrocytic ALA dehydratase is considered more diagnostic than measuring urinary ALA dehydratase content.[65,86,122] Increased blood concentration of zinc protoporphyrin also has been documented in an affected horse.[124] 1478

1479

Hematologic abnormalities in affected horses can include a marginal anemia frequently accompanied by metarubricytes and Howell-Jolly bodies in peripheral blood.[86,123] Anisocytosis, poikilocytosis, hypochromasia, polychromasia, and basophilic stippling also can occur. These changes are suggestive but not pathognomonic of lead toxicity in horses.[86] One also can measure the concentration of lead in soil and in forages. Poisoning in horses has been reported when grazed forage contained greater than 300 ppm lead.[86,122]

Treatment of affected horses should include immediate elimination of the lead source, if possible, and prompt initiation of chelation therapy. Calcium disodium EDTA is the chelator of choice; it chelates osseous lead but not tissue-bound lead. The chelated lead then becomes soluble and is excreted by the kidney. The unsaturated bone stores then reequilibrate with the lead in soft tissues. One can give calcium disodium EDTA by slow intravenous infusion at a dosage of 75 mg/kg body mass daily for 3 to 5 days. One may follow a 2-day nontreatment period to allow reequilibration of soft tissue and bone lead by an additional 5-day treatment regimen if needed. An alternative dosage schedule is 110 mg/kg intravenously twice daily for 2 days. Following a 2-day nontreatment period, one may repeat this same regimen. One should base the decision to continue therapy with EDTA on posttreatment blood lead concentrations and renal function tests.[86]

Additional therapy should include fluid and nutritional support. Although thiamine administration has been advocated along with chelation therapy in ruminant lead toxicosis,[86] its efficacy in treating horses with lead poisoning has not been investigated. Dietary calcium supplementation may have some beneficial effect by helping to reduce further gastrointestinal absorption of lead.

Methods aimed at reducing exposure to lead and preventing intoxication include appropriate cutting and disposal of contaminated forage, tilling or burning the stubble, and addition of lime to the soil.[122] Use of alfalfa as a roughage might also be beneficial, possibly because of the high calcium content in alfalfa because intoxication is more difficult to produce experimentally in horses when alfalfa is being fed and because increased dietary calcium decreases the gastrointestinal absorption of lead.[86,122]

20.4 Toxicoses Causing Signs Relating to the Cardiovascular System

Toxicoses affecting the heart are relatively uncommon. Far more frequent are manifestations associated with hemolysis and anemia or the development of abnormalities.

20.4.1 PLANTS

20.4.1.1 Red Maple *(Acer rubrum)*

Intoxication of the horse by leaves of the red maple tree is a seasonal disorder that occurs during the summer and fall months, primarily in the eastern United States.[125,126] Fresh leaves present no problem to the horse, but wilted or dried leaves are toxic, and overnight freezing and storage of dried leaves for 30 days does not alter their toxicity.[127] Experimentally, dried leaves are toxic when administered at a dose of 1.5 mg/kg body mass.[126,127] The toxin present in red maple leaves is unknown, but the clinical syndrome produced is one of acute hemolytic anemia with methemoglobinemia and Heinz body production.[125–128] Red maple toxicity has been recognized in horses[128] and Grevy's zebras,[129] and horses electively ingest the leaves when other suitable forage is available.

20.4.1.1.1 Clinical Signs

Signs of toxicity generally commence within 48 hours of ingestion. Affected horses show acute onset of lethargy, anorexia, weakness, and depression. Increased heart and respiratory rates are typical, and the animals are afebrile. Two outstanding characteristics of most affected horses are the obvious presence of icterus or brown discoloration of mucous membranes, and a brownish discoloration of blood and urine. Many horses may appear cyanotic, and petechiation of mucous membranes has been reported. Signs of secondary acute renal failure also have been documented.[128] Death, when it occurs, generally happens 3 to 7 days following ingestion.[125–128] The mortality in naturally occurring and experimental cases is approximately 60%.[130]

The aforementioned signs are representative of naturally occurring instances of toxicity. In an experimental study, however, two patterns of toxicity were recognized. One group of ponies given dried leaves accumulated before September 15 exhibited signs of the typical hemolytic syndrome and died 3 to 5 days later. Ponies given leaves collected after September 15 died within 18 hours of dosing and exhibited clinical signs only of cyanosis and depression.[127] The reason(s) for this disparity of signs was not offered.

20.4.1.1.2 Pathophysiology

Although the toxin in wilted or dried red maple leaves has not been identified, it produces an acute hemolytic anemia with methemoglobinemia and Heinz body production in affected horses. The mechanism of erythrocyte damage has not been determined, but these hematologic abnormalities are characteristic of an oxidant.[126–128]

Heinz bodies are intracellular precipitates of oxidized hemoglobin that result from oxidant injury to erythrocytes. They damage the erythrocyte membrane and produce intravascular and extravascular hemolysis. Intravascular hemolysis results when erythrocyte membrane functions involving active and 1479
passive ion transport become impaired. The hyperpermeability changes that then occur alter the osmotic 1480
gradient of the erythrocyte and cause rupture of the affected red blood cell. Extravascular hemolysis occurs when the red blood cell remains intact, but the damaged erythrocyte is removed from circulation by cells of the reticuloendothelial system.[125,126,128]

Methemoglobin is formed when hemoglobin is oxidized from the ferrous to the ferric form of iron. A certain amount of direct oxidation of hemoglobin to methemoglobin occurs naturally, but erythrocytes are able to reduce methemoglobin back to hemoglobin. Excessive production of methemoglobin occurs under conditions of excessive oxidative stress or when methemoglobin reduction is impaired.[125,127] Although methemoglobin itself does not produce hemolysis, it is incapable of transporting oxygen and therefore contributes to hypoxia. When present in sufficient quantities, methemoglobin imparts a brown discoloration to peripheral blood and mucous membranes.

20.4.1.1.3 Diagnosis and Treatment

Red maple leaf toxicosis occurs under rather specific conditions but is characterized by an acute onset of hemolytic anemia with methemoglobinemia and Heinz body production. The typical clinical signs and conditions supporting red maple leaf intoxication should be in evidence before making a diagnosis. Because the toxic principle is yet unidentified, no specific assay of feed or tissue specimens is available.

Hematologic abnormalities noted in affected horses include moderate to severe anemia (PCV often <10% in severely affected horses), hemoglobinemia, methemoglobinemia, Heinz bodies, anisocytosis, hyperbilirubinemia, and increased erythrocyte fragility. Blood chemistry analysis may reveal depletion of erythrocyte-reduced glutathione and increased serum concentrations of LDH, creatine phosphokinase, AST, and sorbitol dehydrogenase.[125–128] Transient hypercalcemia has been recorded in some horses,[125] and one can expect increases in BUN and creatinine concentrations in horses undergoing significant renal insult secondary to hemolysis.[128] Urine analysis may indicate varying degrees of hemoglobinuria, hematuria, bilirubinuria, and proteinuria.[125–127]

Treatment of affected horses is primarily symptomatic. Exposure to red maple leaves should be eliminated immediately, and one should maintain affected horses in a quiet, calm environment. Oxygen therapy may be beneficial in selected cases, and one can administer blood transfusions to severely affected individuals.

Balanced, polyionic fluid administration is important to maintain renal function and to aid in diuresis because affected horses may be at great risk of developing hemoglobin nephrosis and acute renal failure. One should monitor blood electrolyte and acid-base parameters and correct abnormalities as needed. One should treat acute renal failure appropriately if it develops.

Other symptomatic therapies suggested include nasogastric intubation with activated charcoal to aid in binding toxin; dexamethasone to aid in stabilizing red blood cell membranes and decrease phagocytosis of damaged red blood cells; and ascorbic acid (30 mg/kg) given in intravenous fluids twice daily potentially to reduce oxidative damage to red blood cells. Whether dexamethasone increases the risk of laminitis in affected horses is unknown, and the efficacy of ascorbic acid is questionable.[130]

Prevention of the disease is accomplished by preventing exposure of horses to leaves from red maple trees.

20.4.1.2 Onion (*Allium* Species)

Onion toxicosis in horses is a rare event and occurs when horses are fed large amounts of culled onions (from commercial onion farms) or when forced to eat wild onions because of inadequate available forage. Horses appear to avoid these plants when suitable forage is available.[131,132] The toxic principle in onions, *n*-propyl

disulfide, only affects circulating erythrocytes, in which it causes oxidant injury to the red blood cell with resultant Heinz body formation and subsequent hemolytic anemia. Severely affected horses can succumb to onion toxicosis because of severe anemia or secondary renal failure caused by hemoglobin nephropathy.

The clinical signs, laboratory values, and pathogenesis of the anemia are all similar to those of red maple leaf toxicosis, with the exception that methemoglobin formation does not seem to be nearly as pronounced in onion toxicity as in the former. Horses dying from onion toxicosis may have an onion odor of the carcass noticeable at necropsy.[131]

Affected horses may recover from onion toxicity if they are removed from the onion source soon enough and the anemia is not pronounced. Treatment is largely symptomatic and should include removal of the onion source and provision of adequate, suitable forage. Hematinics are of little value, but oxygen therapy and blood transfusions may be indicated in more severely affected animals.

Maintenance of renal function is of primary concern because affected horses are at risk of developing secondary hemoglobin nephropathy. One should give balanced, polyionic fluids to promote diuresis and should correct electrolyte and acid-base abnormalities. One should use diuretics with caution, but they may be of value in the volume-loaded horse.

20.4.2 MEDICATIONS

20.4.2.1 Heparin

Heparin is an anionic sulfated glycosaminoglycan polysaccharide found normally in mast cells. Heparin is 1480
commercially available as a calcium or sodium salt and is prepared from bovine lung tissue or porcine 1481
intestinal mucosa.[7] Its use in human beings and animals is primarily as an anticoagulant, and in horses heparin has been used as adjunct therapy to treat acute laminitis, septic peritonitis, and disseminated intravascular coagulation and in horses at risk of developing thrombosis. Heparin also has been used to prevent peritoneal fibrin deposition following abdominal surgery and in attempts to prevent disseminated intravascular coagulation in horses suffering from various systemic diseases.[133–135]

Heparin is strongly acidic and is not absorbed from the gastrointestinal tract. Intramuscular injection often leads to painful, large hematomas at the injection site, so administration is limited to the intravenous and subcutaneous routes.[7,136] Heparin is bound primarily to low-density lipoproteins, globulins, and fibrinogen and is metabolized in the liver by heparinase. In human beings the inactive metabolic products are excreted in urine along with a small fraction of unchanged drug. Heparin apparently is removed from the circulation primarily by the reticuloendothelial system and localizes on arterial and venous endothelium.[7] The plasma half-life of heparin is approximately 1½ hours.[136]

20.4.2.1.1 Clinical Signs

One adverse effect of heparin overdose is hemorrhage because heparin acts as an anticoagulant, but the primary problem associated with heparin therapy in the horse is that of induced reduction of red blood cell mass.[133–135] In experimental studies, dosages of 160 units per kilogram of body mass or greater given subcutaneously twice a day resulted in reduction of the measured circulating red blood cell mass and an increase in measured mean corpuscular volume. The red blood cell mass was reduced as much as 57% in

some horses. A dosage of 40 units per kilogram twice daily had no effect on measured blood parameters.[133] In reports of clinical cases, a dosage of 80 units per kilogram subcutanously twice daily also resulted in reduced PCV and increased mean corpuscular volume.[134]

In affected horses the PCV begins to decrease by 12 to 24 hours after initiation of a toxic dose and appears to remain depressed while the horse remains on heparin therapy. The PCV begins to increase by 24 hours after cessation of heparin therapy and approaches baseline values in 72 hours.[133,137] One anecdotal report indicated that horses maintained on heparin therapy also would exhibit a rebound in PCV in several days, even though heparin administration was continued.[138] Other hematologic abnormalities that may be apparent in affected horses include an increase in serum bilirubin concentration[137] and decreased serum concentrations of hemoglobin and platelets. These hematologic discrepancies have been induced in horses given heparin at a dosage that maintained the activated PTT within a therapeutic range of 1.5 to 2 times the normal mean.[133]

20.4.2.1.2 Pathophysiology

Heparin by itself is inactive and produces its anticoagulant action by combining with antithrombin III, an endogenous α_2-globulin that must be present in adequate amounts for the process to proceed. The heparin–antithrombin III complex greatly accelerates the antagonistic action of antithrombin III on thrombin, thereby restricting the conversion of fibrinogen to fibrin. This complex is also inhibitory to kallikrein and to the activated forms of clotting factors IX, X, XI, and XII.[136] In addition, heparin acts as an opsonin and may augment certain phagocytic activities of the reticuloendothelial system.[133,137]

The mechanism whereby heparin causes a reduction in circulating red blood cell mass is still under scrutiny. Initially, heparin was suggested to cause extravascular hemolysis by enhanced phagocytosis of red blood cells by the reticuloendothelial system.[133,137] More recent evidence, however, strongly suggests that the deleterious effects of heparin on formed blood elements are largely artifactual and that the measured abnormalities result from erythrocyte agglutination.[134,135] Further investigation is required to determine if this agglutination also occurs in vivo in heparinized horses.

20.4.2.1.3 Diagnosis and Treatment

The rapid onset of dramatic reduction in PCV in horses treated with heparin implies acute hemorrhage or the previously described adverse reaction of heparin on the formed blood elements of the horse. The specific antidote for heparin is protamine sulfate, which one can give at a dose not to exceed 1 mg for every 90 to 100 units of heparin administered.[136,139] One also can give whole, fresh blood or plasma transfusions to reverse or antagonize the anticoagulant effect.[136]

The foregoing treatments are not likely to be required except in instances of hemorrhage caused by heparin overdose. Removal of heparin from the treatment regimen is usually all that is necessary to stop the decline in PCV. Normal numbers of circulating erythrocytes should reappear by approximately 72 hours after cessation of heparin therapy, if this adverse reaction to heparin is the cause of the lowered PCV.

20.4.2.2 Coumarin Derivatives

Coumarin is normally present in some species of sweet clover and has no anticoagulant action. However, coumarin derivatives are used widely as anticoagulants, with bishydroxycoumarin (dicumarol) and 3-(α-acetonylbenzyl)-4-hydroxycoumarin (warfarin sodium) being the first oral anticoagulants developed from coumarin.[140] These first-generation compounds are used therapeutically as anticoagulants and as rodenticides. In horses, warfarin has been used therapeutically to treat thrombotic disorders such as thrombophlebitis and more recently to treat navicular disease.[141,142] Horses may exhibit signs of toxicity 1481
while being medicated with warfarin if a therapeutic concentration is exceeded. Rarely are horses exposed to 1482
warfarin or other coumarin derivatives used as rodenticides around buildings or feed storage areas. Second-generation anticoagulant rodenticides (brodifacoum and bromodiolone) have been developed because of acquired rodent resistance to the first-generation compounds. Because no indications exist for human or veterinary medicinal use of brodifacoum, toxicity associated with this compound is caused by accidental ingestion.[143]

20.4.2.2.1 Clinical Signs

The clinical signs of toxicity noted with first- and second-generation anticoagulant rodenticides are largely those of a hemorrhagic diathesis. Onset of signs is usually acute and may include hematoma formation, epistaxis, anemia, weakness, pale mucous membranes, or ecchymoses of mucous membranes. Hematuria and melena can occur, and hemorrhage into various body compartments may result in secondary signs caused by malfunction of the involved organ or tissue. Occasionally, affected animals may be found dead.[15] Multiple fractional doses of first-generation compounds given over several days may be more toxic to horses than a single larger dose.[141] Brodifacoum, however, differs from warfarin in that a single oral dose has the potential of causing illness and may even be lethal (estimated LD_{50} of 1 to 2 kg per adult horse).[143,144]

The onset of clinical signs for first-generation compounds is delayed from the time of ingestion. In an experimental model the hypothrombogenic effect of warfarin anticoagulation was noticed 60 hours following an acute dose.[145] This effect persisted for approximately 30 hours. The effect of brodifacoum, however, is much longer and may persist for weeks, despite its half-life of 1.22 days.[143] In an experimental study, horses given a single dose of brodifacoum (0.125 mg/kg body mass) showed increased PTT at 24 hours and one-stage PT at 48 hours after exposure. These values returned to pretreatment levels by day 12. However, two horses required 23 days for clotting time to return to normal. In these horses, peak plasma concentration of brodifacoum occurred 2 to 3 hours after oral administration. Additionally, four of the six experimental horses showed clinical signs of depression, anorexia and weight loss.[144]

20.4.2.2.2 Pathophysiology

Warfarin is absorbed readily from the gastrointestinal tract but also may be administered intravenously for therapeutic purposes.[140,141] Once absorbed, warfarin is highly bound (>90%) to plasma proteins and some is stored in the liver.[140,141,145] The degree of brodifacoum protein binding in horses is not known but may be similar.[143] Warfarin is hydroxylated by hepatic enzymes to inactive compounds that are eliminated by

the kidney. The metabolites have no anticoagulant effect,[140] and the biologic half-life of warfarin in the horse is approximately 13 hours.[145] Coumarins also cross the placenta and are secreted in milk.[140]

Protein binding of warfarin is reversible, and the protein-bound, pharmacologically inactive warfarin serves as a reservoir. The unbound portion remains fairly constant in plasma.[140,141]

A number of factors can influence the amount of unbound free warfarin in serum. Protein-bound drugs such as phenylbutazone, chloral hydrate, and sulfonamides may enhance the toxicity of warfarin by displacing warfarin from protein-binding sites. This allows a greater proportion of the drug to be in the free form and thus able to exert its pharmacologic effect.[15,140] Corticosteroids and thyroxine may lower the therapeutic dose of warfarin by increasing clotting factor catabolism and receptor site affinity.[141]

Certain physiologic factors also may enhance the toxicity of warfarin. Hypoalbuminemia may result in fewer binding sites available, thereby increasing the amount of free drug. Hepatic dysfunction may impede metabolism of warfarin, and reduced amounts of vitamin K in the diet may predispose to toxicity.[140]

Some drugs reduce the therapeutic response to a given dose of warfarin. Barbiturates, rifampin, and chloramphenicol induce hepatic microsomal enzyme activity, thereby accelerating metabolism of warfarin. If these drugs are withdrawn during warfarin therapy, toxicosis may result.[140,141] Likewise, excessive dietary intake of vitamin K (such as alfalfa) or vitamin K administration can reduce or inhibit the anticoagulant effects of warfarin.[141]

Warfarin acts as an anticoagulant by inhibiting production of the vitamin K–dependent clotting factors II, VII, IX, and X. Vitamin K is a co-factor in the synthesis of clotting factors II, VII, IX, and X and also acts on all clotting factor precursors to convert glutamyl residues to α-carboxyglutamyl residues. During this carboxylation process, vitamin K_1 is converted to vitamin K_1 2,3-epoxide, an inactive metabolite. This epoxide returns to active vitamin K_1 by action of the microsomal enzyme vitamin K_1 epoxide reductase. The coumarin anticoagulants inhibit vitamin K_1 epoxide reductase, thereby creating a vitamin K_1 deficiency. The result is decreased production and subsequent deficiency of the vitamin K–dependent clotting factors.[7] The delayed onset of action of the coumarin derivative anticoagulants results from this impediment to clotting factor synthesis rather than from a direct effect on the clotting mechanism per se.

The various blood clotting factors have different plasma half-lives. Factor VII has a shorter half-life than the other clotting factors, so the earliest laboratory indication of warfarin toxicity is a prolonged PT. As the other clotting factors become depleted, activated PTT also increases as toxicity continues.[141] One should 1482
monitor horses given warfarin therapeutically for clotting abnormalities. Prolongation of PT by 1.5 to 2 1483
times baseline has been suggested as the effective range of anticoagulation. However, some horses maintained in this range may show signs of hemorrhage.[142]

20.4.2.2.3 Diagnosis and Treatment

The diagnosis of warfarin or other coumarin derivative anticoagulant intoxication is based on evidence of exposure to the drug, presence of a bleeding diathesis, and prolongation of PT with or without prolonged activated PTT. Because warfarin only affects certain clotting factors, other elements of the clotting profile are not affected. The platelet count, fibrinogen concentration, fibrin degradation product concentration, and antithrombin III activity remain within their respective normal ranges of activity.[141,142]

One also can evaluate tissue concentration of warfarin on postmortem specimens. One can submit liver, kidney, spleen, stomach and intestinal contents, feces, and unclotted blood for evaluation. One should freeze specimens for transportation to the laboratory.[15,145] Certain laboratories can evaluate serum for brodifacoum content.[143]

In horses intoxicated by warfarin, discontinuance of warfarin therapy or removal of the product from the environment of the horse is of primary importance. Vitamin K_1 is the specific antidote for coumarin derivatives and should be given at a dosage of 300 to 500 mg subcutaneously every 4 to 6 hours until PT returns to the baseline value. Thereafter, one should monitor PT daily for 3 to 4 days to ensure stability. The subcutaneous route of vitamin K_1 administration is preferable because of the possibility of adverse reactions if the product is given intravenously or intramuscularly. Intravenous injection of vitamin K_1 may result in transient restlessness, tachypnea, tachycardia, sweating, and anaphylactic reactions.[146,147] Intramuscular administration of vitamin K_1 results in erratic response times and therefore may be inappropriate for a hemorrhaging patient.[146] Alternative doses for vitamin K_1 administration are 0.3 to 0.5 mg/kg intravenously, and a dosage of greater than 0.5 mg/kg intravenously has been suggested to inhibit coumarin activity for several days.[141] If one chooses the intravenous route, one should dilute vitamin K_1 in 5% dextrose or saline and give it slowly.[15]

In horses intoxicated with brodifacoum, treatment with vitamin K_1 may be required for 3 to 5 weeks or until serum brodifacoum concentration decreases to less than 30 ng/ml and coagulation status is normal 2 to 3 days following the last dose of vitamin K_1. Affected horses were given vitamin K_1 (2.5 mg/kg) subcutaneously every 12 hours for 36 hours and then orally twice daily for the duration of treatment.[143]

If bleeding is serious, fresh blood or fresh plasma given intravenously may be necessary to control hemorrhage and to help correct hypovolemia. Other supportive measures such as bandaging and keeping the animal in a quiet environment may be helpful. Alfalfa hay added to the diet may provide a natural source of vitamin K_1.[143] One should treat organ dysfunction induced by hemorrhage appropriately. The prognosis for horses intoxicated with first-generation compounds is good if the disorder is recognized early and appropriate therapy is instituted. The prognosis for brodifacoum toxicosis is much more guarded because of the prolonged and severe course of clinical signs with this substance.

Prevention of warfarin toxicosis should aim to minimize exposure of horses to the product and to monitor the therapeutic use of the drug in the horse carefully. Contraindications to its use include any clinical or laboratory suggestion of hepatic disease, hypoproteinemia, or other condition that might increase the risk of toxicity. One should evalute the concurrent use of other medications because they may affect the potential for toxicosis to develop, and one should reevaluate the dosage of warfarin administered if other concurrent medications are changed. Additionally, one should minimize the potential for traumatic injury in horses undergoing warfarin therapy.

20.4.2.3 Dimethyl Sulfoxide

Dimethyl sulfoxide is a by-product of the papermaking industry and is a colorless liquid originally used as an industrial solvent. The chemical is a polar compound that readily mixes with ethyl alcohol and many organic solvents, is extremely hygroscopic, and can absorb more than 70% of its weight of water from air. Dimethyl

sulfoxide possesses some antimicrobial and antifungal activity, but its primary medical use has been as an antiinflammatory agent and as a transdermal transport agent.[148] More recently, DMSO has found use as a diuretic and has shown promising results when used to treat acute cranial and spinal cord trauma.[148,149]

The systemic toxicity of DMSO is considered to be low, and its greatest toxic potential appears to result from its combination with other toxic agents.[150] However, in an experimental study, rapid infusion of DMSO in 20% and 40% concentrations caused hemolysis, hemoglobinuria, diarrhea, muscle tremors, and signs of colic in some horses.[151] The LD_{50} of DMSO has not been established for horses but ranges from 2.5 to 9.0 g/kg as a single intravenous dose in a number of animal species.[150] A dose of 1 g/kg intravenously has been suggested for use in horses. One should dilute this dose to a 10% to 20% solution and administer it slowly intravenously.[149,150]

Dimethyl sulfoxide produces hemolysis when given intravenously in concentrations of 20% to 50% or greater.[148–150] If hemolysis is severe, affected horses may be at increased risk of developing hemoglobin 1483
nephrosis. Concentrations of 10% or less are considered suitable for intravenous injection in horses. 1484
[148,149,151] Additionally, increased white blood cell adherence and fibrinogen precipitation have been reported when concentrations greater than 50% were administered.[148]

Dimethyl sulfoxide is a mild cholinesterase inhibitor, and its concurrent use with organophosphates or other cholinesterase inhibitors is not recommended.[148,150] Dimethyl sulfoxide also is known to induce histamine release from mast cells, but the significance of this phenomenon is uncertain.[150]

Skin reactions to topically applied DMSO can occur. Varying degrees of erythema, pruritus, drying, hardening, and desquamation of normal skin may be evident. These reactions are usually self-limiting and typically diminish with repeated applications.[150]

The greatest risk of toxicity resulting from DMSO is probably a consequence of its concomitant use with other toxic or potentially toxic agents. Dimethyl sulfoxide may aid transport of a variety of toxic compounds across skin, thereby inducing toxicosis from the transported agent. For example, mercury toxicity has been reported in a horse that had a blister from DMSO and mercury applied topically to a leg.[152] In such instances, one should treat the specific toxic reaction(s) appropriately. No specific antidote is recognized for DMSO, other than its judicious and conscientious use when being administered to horses. One should heed the aforementioned precautions when administering the drug.

20.4.2.4 Phenothiazine

Phenothiazine is an anthelmintic that has a narrow spectrum of activity in horses. Phenothiazine is active only against strongyles, is moderately effective in removing large strongyles, but is more effective against small strongyles.[153] This drug rarely is used as an equine anthelmintic because of its side effects and because safer and more efficacious anthelmintics with broader spectra of activity have been developed.

20.4.2.4.1 Clinical Signs

Phenothiazine administration has resulted in acute and unpredictable signs of toxicity in horses. Debilitated, weak, and anemic animals are reported to be more susceptible to toxicosis, yet toxic reactions

have been seen at normal therapeutic doses.[77,153] Fifteen grams may be toxic to horses, and 30 g may be lethal.[15] The common signs of toxicity reported are hemolytic anemia with subsequent hemoglobinuria and icterus, anorexia, weakness, CNS disturbances, and photosensitization.[77,153]

20.4.2.4.2 Pathophysiology

Phenothiazine is absorbed from the intestinal tract after being converted to phenothiazine sulfoxide by cellular enzymes of the intestinal epithelium. Once absorbed, phenothiazine is oxidized further in the liver to leukophenothiazone and leukothionol. These two substances then are excreted primarily in urine.[77]

Phenothiazine acts as an oxidant to red blood cells, resulting in the production of Heinz body anemia. For a detailed description of the pathogenesis of this condition, see Red Maple *(Acer rubrum)*.

Photosensitization may not be a problem in horses intoxicated by phenothiazine. However, in other species, photosensitization can occur because of abnormal accumulation of phenothiazine sulfoxide. Normally, this product is converted to phenothiazone by the liver. When high doses of phenothiazine are given or if liver dysfunction impairs the conversion of phenothiazine sulfoxide to phenothiazone, the sulfoxide diffuses into the general circulation and ultimately into the aqueous humor of the eye. On exposure to ultraviolet light, the phenothiazine sulfoxide produces photosensitization in unpigmented skin and a photosensitive keratitis. The keratitis may be manifested by corneal ulceration and blindness.[77]

The mechanism whereby phenothiazine may induce CNS derangements has not been elucidated.

20.4.2.4.3 Diagnosis and Treatment

Diagnosis of phenothiazine toxicity is based primarily on history of exposure to the drug and compatible clinical signs. No specific antidote is available, so symptomatic therapy is indicated. One should treat Heinz body anemia as previously described. One can prevent photosensitization by keeping treated animals out of direct sunlight for 2 to 3 days following therapy. One should not give phenothiazine to debilitated, weak animals and those with evidence of liver disease.

20.4.2.5 Digoxin

Digoxin has been used to treat congestive heart failure in many species, including horses. However, reports of digoxin toxicity in horses are rare. The margin between therapeutic and toxic doses in many species is narrow, and wide variations in individual tolerance to the drug occur. Therefore signs of toxicity may develop in horses given a normal dose of digoxin.[154]

20.4.2.5.1 Clinical Signs

The clinical signs reported in one instance of digoxin intoxication were anorexia, depression that became more pronounced over time, muscle tremors, and colic. Cardiac abnormalities included a slower, irregular pulse rate and electrocardiographic findings of a prolonged PQ interval with increased duration (widening) of the P wave. The horse also was hyponatremic and hypochloremic.[154]

Signs of toxicity reported in other species, including human beings, are anorexia, vomiting, salivation, diarrhea, abdominal pain, depression, skin rashes, neurologic signs, nausea, and various cardiac arrhythmias.[154,155] The most frequently encountered arrhythmias include incomplete or complete heart block, junctional escape rhythms and ventricular premature beats, ventricular bigeminy, and paroxysmal ventricular or atrial tachycardia with block. Death, when it occurs, is caused by cardiac arrhythmias.[155] 1484

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20.4.2.5.2 Pathophysiology

One can administer digoxin intravenously or orally, but the latter is preferred in the horse. Intravenous administration of the drug results in an initial high serum concentration and is an unsatisfactory means of providing long-term therapy. Absorption of digoxin is rapid and occurs largely in the small intestine, but the bioavailability of the drug is low (approximately 20%), and considerable variation in absorption occurs among horses.[155,156] Following absorption, digoxin is bound primarily to serum albumin, but again individual variation in the amount of protein binding in normal horses is considerable.[157] The biologic half-life of digoxin in the horse is reported to range from 17 to 23 hours,[156,158] and elimination is largely through urinary excretion of unchanged drug.[157]

The suggested therapeutic range for digoxin in the horse is a serum concentration of 0.5 to 2.0 ng/ml. This concentration has been achieved by administering an oral dose of 35 μg/kg every 24 hours. However, one must remember that no dosage regimen is absolute because of the extreme individual variation in absorption and protein binding of the drug. Repeated clinical monitoring of treated horses is imperative, with dosage adjustments based on clinical improvement, development of early signs of toxicosis, and assay of plasma digoxin concentration.[156]

Digoxin acts as a positive ionotrope and a negative chronotrope. The cellular mechanisms of inotropic action of digoxin still are being evaluated, but digoxin is known to inhibit the Na^+,K^+-ATPase–dependent transport system. This results in an intracellular loss of potassium and an intracellular elevation of sodium and calcium. The resting membrane potential therefore is reduced, making the cardiac cells more excitable. Another hypothesis is that the increased intracellular sodium concentration augments transmembrane exchange of intracellular sodium for extracellular calcium. The increased calcium delivered to the contractile proteins may be partially responsible for the positive inotropic effect.[155]

Digoxin acts as a negative chronotrope by decreasing sinus rate and slowing conduction of the atrioventricular impulse. These effects result from increased vagal tone and secondary hemodynamic improvement in digitalized animals. The increased vagal tone has been attributed to at least three mechanisms: (1) direct stimulation of vagal centers in the brain, (2) sensitization of baroreceptors in the carotid sinus to blood pressure, and (3) enhanced pacemaker response to acetylcholine at the myocardial level. The circulatory improvement following digitalization tends to remove the stimuli responsible for reflex sinus tachycardia, a compensatory effort in many instances of congestive heart failure. As a result the sinus rate can return to normal.[155]

Toxic effects of digoxin on cardiac muscle occur when the aforementioned mechanisms proceed beyond a beneficial level. Increased cardiac automaticity and excitability, coupled with excessive refractoriness at the atrioventricular node and decreased refractoriness of the atria and ventricles, can lead to extrasystoles and tachyarrhythmias. These dysrhythmias can be severe enough to result in cardiac failure and death.[7]

Many factors may enhance digoxin toxicity in human beings. Diseases such as renal failure, hypothyroidism, myocardial infarction, and myocarditis can have an additive effect on toxicity. Certain drugs—including quinidine, verapamil, amiodarone, spironolactone, and indomethacin—act to increase serum digoxin levels, and abnormal electrolyte concentrations can enhance digoxin toxicity. Hypokalemia, hyperkalemia, hypomagnesemia, hypernatremia, and alkalosis can contribute to digoxin toxicity.[7]

The mechanisms responsible for the noncardiac signs of digoxin toxicity have not been elucidated.

20.4.2.5.3

Diagnosis and Treatment

Diagnosis of digoxin toxicity may be difficult to differentiate from continuing cardiac compromise. If compatible clinical and electrocardiographic findings of toxicity are present, measurement of serum digoxin concentration should verify the diagnosis. However, some horses may exhibit signs of toxicity even though the serum concentration is within the suggested nontoxic range of 0.5 to 2.0 ng/ml.

Treatment of digoxin toxicosis should begin with drug withdrawal. One should evaluate serum electrolyte concentrations and acid-base status and correct abnormalities.[7,155] Oral charcoal administration has been advocated in instances of human toxicosis,[7] but its efficacy in equine poisoning remains undetermined. For horses that require digoxin therapy, one should start at a low dose and increase the dosage as needed over time.

20.4.2.6

Bicarbonate

Sodium bicarbonate is one of the most common alkalizing agents used in animals, including horses. Sodium bicarbonate is indicated specifically to treat acute, severe metabolic acidosis because of its rapid effect on blood pH when given intravenously.[159] Sodium bicarbonate also has been used in performance horses to treat exertional myopathies and in attempts to prevent rhabdomyolysis. More recently, sodium bicarbonate administration in performance horses has been investigated because of its suggested role in limiting or preventing systemic lactic acidosis, thereby enhancing the performance level of the horse.[160,161] The adverse effects of bicarbonate administration occur when it is given too rapidly or in excessive quantities or when it is given in the presence of certain other systemic abnormalities.

20.4.2.6.1

Clinical Signs

Animals, including horses, that suffer from an acute overdose of bicarbonate may exhibit signs of delirium, depression, and coma. Rapidly induced alkalosis also has been associated with cardiac dysrhythmias.[159] 1485

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Horses such as endurance horses or racehorses that are volume depleted and have sustained excessive electrolyte loss through sweat often have clinicopathologic changes of hypokalemia, hypochloremia, hypocalcemia, and metabolic alkalosis. When such horses are given bicarbonate, dramatic deleterious effects occur. These animals may exhibit signs of muscle fasciculation, synchronous diaphragmatic flutter, bruxism, and decreased respiratory rate. One also may note clinical evidence of further dehydration, such as increased capillary refill time, delayed jugular distensibility, diminished skin turgor, and decreased arterial pulse.[161]

20.4.2.6.2

Pathophysiology

The rapid administration or overdose of sodium bicarbonate has been associated with extracellular hyperosmolality, intracranial hemorrhage, and CSF acidosis. Hyperosmolality results from hypernatremia because sodium bicarbonate dissociates into sodium ions and bicarbonate ions. An abrupt increase in serum osmolality can lead to intracranial hemorrhage as intracellular water moves into the extracellular space. This extracellular fluid accumulation may result in engorgement of perivascular spaces, with subsequent tearing of bridge veins and resultant hemorrhage. The CSF acidosis results from the rapid diffusion of generated carbon dioxide into the CSF. Carbon dioxide enters the CSF almost instantaneously, establishing new steady-state levels within minutes. Bicarbonate ion, however, is slow to enter the CSF and requires hours to days to achieve new steady-state levels. With sodium bicarbonate administration, the increasing amount of carbon dioxide generated readily enters the CSF disproportionately more than does the bicarbonate ion. As a consequence the CSF becomes acidic.[162] These mechanisms, individually or collectively, are thought to be responsible for development of the clinical signs observed with rapid or excessive administration of sodium bicarbonate solution.

Another effect of alkalosis is a shift to the left of the oxyhemoglobin dissociation curve. This left shift indicates an increased affinity of hemoglobin for oxygen, with a resultant decrease in the amount of oxygen available for cellular use. This change in hemoglobin affinity for oxygen has been associated with cardiac dysrhythmias following rapidly induced alkalosis.[159]

Horses undergoing extensive exercise or horses that are treated with furosemide (primarily as prerace medication for exercise-induced pulmonary hemorrhage) are at risk of developing hypochloremic, hypokalemic metabolic alkalosis. When sodium bicarbonate is given to these volume-depleted horses with electrolyte loss and concurrent metabolic alkalosis, another deleterious series of events can result. Further reduction of the circulating fluid volume may occur, along with development of serum hyperosmolality, electrolyte abnormalities of hypernatremia, hypokalemia, hypochloremia, and hypocalcemia, and further development of metabolic alkalosis.[161]

Excess sodium bicarbonate produces hyperosmolality caused by hypernatremia because sodium bicarbonate dissociates into sodium and bicarbonate ions. The hypokalemia is explained partially by the intracellular shift of potassium in response to metabolic alkalosis, and the bicarbonate ion adds further to the alkalosis already present. Furosemide administration causes urinary loss of potassium, chloride, and calcium, and sweating also can result in significant loss of chloride and calcium. Additionally, rapid intravenous infusion of 5% sodium bicarbonate in normal horses results in hypochloremia.[163]

The muscle fasciculations and diaphragmatic flutter noted in some horses likely result from hypocalcemia.[161] The total serum calcium concentration may be within a normal range, but alkalosis causes a reduction in the amount of circulating ionized calcium. Because ionized calcium is responsible for neuromuscular function, reduction in this fraction can cause clinical signs of hypocalcemia.

20.4.2.6.3

Diagnosis and Treatment

One should monitor horses being treated with sodium bicarbonate solutions closely for clinical signs of alkalosis. Blood pH obviously is elevated, but rapid infusion of sodium bicarbonate or excessive use in horses already sustaining fluid and electrolyte loss can result in a number of clinicopathologic alterations.

Affected horses typically exhibit increases in PCV, total serum protein, and serum bicarbonate and sodium concentrations. Additional findings of hypokalemia, hypochloremia, and hypocalcemia are usually present.

Treatment of affected horses involves cessation of bicarbonate administration and correction of the alkalosis and electrolyte abnormalities. Potassium chloride administration is indicated in horses with hypokalemic, hypochloremic metabolic alkalosis, and is much more effective in correcting these electrolyte abnormalities than is sodium chloride. Potassium chloride also has been shown to cause a prompt, significant decline in venous blood pH in these horses.[161]

Apparently, one should not use hypertonic sodium bicarbonate solutions in dehydrated horses. The concurrent use of furosemide and sodium bicarbonate also appears to be contraindicated or at best must be used with extreme caution and close observation with laboratory assessment. One should not mix sodium bicarbonate with fluids containing calcium because insoluble complexes may form. The use of sodium bicarbonate orally in horses subjected to short-term, intense exercise is still controversial.

20.4.3 MISCELLANEOUS AGENTS

20.4.3.1 Nitrates and Nitrites

Nitrates are an important component in the naturally occurring nitrogen cycle and as such are present in soils, 1486
groundwater, forages, row crops, weeds, animal tissues, and excreta. Nitrates also are used widely in 1487
fertilizers. Toxicity problems can arise when animals consume plants, feed, or water containing excessive amounts of nitrates or when they ingest nitrate fertilizers or residues. Nitrates naturally undergo microbial decomposition to nitrites, so nitrite toxicity can occur when animals ingest feed or water in which the nitrates have decomposed to yield large amounts of nitrites. This decomposition can occur in moist haystacks, water troughs, farm ponds, silages, and pig swills. Nitrite also is administered intravenously to treat cyanide poisoning, and overzealous use of this therapy can result in toxicity.[42]

The primary exposure to nitrate or nitrite for most animals is the plants they consume. Many plants and forages are known to be nitrate accumulators, and a number of factors influence the uptake of nitrates in plants. Nitrate concentration by plants is enhanced by low soil pH; low soil molybdenum, sulfur, or phosphorus content; low soil temperature; drought; soil aeration; decreased light; and use of phenoxyacetic acid herbicides such as 2,4-dichlorophenoxyacetic acid. Nitrate and nitrite accumulation in ponds and groundwater is caused by water runoff from nitrate-rich soils or by direct contamination with nitrates and nitrites.[15,42] Nitrates and nitrites are water soluble and are carried easily from feedlots, pigpens, and fertilized areas into the soil and subsequently into plants, wells, and ponds.

The most important aspect of nitrates is their ease of microbial conversion to nitrites. Nitrite is responsible for the primary signs associated with toxicity in nitrate poisoning.

20.4.3.1.1 Clinical Signs

Although apparently rare, horses are reported to be susceptible to nitrate intoxication. Experimentally, an oral dose of 1 g/kg potassium nitrate caused illness but not death in horses. However, nitrates have been associated with death of horses under field conditions.[42]

In monogastric animals, nitrate ingestion is reported to produce gastrointestinal irritation with resulting emesis or enteritis. Salivation, diarrhea, colic, and frequent urination may be evident if the nitrate is concentrated enough. Because no mechanism are known whereby these animals can rapidly reduce nitrate to nitrite, they are generally much more tolerant of nitrate than are ruminants.[15]

Signs of acute poisoning with nitrites usually commence within 30 minutes to 4 hours following ingestion of high-nitrite feed or water. The most characteristic signs are those referable to respiratory insufficiency and include dyspnea, cyanosis, rapid and weak pulse, and anxiety. Exertion may exacerbate these signs and induce muscle tremors and collapse. The horse may have terminal clonic convulsions. The blood of affected animals is usually brown or chocolate-colored and imparts a cyanotic appearance to mucous membranes. Death may occur within several hours or be delayed until 12 to 24 hours after ingestion.[15,42]

20.4.3.1.2

Pathophysiology

Nitrites are absorbed rapidly from the gastrointestinal tract into the bloodstream. The nitrite ion acts directly on vascular smooth muscle to cause relaxation, and easily enters erythrocytes in exchange for chloride ion. Nitrite also can pass the placenta to enter fetal erythrocytes, which are especially sensitive to nitrite. The biologic half-life of blood nitrate in horses is 4.8 hours. Only small amounts of nitrate or nitrite are bound to plasma proteins.[42,164]

Nitrite causes acute poisoning by two mechanisms. The primary action of nitrite is to interact with hemoglobin to form methemoglobin. One molecule of nitrite interacts with two molecules of hemoglobin, causing the oxidation of normal ferrous hemoglobin to ferric hemoglobin, which is called methemoglobin. Methemoglobin is incapable of transporting oxygen to tissues, and if sufficient quantities are formed, severe oxygen deficiency can occur. Clinical signs become evident when methemoglobin levels approach 30% to 40%, and death occurs when 80% to 90% of hemoglobin is oxidized to methemoglobin.[15,42] However, death can occur in active animals with only 50% to 60% methemoglobin.[42]

Normally, methemoglobin is converted back to ferrous hemoglobin by two reducing enzyme systems: nicotinamide adenine dinucleotide–dependent diaphorase I and nicotinamide adenine dinucleotide phosphate–dependent diaphorase II. This conversion occurs slowly, and in instances of nitrate toxicity, methemoglobin formation far exceeds the ability of these enzyme systems to regenerate hemoglobin.[42]

The second action of nitrite is to cause direct relaxation of smooth muscle, particularly vascular smooth muscle. The mechanism by which this occurs is unknown, but the physiologic changes brought about by the vasodilating action of nitrite include pulmonary arterial, central venous, and systemic arterial hypotension and decreased cardiac output. These changes may contribute to tissue anoxia and act to enhance tissue oxygen starvation already initiated by methemoglobin.[42]

20.4.3.1.3

Diagnosis and Treatment

The diagnosis of nitrate or nitrite toxicity is based on compatible clinical signs; methemoglobinemia; history of exposure to nitrate- or nitrite-containing plants, water, or fertilizers; and nitrate quantitation in blood. One should perform serum nitrate and methemoglobin determinations quickly following collection because these are not stable in refrigerated, heparinized blood for more than a few hours. However, blood mixed with a phosphate buffer preserves the methemoglobin and allows shipment to a diagnostic 1487

laboratory. One can analyze forage, hay, and water samples for nitrate or nitrite content, as well as other body fluids such as urine, aqueous humor, and intestinal contents.[15]

Treatment aims to reduce methemoglobin back to hemoglobin. Mildly affected animals may recover spontaneously if the toxic source is removed and if they are given sufficient time for normal methemoglobin reduction processes to occur. One may use methylene blue to treat more severely affected horses at a suggested dose of 4.4 mg/kg intravenously given slowly as a 1% solution in isotonic saline. One can repeat the dose in 30 minutes if clinical response is unsatisfactory. One should exercise caution with this product, however, because excess methylene blue may directly oxidize hemoglobin to methemoglobin. Methylene blue is converted to leukomethylene blue by an $NADPH_2$-dependent system. Leukomethylene then reduces methemoglobin to hemoglobin. In this reaction, leukomethylene blue is oxidized back to methylene blue but can be reconverted to leukomethylene blue as long as sufficient $NADPH_2$ is available. If this $NADPH_2$ system becomes saturated with methylene blue, excess methylene blue may oxidize hemoglobin directly to more methemoglobin.[42]

Other nonspecific therapies one may use include blood transfusion, oxygen therapy, and laxatives such as mineral oil to aid evacuation of the gastrointestinal tract.[42]

20.4.3.2 Cyanide

Hydrogen cyanide, cyanide, hydrocyanic acid, and *prussic acid* are terms relating to the same toxic substance. Horses become exposed primarily through ingestion of certain plants that contain cyanogenic glycosides, but compounds containing cyanide also are used as fumigants, rodenticides, and fertilizers.[15,42]

A number of plants or plant parts may accumulate large quantities of cyanide or cyanogenic glycosides, and a more complete description of them can be found elsewhere.[165] When these cyanogenic glycosides undergo hydrolysis, free HCN is formed. Plant cells contain degradative enzymes that can hydrolyze these glycosides, but under natural conditions the enzymes are kept separated spatially from the glycosides in intact cells. Damage to the plant cells by wilting, freezing, or stunting allows enzymatic degradation of the glycoside.[15] Rapid hydrolysis and release of HCN occurs only when the plant cell structure is disrupted. When the glycoside is exposed to an acid medium, or when maceration of the plant occurs within the intestinal tract, hydrolysis and subsequent formation of HCN also occurs.

A number of factors can influence the toxic potential of cyanogenic plants. Because plant cyanogenic glycosides and degradative enzymes are controlled genetically by a dominant gene, selectively breeding plants with low cyanogenic potential is possible. Therefore, species and varieties of forage affect cyanogenic content. High-nitrogen fertilization, nitrogen and phosphorus imbalance in soil, and drought conditions also can influence cyanide potential in plants.[15]

Most of the cyanogenic activity in the plant is located in the leaves and seeds, and immature and rapidly growing plants have the greatest potential for high glycoside levels. Conditions that damage the plant, such as drought, wilting, or freezing, may allow for more rapid combination of glycoside and enzyme, thereby enhancing plant toxicity. Other factors that may affect toxicity are the size of the animal, speed of ingestion, the type of food ingested along with the cyanogen, and the presence of active degradative enzymes in the plant and in the digestive tract of the horse.[15]

20.4.3.2.1

Clinical Signs

Because cyanide is such a potent, rapidly acting poison, affected animals commonly are found dead. On observation, clinical signs may range from mild tachypnea and anxiousness to severe panting, gasping, and behavioral excitement. Salivation, lacrimation, muscle tremors, defecation, urination, and mydriasis may be evident. These signs are followed by prostration, clonic convulsions, and death. The mucous membranes typically have a bright red appearance, and blood color is a bright cherry red. Clinical signs may last from only several minutes to a few hours, but horses that survive longer than 90 to 120 minutes following exposure usually survive.[6,15,42,165]

20.4.3.2.2

Pathophysiology

HCN is absorbed rapidly from the gastrointestinal tract or from the lungs. Following absorption, endogenous thiosulfate combines with the cyanide ion to form thiocyanate, which is relatively harmless. This reaction occurs in the liver and other tissues, and the generated thiocyanate is excreted in urine. Another inherent detoxification mechanism involves inactivation of HCN in the bloodstream by combining with the ferric iron of methemoglobin. However, because asmall amount of methemoglobin normally is present in blood and endogenous stores of thiosulfate can be depleted rapidly, these two endogenous mechanisms of detoxification are overcome rapidly in cases of clinical toxicity.[15]

Excess cyanide ion reacts readily with the trivalent (ferric) iron of cytochrome oxidase to form a stable cyanide–cytochrome oxidase complex. When iron is maintained in the ferric form, electron transport can no longer occur, and the chain of cellular respiration is brought to a halt. As a consequence, hemoglobin is unable to release its oxygen to the electron transport system, and cellular hypoxia results. This action occurs despite a large concentration of oxygen in the bloodstream. Cytochrome oxidase is most concentrated in tissues that have a high oxidative metabolic rate, such as the CNS and cardiac muscle. All tissues can be affected from this lack of usable oxygen, but death primarily is caused by anoxia in the brain.[15,42] 1488

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The acute oral LD_{50} of HCN is 2.0 to 2.3 mg/kg, and rapid intake of plant material equivalent to about 4 mg/kg is thought to be a lethal amount.[42]

20.4.3.2.3

Diagnosis and Treatment

One should consider cyanide poisoning when animals consuming cyanogenic plants are affected with acute signs of oxygen starvation and bright red blood. Chemical confirmation can be accomplished, and one may submit samples of forage, blood, liver, muscle, brain, and heart. One should quick-freeze all samples as soon as possible, and ship them frozen.[15,42] Plant materials containing greater than 200 ppm HCN, and concentrations in brain and ventricular myocardium greater than 100 µg/100 g wet tissue are considered significant.[42]

Treatment aims at splitting the cyanide–cytochrome oxidase complex, with subsequent removal of the cyanide complex, and augmenting available thiosulfate in the bloodstream. Sodium nitrite displaces the cyanide molecule from the cytochrome enzyme and changes some of the hemoglobin to methemoglobin, which then competes with cytochrome oxidase for the cyanide ion. In this process, methemoglobin and the

cyanide ion form cyanomethemoglobin, and cytochrome oxidase subsequently is regenerated. One should use sodium nitrite cautiously because of the possible danger of producing nitrite toxicosis, but it can be administered intravenously at doses ranging from 6 mg/kg, given as a 20% solution,[157] to 15 to 25 mg/kg.[42]

Sodium thiosulfate reacts with the cyanide ion in the blood or liberated from cyanomethemoglobin and forms thiocyanate, which is essentially harmless and is excreted in urine. One also can give sodium thiosulfate intravenously at doses ranging from 60 to 660 mg/kg, as a 20% solution,[6] to 1.25 g/kg. Additional recommended therapies include large doses of hydroxycobalamin (vitamin B_{12}) and mineral oil. The cobalt in the vitamin B_{12} preparation may bind additional cyanide in the circulation, and mineral oil aids evacuation of the gastrointestinal tract. Animals that survive 24 hours usually do not require further treatment.[42]

20.4.3.3 Sodium Fluoroacetate (1080)

Sodium fluoroacetate and fluoroacetamide are highly toxic to many animal species. They are used as rodenticides and in predator control, and because of their toxicity are available only from licensed exterminators. The compounds are odorless, tasteless, and water soluble and are typically incorporated into baits composed of carrot chunks, bread, bran, or meats. In the United States, these compounds are mixed with a black dye before being placed in baits. Horses may become intoxicated by inadvertent exposure to these baits.[15,65]

20.4.3.3.1 Clinical Signs

Sodium fluoroacetate causes signs primarily related to cardiac dysfunction in herbivores. The onset of signs usually occurs from 30 minutes to 2 hours following ingestion. When signs begin, their onset is usually acute and follow a rapid, usually violent course. Significant cardiac arrhythmias with rapid, weak pulse and eventual ventricular fibrillation are typical findings. Horses may exhibit staggering, trembling, restlessness, urination, and defecation. Moaning and bruxism may occur along with profuse sweating and signs of colic. Terminal convulsions also may occur. Ventricular fibrillation is the cause of death, and some horses may be found dead with no outward signs of struggle.[15,65]

20.4.3.3.2 Pathophysiology

Fluoroacetate is absorbed readily from the gastrointestinal tract, lungs, or open wounds, but not through intact skin. Following absorption, fluoroacetate is distributed throughout the body and does not accumulate in any specific tissue. The acute oral LD_{50} of fluoroacetate in horses is 0.35 to 0.55 mg/kg.[65]

Following entry into cells, fluoroacetate can replace acetyl coenzyme A, combining with oxaloacetate to form fluorocitrate. Fluorocitrate then competes with citrate for the active site of aconitase, a Krebs cycle enzyme, and also inhibits succinate dehydrogenase, which catalyzes succinate metabolism. The inhibition of these two enzymes and the subsequent accumulation of citrate block the Krebs cycle leads to decreased glucose metabolism, energy stores, and cellular respiration. These actions occur in all cells, but organs with high metabolic rates (such as brain and heart) are affected most severely.[7,15,65]

The short latent period between ingestion and onset of signs is because fluoroacetate must be converted to the more toxic fluorocitrate. The accumulation of toxic levels of fluorocitrate therefore requires some time. [15]

20.4.3.3.3 Diagnosis and Treatment

Diagnosis of fluoroacetate toxicity must rely heavily on history of exposure, compatible clinical signs, and absence of other pathologic findings. Laboratory detection of these compounds can be difficult, but specimens can be subjected to analysis by gas-liquid and high-performance liquid chromatography.[7,15] Fluoride ion–specific electrodes also can detect the compound in suspect samples. Suspect baits, stomach contents, liver, and kidney are the best samples to submit for evaluation.[15,65] Significantly increased kidney citrate levels suggests 1080 toxicity.[65]

Laboratory abnormalities in affected animals might include hyperglycemia and lactic acidemia.[15] However, these findings are inconclusive and accompany many disease states in the horse.

No specific antidote is available. Therapy is largely supportive and apparently unrewarding in horses already showing signs of toxicity. One may attempt intestinal decontamination using orally administered mineral oil and activated charcoal. If hypocalcemia develops, one may administer calcium gluconate or 1489
calcium chloride. Glycerol monoacetate at 0.1 to 0.5 mg/kg intramuscularly with hourly repeat treatments 1490
has been suggested but may not be effective after onset of clinical signs.[15,24]

20.5 Toxicoses Causing Signs Relating to the Epithelium, Skeletal System, and General Body Condition

Toxicoses causing these clinical signs can be among the most difficult to diagnose. Signs are often mild-moderate and quite nonspecific. In some cases the history is nonspecific and the possibility of exposure to a toxin is difficult to evaluate.

20.5.1 PLANTS

20.5.1.1 Black Walnut *(Juglans nigra)*

Shavings and aqueous extracts from black walnut trees are responsible for a toxic syndrome in horses characterized by acute onset of laminitis and variable degrees of limb edema.[166–168]

20.5.1.1.1 Clinical Signs

Horses begin showing signs of toxicity within 10 to 12 hours of being bedded with black walnut shavings. The primary signs are those of laminitis and include reluctance to move, shifting weight from limb to limb, increased digital pulse and temperature of the hoof, and positive response to hoof testers. The laminitis can vary from mild to severe.[166–168] Another characteristic finding is that of limb edema, which can become pronounced. Additional signs noted may include increased respiratory rate with flared nostrils, anorexia

and lethargy, and abdominal pain.[167] Horses removed from the bedding after clinical signs have developed have a good prognosis for full recovery.[166,167]

20.5.1.1.2

Pathophysiology

The toxic principle involved in this toxicosis is yet unidentified. Earlier, juglone, a naphthoquinone found in roots, bark, and nuts of black walnut trees, was suggested to be the causative agent.[166–168] Further work has shown, however, that an aqueous soluble toxin other than juglone that is found in the heartwood is more likely to be responsible for generation of clinical signs.[168] The mechanism of action of this soluble toxin has not been elucidated fully, but it has been shown reversibly to enhance vasoconstriction of isolated digital vessels in vitro induced by administration of epinephrine potentiated with hydrocortisone.[169]

20.5.1.1.3

Diagnosis and Treatment

Diagnosis of black walnut– induced laminitis is based primarily on known exposure to shavings containing the plant and subsequent development of clinical signs. The treatment of laminitis is covered elsewhere, but horses have a good prognosis for recovery if they are removed from the offending bedding. Black walnut shavings should not be used as bedding for horses.

20.5.1.2

Wild Jasmine *(Cestrum diurnum)*

Cestrum diurnum (wild jasmine, day cestrum, day-blooming jessamine, king-of-the-day, Chinese inkberry) is a tropical to subtropical plant native to the West Indies that has been introduced and cultivated widely as an ornamental in southern parts of the United States, including Florida, Texas, and California. The plant grows rapidly from seeds, and birds may contribute to spread of the plant because of their appetite for ripe berries that contain seeds. The plant is naturalized in Hawaii and India also and tends to multiply along fence rows, roadsides, and in neglected pastures and fields. Horses show signs of disease following ingestion of the plant for several weeks to months.[170]

20.5.1.2.1

Clinical Signs

Affected horses show signs primarily of weight loss and lameness. Weight loss occurs over several weeks to months in spite of normal appetite. Lameness is usually of increasing severity and may begin with signs of generalized stiffness. Eventually, the fetlock joints may become overextended and horses may exhibit kyphosis. The flexor tendons and particularly the suspensory ligament become sensitive to palpation. The lameness can become so severe as to require euthanasia.[170] Signs of renal failure also may become evident in isolated cases.

20.5.1.2.2

Pathophysiology

Cestrum diurnum and certain other members of the Solanaceae family contain a potent steroid glycoside with vitamin D–like activity. The toxic agent is a 1,25-dihydroxycholecalciferol (1,25[OH]2D_3) glycoside found in the leaves of the plant.[171,172] Normally, vitamin D_3 is acquired from the diet or is produced in the

skin by a reaction dependent on ultraviolet light. Vitamin D_3 is hydroxylated in the liver to yield 25-hydroxycholecalciferol, which subsequently is hydroxylated in the kidney to $1,25(OH)2D_3$. This compound is the most active form of the vitamin and acts to increase calcium absorption and stimulate production of calcium-binding protein in the intestine.[171]

The normal rate of production of $1,25(OH)2D_3$ is regulated by a negative feedback mechanism. Calcium or phosphorus deprivation stimulates the production of $1,25(OH)2D_3$, and a decreased rate of production occurs when calcium or phosphorus is present in adequate amounts.[171]

This natural feedback mechanism is bypassed when $1,25(OH)2D_3$ is supplied exogenously, as in the case of ingestion of *C. diurnum*. As a consequence, excessive calcium-binding protein is synthesized in the intestine, and excessive amounts of calcium and phosphorus are absorbed. If the calcium load exceeds the capacity of the kidney to excrete it, soft tissue mineralization (dystrophic calcification) and osteopetrosis occur.[171] In horses ingesting toxic amounts of *C. diurnum,* calcification of the flexor tendons, suspensory
ligament, and other elastic tissues appears to predominate over deposition of calcium into other soft 1490
tissues. Osteopetrosis is thought to result from sustained hypercalcemia and secondary elevation of 1491
calcitonin.[170]

20.5.1.2.3 Diagnosis and Treatment

One should suspect a diagnosis of toxicosis when horses exhibit signs of weight loss and lameness and they have known prolonged access to *C. diurnum*. Affected horses typically are hypercalcemic, but serum phosphorus concentration is usually within a normal range. Renal dysfunction, if present, may be characterized by elevations in BUN and creatinine concentrations. Urine analysis may indicate an increased fractional excretion of sodium, potassium, and phosphorus, along with other laboratory findings associated with renal failure.

No treatment for vitamin D toxicosis in known. Horses should be denied access to *C. diurnum* plants.

20.5.1.3 Hairy Vetch *(Vicia villosa)*

One report of toxicosis suspected to result from ingestion of hairy vetch is described.[173] The affected horse was a 1-year-old crossbred female presented for euthanasia because of severe weight loss and bilateral corneal ulceration with perforation.

The clinical signs reported were weight loss despite good appetite, fluctuating body temperature, subcutaneous swelling that started around the lips and spread to involve the rest of the body, and bilateral corneal ulceration with eventual perforation.

The only recorded abnormal laboratory findings consisted of elevated serum concentrations of LDH and AST measured 2 weeks after onset of clinical signs. Histologic lesions consisted of multifocal to diffuse granulomatous inflammation of the heart, lungs, kidneys, skin, lymph nodes, ileum, colon, skeletal muscle, and choroid.

The toxic substance has not been identified, and no specific therapy has been recommended. A similar toxic condition occasionally occurs in cattle grazing hairy vetch, with most cases of toxicosis occurring between April and July.

20.5.1.4

Photosensitizing Plants

Many plants can cause photosensitive reactions in horses. Some plants contain photodynamic substances that are absorbed from the gastrointestinal tract intact or in a form metabolically altered into an active compound (primary photosensitizing plants). Other plants may cause photodermatitis following liver dysfunction, in which the photodynamic toxin is a metabolite normally excreted in bile (secondary photosensitivity). Because of hepatic damage induced by the plant, these metabolites enter the circulation and subsequent interaction with light results in clinical manifestation of disease. Box 20-1 lists plants[6,165,174] known to induce photosensitization in herbivores.

20.5.1.4.1

Clinical Signs

1491

Signs of photosensitization are similar, regardless of the cause, and vary in degree. Factors that influence 1492 the severity of signs include the amount of reactive pigment present in the skin at a given time, the degree of exposure to light of appropriate wavelength, and the severity of hepatic damage, if hepatogenous photosensitization is the cause.[174]

20.5.1.4.1.1

BOX 20-1 PLANTS INDUCING PHOTOSENSITIZATION IN HERBIVORES

20.5.1.4.1.1.1

Primary Photosensitizers

Ammi majus (bishop's weed): Contains furocoumarins

Avena fatua (oatgrass)

Brassica (rape)

Cooperia pedunculata

Cymopterus watsonii (spring parsley): Contains furocoumarins

Erodium (trefoil)

Fagopyrum sagittatum (buckwheat): Contains the naphthodi-anthrone derivative fagopyrin

Hypericum perforatum (St. John's wort, Klamath weed): Contains the naphthodianthrone derivative hypericin

Perennial ryegrass

Ricinus communis (castor bean)

Rutaceae

Trifolium (clover)

Umbelliferae

20.5.1.4.1.1.2 Secondary or Hepatogenous Photosensitizers

Agave lecheguilla (lecheguilla)

Blue-green algae

Brachiaria brizantha

Brassia hyssopifolia

Brassica napus (cultivated rape)

Holocalyx glaziovii

Lantana spp.

Lippia rehmanni

Myoporum laetum (ngaio)

Narthecium ossifragum (bog asaphodel)

Nolina texana (sacahuiste)

Panicum spp. (panic grass, kleingrass)

Pithomyces chartarum and *Pithomyces minutissima*

Senecio spp. (ragwort, groundsel)

Tetradymia canescens (gray horsebrush)

Tetradymia glabrata (spineless horsebrush)

Tribulus terrestris (puncture vine)

20.5.1.4.1.1.3 Other Photosensitizers

Avena (oats)

Euphorbia maculata (milk purslane)

Kochia scoparia (summer cyprus, fireweed)

Medicago (alfalfa)

Polygonum spp. (smartweed)

Sorghum vulgare (Sudan grass)

Trifolium (clover)

Vicia spp. (vetches)

Restlessness and discomfort are usually the first signs one notes. Erythema may be apparent, followed by edema of affected areas. Blister formation and subsequent serum exudation and scab formation occur as the condition progresses. Affected sites are usually painful to touch, and animals often attempt to protect themselves from direct sunlight.[6,174]

The light-colored or nonpigmented areas of skin are affected most severely, particularly those areas involving the face, nose, back, escutcheon, and coronary band. Severely affected animals also may have involvement of pigmented areas of skin, and secondary self-trauma and bacterial infection may arise from the attempts of the animal at rubbing the affected areas. Horses may lose patches of skin that slough in large, leathery plaques.[6,174]

20.5.1.4.2 Pathophysiology

Plants causing primary photosensitization contain a photodynamic agent that is absorbed from the gastrointestinal tract intact or that is later metabolically altered into an active compound. Secondary photosensitizing plants induce hepatic damage of sufficient magnitude to inhibit adequate clearance of photodynamic agents. Normally, these photodynamic toxins are metabolites excreted in bile. When the liver is damaged sufficiently or bile flow is hindered, these metabolites enter the circulation. Phylloerythrin, a normal chlorophyll breakdown product, is considered the only important photodynamic substance in instances of secondary photosensitization.[15] These photodynamic agents then are circulated throughout the body, eventually reaching the dermal capillaries.

Interaction of long-wave ultraviolet light with these photodynamic agents circulating in the skin capillaries results in chemical excitement of these substances. As a consequence, free radicals form that are highly inflammatory and cause degradation of cell phospholipid membranes, polypeptide proteins, and nucleic acids.[6,175] These processes disrupt the cells and ultimately result in the dermal lesions noted with this toxicity. One should remember that drugs and diseases that cause liver damage also potentially can cause photosensitivity.

20.5.1.4.3 Diagnosis and Treatment

Clinical signs of photosensitivity are fairly typical and seldom are confused with other afflictions of skin. When clinical signs become evident, one should evaluate the horse thoroughly for evidence of hepatopathy because primary and secondary photosensitivity produce the same clinical signs. If hepatic disease is present, one should ascertain the cause and institute appropriate therapy.

One can identify primary photodynamic agents with several biologic assay systems. A mouse assay system and a microbial assay using *Candida albicans* are available for this purpose.[6]

Specific therapy for photodermatitis is not available. Horses should be removed from direct sunlight until skin lesions are healed and the offending plant is removed from the environment. One may use various topical agents to help skin healing. One should treat superficial bacterial dermatitis with appropriate antibiotics, and nonsteroidal antiinflammatory therapy may be beneficial during initial stages of the disease process.

20.5.1.5 Pyrrolizidine Alkaloids

Pyrrolizidine alkaloid toxicity results from consumption of plants that contain various pyrrolizidine alkaloids. Horses can become intoxicated by ingestion of fresh plants and dried plants incorporated into hay. As with many toxic plants, though, these plants are frequently unpalatable to most horses, yet certain conditions may render them more appetizing. This toxicity is characterized by a chronic, progressive disorder manifested by signs of liver failure. The more common plants that cause toxicosis in horses include *Senecio jacobaea* (tansy ragwort), *S. vulgaris, S. longilobus* (groundsel), *Amsinckia intermedia* (fiddleneck), *Crotalaria* species (rattlebox), *Echium plantagineum* (Viper's buglos), *Heliotropium europaeum* (common heliotrope), and *Cynoglossum officinale* (houndstongue).[176,177]

20.5.1.5.1 Clinical Signs

Signs of pyrrolizidine alkaloid intoxication in horses are essentially those of liver failure. The more frequent signs noted include weight loss of weeks to months in duration, icterus, and behavioral abnormalities. The behavioral changes indicate hepatoencephalopathy and may include aimless pacing or wandering, ataxia, licking inanimate objects, blindness, head pressing, and uncharacteristic aggression. Convulsions and coma may precede death. Clinical signs of abnormal behavior usually are a terminal event in the disease process and typically have an acute onset.[177,178] Other signs less frequently reported include diarrhea, photosensitization of nonpigmented areas of skin, hemoglobinuria, and inspiratory dyspnea.[177,179] Abortion and poor exercise tolerance (reduced athletic performance) also have been observed in horses following ingestion of sublethal amounts.[180] Because toxicity of these plants is related to liver dysfunction, clinical signs of disease may not become apparent for weeks to months following ingestion.

20.5.1.5.2 Pathophysiology

A number of pyrrolizidine alkaloids are present in various plant species, and some plants may contain
multiple alkaloids. These substances are absorbed from the gastrointestinal tract and carried to the liver 1492
where they are metabolized by hepatic microsomal enzymes to pyrroles. These pyrroles then can cross- 1493
link double-stranded DNA and bind to proteins and nucleic acid within hepatocytes.[176,177]

The cross-linking of DNA has an antimitotic effect on hepatocytes. The hepatocytes are unable to divide and subsequently become megalocytes. As these cells die, they are replaced by fibrous tissue rather than normal hepatocytes. Binding of protein and nucleic acid results in inhibition of cytoplasmic protein synthesis. These changes may lead to more rapid death of hepatocytes and cause centrilobular necrosis. Eventually, liver function begins to fail because of progressive hepatocellular death and subsequent fibrosis.[176,177] As the disease progresses, generalized fibrosis develops. Once connective tissue bridges form between portal areas, the disease is fatal.[176]

Variation in the dosage and frequency of administration of alkaloids results in a wide spectrum of hepatic lesions. Acute toxicosis resulting from massive doses is more likely to produce centrilobular necrosis with hemorrhage. Chronic doses tend to produce hepatocellular death in the portal areas, along with megalocytosis, fibrosis, biliary hyperplasia, and occlusion of hepatic veins.[176,177] Liver failure is thought to be ultimately responsible for the clinical signs observed in pyrrolizidine alkaloid toxicosis.

The toxic dose of dried *Senecio* is estimated to be 5% of the body weight of the horse. This amount does not need to be ingested all at once, however, because the effects are cumulative. The total dose of alkaloids consumed determines the toxic effect, regardless of the amount of time in which the alkaloids were consumed.[176,177]

20.5.1.5.3 Diagnosis and Treatment

Most cases of pyrrolizidine alkaloid toxicosis are diagnosed based on history, compatible clinical signs, serum liver enzyme activities, and liver biopsy findings. When active hepatocellular damage is occurring early in the disease process, sorbitol dehydrogenase and LDH usually are elevated, but they often decline to normal values by the time the horse is showing clinical signs. Serum GGT, alkaline phosphatase, and AST activities tend to be elevated throughout the disease course. Serum concentration of bile acids also is reported to be elevated in affected horses. Serum bilirubin concentration tends to be elevated in later stages of disease, and hypoglycemia and hypoalbuminemia rarely are seen except in cases of severe hepatic disease.[176,177,179,180]

Other diagnostic aids that may be useful from a diagnostic and prognostic standpoint include measurement of the ratio of branched-chain amino acids to aromatic amino acids in serum and liver biopsy. The branched-chain amino acids isoleucine, leucine, and valine are catabolized primarily in muscle, and the aromatic amino acids phenylalanine and tyrosine are catabolized mainly in the liver. The ratio of the sums of these amino acids (branched-chain to aromatic) has been shown to decrease progressively from normal in horses affected with pyrrolizidine alkaloid toxicosis. If the ratio is below normal range and the horses are exposed continually to alkaloid-containing plants, they have a poor chance of survival. Some affected horses also showed a dramatic decrease in this ratio just before death.[181] Liver biopsy findings of a triad of fibrosis, bile duct proliferation, and megalocytosis are highly suggestive of pyrrolizidine alkaloid toxicosis. Liver biopsy samples also can help in establishing prognosis because the presence of advanced or generalized hepatic fibrosis warrants a grave prognosis.[176,177]

One may analyze feed samples for pyrrolizidine alkaloid content, but the process is often time-consuming and expensive.[176]

No specific treatment for pyrrolizidine alkaloid toxicity exists. Affected horses may survive if they are denied further exposure to alkaloid-containing plants and are fed a suitable diet. However, some horses still may show signs of liver disease even though they have not had exposure to the plant for some time. Specific treatment of acute and chronic liver failure is discussed elsewhere.

Prevention is accomplished by keeping horses away from pasture or feed contaminated with pyrrolizidine alkaloid–containing plants.

20.5.1.6 Tall Fescue *(Festuca arundinacea)*

The causative agent of the disorder associated with grazing tall fescue is *Neotyphodium coenophialum*[182] (formerly identified as *Acremonium coenophialum*), a fungal endophyte that grows within the stem, leaf sheaths, and seeds of tall fescue.[183,184] This endophyte is highly indigenous to many areas of the United States and may contaminate as much as 90% of fescue pastures in certain geographic areas.[183]

20.5.1.6.1

Clinical Signs

Horses grazing or being fed tall fescue hay preparations may develop a condition termed *summer slump* or *summer syndrome*.[6,185] The disorder is characterized by anorexia, weight loss, poor hair quality, pyrexia, and hypersalivation. Additionally, mares may sustain a variety of pregnancy- and reproductive-related disorders. Typical findings include the presence of a thick, tough placenta, prolonged gestation, abortion, birth of dead or weak foals, and high perinatal foal mortality.[186] Mares frequently are affected also with agalactia, retained placenta, and rebreeding difficulties.[6,175,183]

20.5.1.6.2

Pathophysiology

The fungus produces multiple toxins, including peramines, lolines, and ergopeptine alkaloids. Peramines have no apparent effect on animal health, but the lolines (*N*-acetyl loline and *N*-formyl loline) present in *Neotyphodium* are pyrrolizidine alkaloids. However, the hepatotoxicity characteristic of pyrrolizidine alkaloids has not been observed with tall fescue toxicosis in any species.[184] 1493

1494

The ergopeptine alkaloids appear to be responsible for most of the abnormalities associated with fescue toxicosis. Ergotamine, ergosine, ergovaline, ergoine, ergocristine, ergocryptine, and ergocornine have been isolated, but ergovaline and ergosine are most prominent. Ergovaline is thought to account for 84% to 97% of the ergopeptine concentration of infected tall fescue. The concentration of ergot alkaloids in tall fescue tends to increase with nitrogen fertilization and drought stress, and toxicity varies from season to season and year to year depending on the percentage of endophyte present, drought stress, nitrogen fertilization, and probably other factors.[184]

Agalactia occurs for a variety of reasons. First, ergopeptines are dopamine D_2-receptor agonists, and dopamine is thought to be the major inhibitor of prolactin secretion in the body. Second, ergot alkaloids inhibit adrenocorticotropic hormone secretion, resulting in reduced fetal cortisol concentration that subsequently causes reduced progesterone secretion by the placenta. Third, these alkaloids decrease tissue binding of estradiol, which can lead to an elevated serum concentration of estradiol-17β[184] (normally, serum estradiol concentrations decline near parturition). The interaction of appropriate levels of prolactin, progesterone, and estradiol-17β plays a major role in preparing the mammary gland for lactation. The combination of reduced concentrations of prolactin and progesterone and elevated concentration of estradiol-17β likely is the major cause of the agalactia and impaired udder development in affected mares.

The gestation period may be prolonged because ergopeptines have been hypothesized to block corticotropin-releasing hormone activity in the foal, which results in a lack of fetal production of adrenocorticotropic hormone and cortisol. Because increased fetal cortisol concentration acts to signal the mare for parturition, lack of fetal production of corticotropin-releasing hormone, adrenocorticotropic hormone, and cortisol may contribute to extended gestation periods in affected mares.[184]

The placental abnormalities frequently noted have been suspected to be associated with vasoconstriction. Edema, fibrosis, and mucoid degeneration of arteries in placentae from affected mares have been observed.[185] These changes were thought to be consistent with anoxia, which was hypothesized to be associated with vasoconstriction. Ergovaline and *N*-acetyl loline, which are present in infected fescue, have vasoconstrictive properties.[184]

Ergot alkaloids also may have a negative effect on fertilized ovum implantation in the endometrium, resulting in reduced reproductive efficiency of affected mares. In the mare the effects of ergopeptines on implantation are inconclusive, but ergocryptine, ergocornine, ergosine, and ergovaline are capable of interrupting early pregnancy in the rat.[184]

Foals born alive to affected mares may be hypothyroid, although the mechanism responsible for this is unknown.[186] High perinatal foal mortality probably also is influenced by agalactia and poor colostral antibody production by the mare, resulting in failure of passive transfer with associated septicemia and failure of the foal to thrive.[184]

20.5.1.6.3 Diagnosis and Treatment

Diagnosis is often empiric, based on clinical signs and access to fescue pasture or hay during late gestation. Affected mares typically have reduced serum concentrations of prolactin and progesterone, and elevated levels of estradiol-17β. Reduced serum concentrations of triiodothyronine, adrenocorticotropic hormone, cortisol, and total progestogens may be present in affected foals. One also may evaluate hay and pasture samples for the presence of endophytes and ergovaline.[184]

Treatment of gravid mares past their foaling date should include removal from fescue as quickly as possible. Domperidone given orally once daily at 1.1 mg/kg during the last 15 days of gestation may be efficacious in helping establish udder development and lactation. One may give postfoaling mares domperidone (1.1 mg/kg orally) twice daily for several days to try to stimulate milk production.

Prevention requires removing mares from fescue pasture or hay during late gestation. In one study, fescue removed from the diet at 300 days of gestation was not associated with any problems in mares.[185] Thus the most critical time for exposure to infected fescue in pregnant mares seems to be during the last 30 days of pregnancy. One should consult local or regional authorities for current methods of managing infected pastures.

20.5.1.7 Hoary Alyssum *(Berteroa incana)*

Toxicosis in horses related to ingestion of hoary alyssum was first reported in 1992.[187,188] This plant is a member of the Cruciferae (mustard) family and can behave as an annual, biannual, or perennial. Hoary alyssum grows primarily in the northeastern and north central United States and Canada but also is reported in the states of Oklahoma, Washington, and California and in Europe. The plant tends to flourish under conditions of drought, frost, and overgrazing, and in areas of poor soil.[188,189] Toxicity has been reported in horses grazing contaminated pasture, eating plant-contaminated hay, and being fed oats containing hoary alyssum seed.[187–190] Reported cases are more prevalent during summer months after ingestion of recently cut hay, but toxicosis has occurred from feeding hay stored for 9 months.[188]

20.5.1.7.1 Clinical Signs

Not all horses exposed to hoary alyssum develop clinical signs of toxicosis. An estimated 45% of horses 1494
exposed to the plant under field conditions did not develop any signs of illness. However, the most 1495
common clinical signs observed under field and experimental conditions were varying degrees of fever,

edema of one to four limbs, and laminitis. Horses affected with laminitis rarely are reported to develop rotation of the distal phalanges. These signs typically commence within 24 hours of ingestion and in most cases subside within 2 to 4 days following removal of the source.[188] Other signs noted have been lethargy, short-term diarrhea, and abdominal discomfort.[189] Early parturition and abortion are rare occurrences in pregnant mares.[187,189] Rarely, individual horses are affected more severely and exhibit clinical signs of endotoxemia, hypovolemic shock, acute renal failure, and ventral rotation of the distal phalanges. Death can occur in these horses.[188,190]

Pregnant mares may be more susceptible to toxicity and develop more severe clinical signs, however. In one report, 23 of 29 broodmares developed signs of fever, tachycardia, tachypnea, distal limb edema, and mild to severe laminitis.[189] Fifteen of these mares subsequently developed moderate to profuse bloody diarrhea, dehydration, abdominal pain, hematuria, and oliguria. Four of these mares were euthanized, and two had necropsy findings of hemoperitoneum, hemothorax, and ventral rotation of distal phalanges. Three of the mares aborted spontaneously, but no abnormalities were noted in the placentae or fetuses.

20.5.1.7.2 Pathophysiology

The toxic agent in hoary alyssum remains unknown,[188] as does the mechanism of action of the toxic substance(s). However, red blood cell destruction appears to occur via hemolysis or some other mechanism.[188,189] No laboratory abnormalities have been noted in an experimental feeding trial and in most field cases.[188] However, laboratory findings in more severely affected horses include significant red blood cell hemolysis; elevations in serum concentrations of creatinine, urea nitrogen, phosphorus, alkaline phosphatase, aspartate transaminase, CK, sorbitol dehydrogenase, and total bilirubin; neutopenia; proteinuria; hematuria; hemoglobinuria; and occult blood in feces and gastric fluid.[188–190] Which of these abnormalities are the direct result of toxin and which result from physiologic deterioration of various organs is unknown.

20.5.1.7.3 Diagnosis and Treatment

One should suspect a diagnosis based on history of exposure to the plant and clinical signs. No specific therapy is indicated, but horses immediately should be denied access to the plant, and all contaminated hay and grain should be removed. One should initiate symptomatic treatment with fluids, nonsteroidal antiinflammatory medications, and gastrointestinal tract evacuation. Activated charcoal (1 to 3 g/kg administered via nasogastric tube) has been suggested to help prevent absorption of the toxin.[190] One should use intravenously administered DMSO with caution because it has potential to cause intravascular hemolysis, thereby compounding the red blood cell destruction caused by the toxin.[188,190] Most horses recover uneventfully in 2 to 4 days following removal of the plant and supportive care.

20.5.2 MEDICATION (IODINE)

Iodine toxicosis or iodism is a rarely reported cause of toxicosis in horses. When iodism occurs, it most likely is caused by iatrogenic administration of iodine-containing substances. Many rations contain iodine in the form of various iodized salts, and sodium iodide and potassium iodide, along with the organic iodide compound ethylenediamine dihydroiodide, are used to treat various medical conditions.[24,191]

20.5.2.1
Clinical Signs

Nonpruritic generalized alopecia and diffuse scaliness of the skin were reported in a horse given 45 g of ethylenediamine dihydroiodide twice daily for 14 days.[192] Other reported clinical signs include goiter following excessive iodine intake, increased secretions of the respiratory tract, nasal discharge, intermittent nonproductive cough, and excessive lacrimation.[15,24,192] Pregnant mares receiving excessive amounts of iodine may produce weak foals with enlarged thyroid glands. Such foals have a high mortality rate.[24]

20.5.2.2
Pathophysiology

Organic and inorganic forms of iodine are absorbed rapidly and almost completely from the gastrointestinal tract in the ionic form and are distributed throughout the body. Iodine is excreted primarily in the urine, but smaller amounts are present in feces, sweat, and milk.[15,191] The only known metabolic role of iodine is involvement in synthesis of the thyroid hormones thyroxine and triiodothyronine.[15]

Oral iodine salts, whether organic or inorganic, taken in higher doses stimulate nerve receptors in the stomach wall. Subsequently, the vagus nerve becomes stimulated and causes reflex secretion by cells in the upper respiratory tract.[15,193] Excessive iodine intake causes thyroidal organic iodine formation to increase to a maximal amount, and then a sharp decline in organic iodine formation occurs. Excessive iodine also inhibits release of organic iodine from the thyroid gland if the gland is stimulated by thyroid-stimulating hormone. The net results are clinical signs of increased amount and viscosity of respiratory tract secretions and occasional goiter development.[193] The mechanism underlying the development of the dermal lesions associated with iodism is unknown.[192]

20.5.2.3
Diagnosis and Treatment

Diagnosis of iodism is based on a history of exposure to high levels of iodine for a prolonged time coupled 1495
with compatible clinical signs of nasal discharge and excessive lacrimation, intermittent nonproductive 1496
cough, and nonpruritic generalized alopecia and scaling. One can measure serum iodine concentrations, which are elevated in cases of iodism, but blood levels decrease rapidly to near background levels within a few days if iodine exposure ceases.[15] Serum concentrations of thyroxine and triiodothyronine may be below normal values in affected horses.[192]

Treatment consists in removing the source of the iodine. Because iodine is mobilized rapidly and excreted from tissues, clinical signs usually subside rapidly when excessive iodine is removed.[15,191,192]

20.5.3
MISCELLANEOUS AGENTS

20.5.3.1
Snake Venom

The venomous snakes in North America belong to the families Crotalidae (pit vipers), Elapidae (cobra), and Viperidae (true vipers). Most poisonous snakebites reported in human beings are inflicted by members of the Crotalidae family, and the same is probably true for horses as well. Of this family, *Crotalus* (rattlesnakes),

Agkistrodon (copperhead, cottonmouth), and *Sistrurus* (pigmy rattlesnake, massasauga) are the three genera most commonly involved. The eastern coral snake *(Micrurus fulvius)* and the Arizona coral snake *(Micruroides euryxanthus)* are two members of the Elapidae family indigenous to the United States, but they account for only about 3% of poisonous bites reported in human beings.[6,7] No documentation of coral snakebite affecting horses could be found in the literature.

Venom injected into prey is an aid to digestion and greatly reduces the time for complete digestion to occur in the snake. The amount of venom injected at a given time is under voluntary control, and larger amounts are injected into larger prey or when the snake strikes for defensive purposes. Not all bites result in envenomation, and rattlesnakes are estimated to fail to inject venom in up to 20% of bites.[6,7]

Snake venom is a highly complex mixture of enzymes, lipids, biogenic amines, free amino acids, metal ions, proteins, and polypeptides. Most snake venoms contain up to 25 different fractions, yet many of them are not yet identified. Venom composition and toxic properties vary among *Crotalus* species and between individuals within the same species. Factors influencing venom composition include age, time from last feeding, and seasonal influences related to changes in feeding patterns or physiologic responses such as hibernation. The LD_{50} in mice exposed to venom of *Crotalus* species ranges from 0.23 mg/kg for the Mojave rattlesnake (*C. scutulatus*) to 3.77 mg/kg for the red diamond rattlesnake (*C. ruber ruber*). The LD_{50} for mice for the copperhead (*Agkistrodon contortrix*) and cottonmouth (*A. piscivorus*) is 10.92 mg/kg and 4.17 mg/kg, respectively.[7] This large variation in dose and chemical composition of venom accounts for the extreme amount of variation in the physiologic responses of animals to these substances.

20.5.3.1.1 Clinical Signs

Snake bites in horses are most common on or near the muzzle, but bites also may occur on the limbs or other parts of the body. The classic signs noted in most horses are acute onset of swelling and edema at the bite site. The muzzle and nasal passages may become swollen to the extent that respiration can become extremely labored, necessitating a tracheotomy. Epistaxis also may be apparent. Initially, one may note fang marks, but they soon disappear with the onset of extensive swelling. Skin discoloration at the injection site is a common occurrence with many rattlesnakes but rarely occurs with copperhead and Mojave rattlesnake bites.[6] Varying degrees of necrosis of local tissue may occur, as well as secondary bacterial infections at the wound site. In the author's experience, bites involving the distal extremities of horses frequently have a prolonged convalescent period that may be accompanied by residual lameness.

Systemic manifestations of snake envenomation may be apparent when venom is injected intravascularly or perivascularly. In a retrospective study of rattlesnake venom poisoning in 32 horses, manifestations included fever, tachycardia, tachypnea, caridac arrhythmia, hemolytic anemia, thrombocytopenia, hemorrhage, thrombosis of venipuncture sites, colic, diarrhea, and prehensile and masticatory dysfunction. Chronic problems included cardiac disease, pneumonia, laminitis, pharyngeal paralysis, and wound complications. The most common chronic problem observed in these horses was cardiac disease.[194] Labored respiration may result from pulmonary edema caused by passive congestion following vascular hypotension or precipitation of pulmonary emboli. Muscle fasciculation may be evident.[6] The venom of the Mojave rattlesnake produces respiratory paralysis that may result in death in human beings.[7] Overall mortality rate in 32 horses acutely affected with bites of prairie rattlesnake was 25%.[194]

The venom of coral snakes produces neurologic signs in affected human beings, with death occurring within 24 hours as a result of respiratory depression, hypotension, and cardiovascular collapse.[7] However, the coral snake requires prolonged contact (30 seconds or greater) to work the venom into the skin of its prey. Therefore for horses to become exposed to coral snake venom except under extremely unusual circumstances seems improbable.

20.5.3.1.2

Pathophysiology

1496

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The venoms of Crotalidae are rich in enzymes. Proteases cause severe tissue damage by digesting tissue proteins and peptides, and hyaluronidase allows rapid spread of venom through tissue by hydrolyzing connective tissue hyaluronic acid. l-Amino acid oxidase, l-arginine ester hydrolases, and 5′-nucleotidase also may contribute to tissue destruction. Phospholipases A, B, C, and D hydrolyze lipids and cause hemolysis by destroying lecithin in the red blood cell membranes. They also disrupt neurotransmission at the presynaptic and postsynaptic junctions. Other enzymes present in crotalid venom include ribonuclease, deoxyribonuclease, transaminase, phosphomonoesterase, phosphodiesterase, ATPase, DNAase, alkaline phosphatase, acid phosphatase, and endonuclease.[7]

Crotalid venom contains a number of polypeptides in addition to the enzymes. These polypeptides are low-molecular-weight proteins that are 5 to 20 times more lethal than crude venom in animal models, and they do not have enzymatic activity. They are present in higher concentrations in cobra venom than in rattlesnake venom and are mainly responsible for blood dyscrasias and coagulopathies. Small peptides are partly responsible for the generation of disseminated intravascular coagulation, and a venom fraction of the timber rattlesnake (*Crotalus horridus horridus)* causes platelet aggregation with resultant thrombocytopenia. Additionally, the venoms of the Mojave and Southern Pacific rattlesnakes contain a direct cardiotoxin.[7]

Rattlesnake venom contains substances with anticoagulant, procoagulant, and plasminogen-induced fibrinogenolytic properties. The coagulopathy that occurs in a given instance varies and depends on the content of the various venom components and on the dose of venom injected. The anticoagulant activity of crotalid venom appears to result from reversible binding of venom to prothrombin. Thrombinlike enzymes produce hypofibrinogenemia and increased concentration of fibrin degradation products. They are also capable of directly converting fibrinogen to fibrin, which may result in excessive fibrin formation and rapid disseminated intravascular coagulation. The fibrinolytic characteristics of crotalid venom occur directly or indirectly through the activation of endogenous plasminogen.[6,7]

20.5.3.1.3

Diagnosis and Treatment

Diagnosis of snakebite usually depends on clinical signs and accessibility to poisonous snakes. Laboratory abnormalities that may be present in affected horses include thrombocytopenia, hypofibrinogenemia, anemia, prolonged PT and PTT, hematuria, proteinuria, and myoglobinuria.[6,7]

Affected horses should be kept calm and quiet. Incision over the fang marks and suction are rarely indicated and probably of minimal value except in the immediate time frame of the bite, because the venom is absorbed almost immediately into the surrounding tissues. Tracheotomy is indicated in those horses that develop excessive edema and swelling of the head and external nares to the point that respiration becomes impaired. Topical cold therapy may have some beneficial effect if applied early and

for short periods. Prolonged or excessive cold application, however, may enhance further tissue necrosis. Antivenom therapy commonly is used in instances of human snakebite but often is considered unnecessary in horses because of the low mortality rate, financial considerations, and availability of other therapies. An exception may be the valuable animal or foals.

One should initiate tetanus prophylaxis and systemic antibiotic therapy. One should use broad-spectrum antimicrobials because gram-positive and gram-negative organisms are found in the mouth of North American pit vipers. The most common organisms isolated include *Proteus vulgaris, Escherichia coli, Corynebacterium, Streptococcus,* and Enterobacteriaceae.[7]

One should not use NSAIDs during the early stages of the bite wound because they may enhance the thrombocytopenia frequently induced by snake venom. One may use NSAIDs in the later stages of the disease to help reduce pain, swelling, and inflammation. One should use corticosteroids with caution because they may diminish the clearance of fibrin degradation products from the peripheral vasculature by the reticuloendothelial system, and they may increase susceptibility to wound infection at the site. Corticosteroids may be beneficial, however, in treating severe hypotensive shock in young animals or in patients with intravenous envenomation. Heparin therapy also has been suggested to be helpful in instances of thrombus formation.[6]

One should treat animals affected with systemic hypotension and cardiac dysrhythmias appropriately. Treatment may include the administration of intravenous fluids and plasma and the use of specific antiarrhythmic medications.

20.5.3.2 Fluoride

Fluoride toxicosis in horses is apparently an rare event.[195] Although acute and chronic fluoride toxicosis have been described in various animals, chronic fluorosis appears more common. Common sources of fluoride in chronic fluorosis include forages subjected to airborne contamination from nearby industrial plants such as aluminum smelters, steel mills, or fertilizer plants that heat fluorine-containing materials and discharge fluorides; drinking water containing excessive fluoride; feed supplements and vitamin and mineral additives with high fluoride concentration; and vegetation grown on soils containing high fluoride levels.[15]

Animals normally ingest small amounts of fluoride throughout their lives, and it accumulates in the body as
long as constant or increasing amounts are ingested. Chronic toxicosis can result from prolonged ingestion of 1497
sufficiently high levels. The long-term dietary tolerance for fluoride in the horse is reported to be 40 to 60 1498
ppm dietary fluoride.

Fluoride is absorbed almost totally from the gastrointestinal tract. Once absorbed, approximately half is excreted rapidly in urine, with the remaining half being stored in bone and teeth. Fluoride accumulates in calcified tissues, but once exposure ceases, bone fluoride is depleted slowly over months to years.

20.5.3.2.1 Clinical Signs

Chronic fluorosis in the horse is a rare event. In one suspected case the affected horse exhibited chronic weight loss of months' duration, poor growth, difficulty in mastication, and deformed, discolored, and absent deciduous incisors. The horse also was missing some deciduous premolars and molars.[195] Classic dental abnor-malities reported in other species include mottled, hypoplastic enamel and brown

discoloration with uneven wear of teeth.[15] Additional signs associated with chronic fluorosis in other animals include hyperostosis, enlargement and roughening of involved bones, intermittent lameness and generalized stiffness, dry and roughened hair coat, and decreased weight and milk production.[195] Because of the insidious nature of chronic fluorosis, one must remember that a time lag may occur between ingestion of excessive fluoride and the appearance of clinical signs.

20.5.3.2.2 Pathophysiology

Excessive fluoride produces dental abnormalities by affecting the teeth during development. The primary effect of fluorine is thought to be a delaying and alteration of normal mineralization of the preenamel, predentine, and precementum. High fluoride levels appear to cause specific ameloblastic and odontoblastic damage. The matrix laid down by these damaged cells fails to accept minerals normally, resulting in faulty mineralization of the tooth bud. Once the tooth is formed fully, the ameloblasts have lost their constructive ability and the enamel lesions cannot be repaired. However, odontoblasts can produce secondary dentine to compensate for deficiencies brought about by excessive fluoride.[15] The brown to black discoloration of affected teeth results from oxidation of organic material in the teeth.

The pathogenesis of bone lesions associated with fluoride toxicosis is still undecided. One theory is that high fluoride levels lead to inadequate matrix and defective, irregular mineralization of bone. Another theory is that hydroxyl radicals in the hydroxyapatite crystal structure are replaced by fluoride ions, resulting in a decrease in crystal lattice dimensions. The pathologic results of skeletal fluorosis include dissociation of normal sequences of osteogenesis, production of abnormal bone, accelerated remodeling of bone, and occasional accelerated bone resorption.[15]

20.5.3.2.3 Diagnosis and Treatment

Diagnosis of chronic fluorosis is based primarily on clinical findings and history of possible exposure to fluorides. One usually confirms fluorosis by analysis of skeletal or dental tissues for fluoride content and evaluation of fluoride concentration in urine. One also should analyze water and feed consumed by the animals for fluoride content.

Treatment of chronic fluorosis aims mainly at dietary restriction of fluoride-containing substances. Aluminum sulfate, aluminum chloride, calcium aluminate, calcium carbonate, and defluorinated phosphate have been used to reduce the toxic effects of fluoride, but no substance completely prevents the toxic effects of ingesting excessive amounts of fluorides.

20.5.3.3 Zinc

Zinc intoxication is a problem of young, growing horses. Sources of excessive zinc are usually soil and forage contamination from nearby smelters and following topdressing of pastures with zinc oxide.[196,197] Classic signs of zinc toxicity also have been produced by experimental feeding of excess zinc to young foals.[198,199] Skeletally mature horses do not appear to be susceptible to the effects of zinc-contaminated pastures.[199]

20.5.3.3.1 Clinical Signs

Typical signs associated with zinc toxicity include swelling at the physeal region of long bones, gradual onset of lameness and stiffness that may become so severe that affected animals are reluctant to rise from lateral recumbency, swollen joints resulting from synovial effusion, unthriftiness, and weight loss despite normal appetite.[196–198] Anemia also may develop in more chronically affected individuals.[198] The joint swellings in affected horses are typical of those of osteochondrosis desiccans, and severe generalized osteochondrosis also develops in zinc-intoxicated horses.[196,197,199]

20.5.3.3.2 Pathophysiology

Zinc toxicity in horses actually appears to be a manifestation of copper deficiency and the subsequent development of hypocupremic-induced articular cartilage disease.[196,199] Copper is an essential co-factor for lysyl oxidase, an enzyme involved in the formation of collagen cross-links. Copper deficiency interferes with collagen metabolism and results in production of weak connective tissue. This allows articular cartilage fractures and growth physeal fractures to occur in the zone of hypertrophic cells, producing the clinical syndrome of osteochondrosis desiccans.

The mechanism of zinc-induced copper deficiency is not understood totally. Experimentally intoxicated foals at necropsy had high hepatic copper content despite low serum copper concentration, suggesting that the hepatic copper was not readily available for production of ceruloplasmin or could not be mobilized rapidly enough for use by other tissues.[199] Excess zinc stimulates production of metallothionein, an
intestinal cell protein that binds to excess zinc, copper, and other divalent metal ions and facilitates their 1498
excretion in bile, feces, and saliva. Copper has a higher affinity for metallothionein than does zinc, and 1499
increased production of metallothionein, with subsequent binding to copper, may lead to copper deficiency via increased copper excretion.[200]

20.5.3.3.3 Diagnosis and Treatment

Once signs of osteochondrosis develop, one should treat the condition appropriately. Diagnosis of zinc toxicity may be difficult in that zinc is excreted rapidly following absorption, and blood and tissue zinc concentrations tend to decline quickly to normal levels with cessation of intake. One can measure liver, kidney, and serum zinc concentrations, but fecal samples collected from affected horses may be more suitable. One also can evaluate feed and water supplies for zinc content.[201]

Treatment of affected horses aims to restore proper copper concentration in the diet and to remove the source of excess zinc. Diets containing 7.7 mg copper per kilogram and 250 mg zinc per kilogram of dry weight were sufficient to maintain normal serum copper and zinc concentrations and did not induce disease in treated foals. Diets containing 1000 mg/kg or greater of zinc caused hypocupremia and subsequent osteochondrosis desiccans when fed to foals over a period of several weeks.[199] Osteochondrosis desiccans is treated surgically.

20.5.3.4
Selenium

Selenium toxicity in horses usually results from prolonged ingestion of plants containing excessive amounts of selenium. Intoxication can result from horses foraging on soil with high levels of selenium and from ingestion of selenium-accumulating plants growing on soils with minimal amounts of selenium.[201–204] Acute toxicosis also can occur through inadvertent overdose of selenium supplements added to rations or given by parenteral injection.[205] The acute single oral toxic dose of selenium given as sodium selenite lies between 3.3 and 6 mg/kg for the horse.[201,206]

20.5.3.4.1
Clinical Signs

Three different syndromes are attributed to selenium intoxication: acute toxicity and two chronic forms described as “blind staggers” and “alkali disease.” Signs of acute toxicity develop within 6 hours of ingestion and include sweating, diarrhea, tachycardia, tachypnea, mild pyrexia, lethargy, and mild to severe colic. Death may occur within 24 hours, and some horses exhibit a dumb attitude before death. Head pressing before death is suggested as a classic sign of acute selenosis.[201,206]

Chronic selenium intoxication described as blind staggers results from frequent ingestion of plants over weeks to months. Signs associated with this syndrome include aimless wandering or circling, muscle weakness, incoordination, respiratory difficulty, and decreased vision. In classic cases, blindness eventually develops and is followed by paralysis and death.[207]

Alkali disease also occurs from ingestion of plants, and signs may develop within 3 weeks. Initially, lameness and swelling of the coronary band regions are apparent, along with anorexia and mild depression. These signs progress to transverse cracking of the hoof wall distal to the coronary band with associated lameness. The hooves eventually may be sloughed. Loss of hair from the mane and tail occurs because the hairs become brittle and are broken easily. Compromised reproductive function also may develop in affected horses.[201–205]

20.5.3.4.2
Pathophysiology

Selenium is absorbed readily from the gastrointestinal tract, but organic forms of selenium generally are retained in greater amounts than are inorganic forms. Elimination occurs rapidly through urine, sweat, feces, and exhaled air. One also must recognize hoof and hair as routes of excretion because excess selenium is deposited in these structures.[205] This latter fact has important diagnostic implications.

The mechanism whereby excess selenium produces these signs is not elucidated fully. Selenium functions in a number of enzymatic and physiologic processes. The toxic effects of selenium have been associated with its affinity for reacting with sulfur-containing amino acid residues such as cystine that are incorporated synthetically into biologically active glycoproteins and polypeptides. As a result, various selenosulfides are formed as a substitute for disulfide bonds.[208]

20.5.3.4.3

Diagnosis and Treatment

One can make a presumptive diagnosis of selenium intoxication based on typical clinical signs and history of possible exposure to selenium iatrogenically in the form of selenium supplementation or via ingestion of appropriate plants. One can attempt to make a definitive diagnosis by assaying serum, hair, and hoof material for selenium content. In acute fatal selenosis, serum selenium concentration may exceed 1 ppm.[201,206] A selenium concentration greater than 5 ppm in hair and hoof wall can be considered diagnostic of selenosis.[201,202,204] One also can measure selenium concentration in liver and kidney samples.

Treatment of affected horses involves removing the selenium source and symptomatic treatment of lesions and good nursing care. Oral dosing of naphthalene at 4.5 g/day for 5 days, waiting 5 days, and then repeating the dose for an additional 5 days has been suggested for treatment of adult horses.[201,202] Prevention of selenosis has been attempted by adding copper,[205] methionine,[209] or sodium arsenite[201,202] to the diet of at-risk animals.

20.5.3.5

Magnesium

Magnesium is an important cation involved in the regulation of many enzyme systems. Magnesium is found primarily intracellularly, with only about 1% of the total body content circulating in the vascular space. Of
this amount, approximately two thirds is unbound in the ionized or active form and one third is bound to 1499
plasma proteins.[210] Magnesium is absorbed primarily from the small intestine, with little or no absorption 1500
occurring in the large intestine. Absorption begins rapidly within 1 hour of ingestion, and the amount absorbed depends on the quantity presented to the intestine, intestinal transit time, interactions with other minerals, and integrity of the intestinal mucosa.[211,212] The serum concentration and total body content of magnesium are regulated primarily by renal filtration.[210] Because of this, hypermagnesemia is difficult to maintain when renal function is normal.

20.5.3.5.1

Clinical Signs

Magnesium toxicosis has not been reported following consumption of natural feedstuffs and occurs only from excessive administration of commercial products. Acute magnesium toxicosis results in signs of impaired motor function in horses, and respiratory paralysis, cyanosis, and cardiac arrest also can occur.[213] One report describes the clinical findings of magnesium toxicosis as a complication in two horses given magnesium sulfate orally for treatment of intestinal impactions.[214] These horses exhibited signs of muscle tremors followed by recumbency. They appeared to maintain normal consciousness but exhibited flaccid paralysis of the neck and limb muscles. Both horses were tachypneic and tachycardic while recumbent, but heart and respiratory rates returned to normal following treatment. Serum magnesium concentration was elevated (14.7 mg/dl and 15.8 mg/dl) in the affected horses at the time of recumbency. Resolution of clinical signs was rapid (within minutes to hours) in both horses following treatment.

20.5.3.5.2

Pathophysiology

When an action potential arrives at a neuromuscular terminal (effecting depolarization), Ca^{2+} enters the presynaptic nerve terminal, causing exocytosis of acetylcholine into the synaptic cleft. Magnesium acts as

a neuromuscular blocking agent by inhibiting this Ca^{2+} influx into the presynaptic nerve terminal, thereby preventing the release of acetylcholine into the synaptic cleft.[215] High concentrations of magnesium also are reported to reduce motor end plate sensitivity and decrease muscle membrane excitability, which also also can result in neuromuscular paralysis.[216] The tachycardia and tachypnea noted in the aforementioned horses was thought to result from secondary reflex responses to hypotension because magnesium infusion capable of causing recumbency in normal horses does not change heart rate or rhythm.

20.5.3.5.3

Diagnosis and Treatment

One can suspect a diagnosis of magnesium toxicosis based on clinical signs and history of recent magnesium administration. One can determine the serum magnesium concentration to confirm the diagnosis. Calcium solutions given intravenously should result in rapid improvement of clinical signs, but additional calcium administration over the course of several hours may be necessary for complete resolution of clinical signs. When possible, one should monitor serum magnesium and calcium concentrations in affected horses and use the results to guide further therapy.

20.5.3.6

Gangrenous Ergotism *(Claviceps purpurea)*

Claviceps purpurea is a fungal parasite that invades the developing ovary of the grass flower and cereal grains. This fungus commonly parasitizes rye, oats, wheat, and Kentucky bluegrass, which are associated most often with outbreaks of gangrenous ergotism. The fungus replaces the seed ovary with a dark brown to purple oblong body called a sclerotium. Sclerotia are slightly larger than the original whole grain seed, and their growth is promoted by warm, moist conditions. Horses rarely are affected with this condition in part because of the distasteful nature of affected feedstuffs and because most fungal elements are removed during commercial grain processing procedures.[15,175]

20.5.3.6.1

Clinical Signs

Signs of intoxication may become apparent if infected feeds are ingested over several days to weeks. Dry gangrene of the extremities is the classic sign associated with *C. purpurea* toxicosis and can affect the limbs, nose, ears, and tail. Early signs of toxicosis are lameness and cool extremities. The hindlimbs often are affected first, with swelling and tenderness in the fetlock area. The involved tissues become dark and discolored and a transverse line of demarcation may occur between normal skin and the distal parts of the limb. Eventually, the hoof and associated bones and tissue may slough. This same sequence can involve the nose, ears, and tail. Gangrenous signs may be preceded by colic and constipation or diarrhea. Subacute effects can include depression, partial anorexia, general unthriftiness, and increased pulse and respiratory rates.[15,175]

20.5.3.6.2

Pathophysiology

The major toxic alkaloids in ergot are divided into three groups: ergotamine, ergotoxine, and ergometrine. They are absorbed slowly and incompletely from the gastrointestinal tract, reaching peak plasma concentrations in approximately 2 hours. The liver is the primary site of metabolism, and approximately 90% of metabolites are excreted in bile. Small amounts of unmetabolized alkaloids are excreted in urine.

The total concentration and proportions of alkaloids present in ergot sclerotia may vary with species and environmental conditions.[15,175]

Ergotamine and ergotoxine are polypeptide derivatives of lysergic acid. The varied physiologic effects of ergot are caused primarily by mixtures of levoisomers of ergotamine, coupled with smaller amounts of acetylcholine, histamine, and tyramine.[15]

Ergotamine is a vasoactive substance that causes arterial and venous constriction. Ergotamine also may
damage capillary endothelium. The combined effects of vasoconstriction and endothelial damage produce 1500
increased blood pressure, decreased blood flow through the extremities, vascular stasis, thrombosis, and 1501
eventually gangrene.[15,175]

The ergotoxine group of alkaloids produce α-adrenergic blockade and antagonize 5-hydroxytryptamine. This produces an increase in blood pressure as a result of peripheral vasoconstriction, particularly in postcapillary vessels.[15]

20.5.3.6.3 Diagnosis and Treatment

Tentative diagnosis of ergotism is based on clinical signs and exclusion of other disease processes. An experienced person can readily identify ergot sclerotia in grains. However, once grinding of feed has occurred, one can recognize ergot only by microscopic examination or chemical analysis for ergot alkaloids. One can identify and quantitate ergot alkaloids by chromatographic methods, and one should obtain a sample of the individual grain components for analysis whenever possible.[15]

No specific treatment exists for gangrenous ergotism. The offending grain should be removed, and affected animals should be kept warm to avoid cold-induced vasoconstriction in the extremities. Supportive therapy in the form of antibiotics and analgesics may be indicated. The use of anti–α-adrenergic pharmaceuticals to effect vasodilation also may be helpful. Such agents might include acepromazine, isoxsuprine, phenoxybenzamine, or similar products.[15,175]

20.6 Toxicoses Causing Signs Relating to the Urinary System

Detection of renal disease can be difficult, regardless of cause. Whereas nephrotoxicity associated with equine administration of medications is well established and usually recognized, that linked to ingestion of plants is less commonly detected and reaching a definitive diagnosis is harder. Although heavy metals like cadmium and mercury have long been known for their nephrotoxic effects, clinical signs are nonspecific and the diagnosis will be made only if the clinician performs diagnostic tests to specifically rule these problems in or out.

20.6.1 PLANTS

20.6.1.1 Oxalate Toxicosis

The most common source of oxalates for livestock is plants, particularly those of the Chenopodiaceae family. These plants contain varied amounts of soluble oxalates, usually in the form of sodium or potassium salts. However, because plants containing oxalates are generally unpalatable to horses, plant-associated oxalate intoxication is rare. The following plants contain large amounts of soluble oxalates:

Amaranthus spp.	Pigweed
Beta vulgaris	Beet, mangold
Chenopodium album	Lambsquarters
Halogeton glomeratus	Halogeton
Oxalis spp.	Wood sorrel, soursob
Portulaca oleracea	Purslane
Rheum rhaponticum	Rhubarb
Rumex spp.	Sorrel, dock
Salsola kali	Russian thistle
Sarcobatus vermiculatus	Black greasewood

Of these, *Halogeton* and *Sarcobatus* seem to be the primary offenders in range animals in the western United States.[15]

Because oxalates accumulate in the plants throughout the growing season, the incidence of toxicosis may be highest in the fall and winter months.[217] The oxalate content is highest in the leaves, with a lesser amount in seeds and a minimal amount present in the stems. The nonfatal toxic dosage of sodium oxalate for adult horses is approximately 200 g/day for 8 days.[15]

20.6.1.1.1 Clinical Signs

Affected horses may begin to show signs of depression, mild to moderate colic, muscular weakness, and irregular gait within 2 to 6 hours of ingestion. Weakness may proceed to lateral recumbency, unconsciousness, and death in 10 to 12 hours. Some animals may exhibit convulsions before succumbing.[15] The observed clinical signs of acute toxicity are typical of those of hypocalcemia.

20.6.1.1.2 Pathophysiology

Oxalates combine with serum calcium ions to form insoluble calcium oxalate. The result is a functional hypocalcemia in acute cases, with associated signs of altered behavior and neuromuscular abnormalities.

More chronic ingestion of oxalates can result in renal failure. Insoluble calcium oxalate crystals can lodge in the renal tubules, producing tubular blockage and necrosis. Oxalates also may crystallize in the vasculature and infiltrate blood vessel walls, producing necrosis and hemorrhage.[15]

20.6.1.1.3 Diagnosis and Treatment

In instances of acute toxicosis, consistent clinicopathologic abnormalities include moderate to significant hypocalcemia and varied electrolyte alterations. In chronic toxicity, urinalysis may reveal the presence of characteristic calcium oxalate crystals on microscopic examination. Impending renal failure also is characterized by increases in BUN and creatinine concentrations.[15]

Treatment is usually of little value once clinical signs have appeared. One can administer calcium gluconate intravenously, but it usually provides only temporary relief of signs. Balanced electrolyte solutions are indicated to aid diuresis, and diuretics also may have benefit in the volume-loaded patient. Prevention primarily aims keeping horses from the plants by means of providing adequate suitable sources of feed.[15]

20.6.1.2 Rayless Goldenrod *(Isocoma wrightii)*

Ingestion of rayless goldenrod may produce renal tubular nephrosis and signs of renal failure, but tremetol is 1501
the most significant toxin present in the plant.[83,218] For a detailed discussion of tremetol toxicity, see the 1502
discussion of white snakeroot toxicity.

20.6.1.3 Sorghum

Ingestion of *Sorghum* species and certain hybrid Sudan grasses has been associated with the development of an ataxia-cystitis syndrome.[219,220] The toxicity occurs when horses graze the plants. More cases occur when the plant is young and rapidly growing, but mature and second-growth plants also have been incriminated. Horses being fed well-cured *Sorghum* species hay have not developed signs of toxicity. Occurrence of toxicity may increase during seasons of medium to high rainfall, but no cases have been recognized following the date of the first frost. Signs of toxicity may develop following a grazing period of 1 week to several months.[219]

20.6.1.3.1 Clinical Signs

The primary clinical signs are those of posterior ataxia and urinary incontinence or cystitis. The neurologic signs usually develop first and begin as posterior ataxia and incoordination. Affected horses may sway from side to side if forced to move, and signs tend to worsen on backing the animal. Occasionally the rearquarters may drop almost to the ground, and flaccid paralysis of the tail and the rear legs may develop within 24 hours of the onset of neurologic signs. Affected horses remain alert and afebrile and have a normal appetite and pulse and respiratory rates. Mares frequently exhibit continual opening and closing of the vulva and relaxation of the perineal muscles. Males typically have a relaxed and extended penis.[219,220]

Urinary incontinence exhibited by continual urine dribbling is prominent in both sexes, and urine scalding on dependent skin becomes pronounced. The urinary bladder typically is distended and atonic, resulting in moderate to severe cystitis. Urethritis and ureteritis also may develop, and in horses that die from the disease, ascending pyelonephritis is usually the cause of death. Other clinical signs include abortion and birth of foals with multiple arthrogryposis.[219,220]

20.6.1.3.2 Pathophysiology

The clinical signs result from axonal degeneration and demyelination of nerve fibers in the spinal cord, particularly in the lumbar and sacral segments. The toxic substance in *Sorghum* species responsible for causing this change is unknown. Most *Sorghum* species are cyanogenic plants and contain various amounts of HCN. Exposure to multiple sublethal doses of HCN has been suggested to induce axonal degeneration and demyelination.[219] Another hypothesis is that sorghum plants contain lathrogenic

precursors and that this toxicosis may be caused by the ingestion of lathrogenic nitriles present in rapidly growing plants.[15,220]

20.6.1.3.3 Diagnosis and Treatment

Diagnosis of sorghum ataxia-cystitis is based primarily on approriate clinical signs, history of grazing the plants, and exclusion of other known causes of posterior ataxia or paresis. No specific diagnostic tests are available. Cystitis and pyelonephritis are diagnosed by standard laboratory methods.

No specific treatment is available. One should remove affected horses from the offending feed immediately. Once the feed is removed, affected horses usually show gradual improvement over several weeks to months, but complete recovery may not occur. Supportive and symptomatic therapy should include appropriate antibiotic treatment of bacterial urinary tract infections and topical treatment of urine scald dermatitis. Periodic manual decompression of the urinary bladder may be helpful. Catheterization and frequent aspiration of bladder contents may be necessary to aid resolution of cystitis.

20.6.2 MEDICATIONS

20.6.2.1 Aminoglycosides

The aminoglycoside antibiotics commonly are used because of their wide spectrum of bactericidal activity against gram-negative organisms. However, these drugs have significant toxic potential. Toxicity in horses is manifested almost exclusively by nephrotoxicosis, but eighth cranial nerve dysfunction, neuromuscular blockade, and direct myocardial depression have been described in other species, including human beings.[221]

The development of toxicity depends on a number of variables. Of the group, streptomycin is least nephrotoxic; neomycin is most nephrotoxic; and gentamicin, kanamycin, and amikacin are intermediate in their ability to cause renal damage. Because the aminglycosides are eliminated primarily by the kidney, any cause of reduced renal function can result in increased serum concentration of the drug and increased potential for nephrotoxicity. Other factors that enhance the toxic potential of these agents include acidosis, dehydration, hypovolemia, endotoxemia, increased dose or frequency of administration of the drug, and prolonged use of the drug. The concurrent use of other potentially nephrotoxic drugs, such as NSAIDs, also may predispose to aminoglycoside nephrotoxicity.[221–223] Although foals have not been shown to be more sensitive than adults to aminoglycoside nephrotoxicity, one should monitor closely use of these products in young animals that have potential for sepsis, hypotension, or dehydration.[224]

20.6.2.1.1 Clinical Signs

The clinical signs of aminoglycoside nephrotoxicity are those of acute renal failure. One may note anorexia and depression while the horse is being medicated or shortly after treatment has stopped. Most cases of acute renal failure caused by aminoglycoside toxicity are nonoliguric, and polyuria is a frequently reported clinical finding. Chapter 17.4 discusses the clinical signs of acute renal failure. 1502

20.6.2.1.2

Pathophysiology

The aminoglycosides are highly polar cations that are poorly lipid soluble but highly water soluble. They are absorbed minimally from the intestinal tract, but rapid absorption occurs from intramuscular and subcutaneous sites of injection.[225,226] Distribution is essentially to the extracellular fluid space in the body, and high concentrations of drug occur only in the renal cortex and the endolymph and perilymph of the inner ear.[226] Binding to plasma proteins is minimal, and excretion occurs almost entirely by glomerular filtration of unchanged drug.

A small portion of filtered aminoglycoside binds to phospholipid receptors on the brush border of cells of the proximal convoluted tubules and pars recta. Subsequently, the drug is reabsorbed, primarily by pinocytosis, and accumulates in lysosomes and other subcellular compartments of proximal tubular cells. This net reabsorption results in high concentrations of aminoglycoside within the renal cortex.[226,227] Concentrated aminoglycoside within the renal cortex forms a poorly exchangeable drug pool, with a renal tissue half-life of several hundred hours. This contrasts to the serum half-life of aminoglycosides, which is several hours. Therefore an aminoglycoside may be excreted slowly in urine for weeks after dosage is discontinued, even if serum levels are undetectable.

Eventually, proximal tubular cell damage and death occur. The mechanisms resulting in cell death are understood poorly, but several have been hypothesized: binding of aminoglycosides to plasma phospholipids, inducing alterations in plasma membrane structure and function; altered phospholipid accumulation and metabolism, resulting in impairment of lysosomal degradation and phagocytosis of cellular debris; activation of cellular phospholipases; damage to mitochondrial respiration, leading to reduced production of cellular ATP; and altered Na^+,K^+-ATPase activity. Although all of these actions may occur, which mechanism is primary still is unclear. The ultimate step leading to cell death may involve changes in subcellular calcium homeostasis, but the precise mechanism is unknown.[227]

In addition to proximal tubular dysfunction, aminoglycoside toxicity also results in a decreased glomerular filtration rate. Proposed mechanisms for this phenomenon include release of vasoactive hormones causing alterations in renal blood flow; damage to proximal tubular cells leading to backleak of fluid and waste products across the damaged epithelium; and obstruction of individual nephrons causing increased hydrostatic pressure within the tubular lumen with subsequent reduction in the net ultrafiltration pressure within the glomerular capillaries.[227] With aminoglycoside nephrotoxicity, acute severe tubular necrosis rarely occurs, and impaired renal function is almost always reversible because of the capacity of the proximal tubular cells to regenerate.[226]

20.6.2.1.3

Diagnosis and Treatment

Aminoglycoside nephrotoxicity initially is manifested by evidence of proteinuria, hematuria, cylindruria, polyuria, and enzymuria. Under experimental conditions, a rise in the urinary GGT ratio was the earliest detectable laboratory change noted with gentamicin toxicity and preceded increases in urinary protein and fractional excretion of phosphate by about 48 hours.[228] Proteinuria and cylindruria may be present 3 to 6 days after initiation of treatment and may precede elevations in serum creatinine and urea nitrogen concentrations by several days.[221,223] From a practical standpoint, monitoring urine for the presence of

protein appears to be a sensitive means of detecting early drug toxicosis. If aminoglycoside toxicity results in acute renal failure, other clinicopathologic findings compatible with acute renal failure will be evident.

Therapeutic drug monitoring has been shown to be an effective means of decreasing the incidence of aminoglycoside nephrotoxicity in human patients. Measuring serum peak and trough concentrations of aminoglycosides allows use of individual dosage regimens, particularly in the high-risk patient. In human patients and foals, elevated trough values have been associated with an increased incidence of nephrotoxicity[229,230] and are more predictable indicators of toxicity than are serum peak concentrations. In high-risk patients, one should monitor serum peak and trough concentrations frequently. In human beings, trough concentrations less than 2 μg/ml for gentamicin and tobramycin and less than 5 μg/ml for amikacin are desirable.[231] Optimal trough concentrations for the aminoglycosides in equine patients have not been established, but extrapolation from human values may provide useful guidelines. In the author's laboratory, trough concentrations of less than 1 μg/ml for gentamicin and tobramycin and less than 2.5 μg/ml for amikacin are considered desired values.

One usually treats aminoglycoside nephrotoxicity by drug dosage adjustment (with the aid of therapeutic drug monitoring) or drug withdrawal, fluid diuresis, and alkalinization of urine. In patients that require aminoglycoside therapy, increased dosage interval or reduced dosage may be necessary to minimize nephrotoxicity. Horses receiving moderate to large volumes of fluid therapy rarely develop nephrotoxicity and in fact usually have less than the expected serum concentration of aminoglycoside when given at recommended dosages.

Diet also may influence the nephrotoxic potential of gentamicin because horses fed oats had a greater degree of gentamicin-induced nephrotoxicosis than did horses fed only alfalfa.[232] Low dietary calcium, sodium, and potassium have been shown to potentiate gentamicin-induced nephrotoxicosis in laboratory animals, and high dietary calcium provided a protective effect against gentamicin-induced nephrotoxicity 1503
in rats.[233] The effect of increased dietary calcium, sodium, and potassium on the nephrotoxic potential of 1504
the aminoglycosides in horses warrants further study. Additional treatments suggested for aminoglycoside-induced nephrotoxicity have included peritoneal dialysis, plasmapheresis, and hemodialysis to reduce the serum concentration of these drugs.[224]

20.6.2.2 Amphotericin B

Amphotericin B is a polyene antibiotic used to treat systemic fungal diseases, including blastomycosis, coccidioidomycosis, histoplasmosis, candidiasis, sporotrichosis, mucormycosis, chromoblastomycosis, aspergillosis, and pithiosis. Its use in the horse rarely is reported, but horses with histoplasmosis and subcutaneous pithiosis have been treated successfully with the drug.[234,235] Amphotericin B is a known nephrotoxin, and therapeutic and toxic levels of the drug overlap.[231]

Clinical signs of toxicity reported in horses include depression, anorexia, weight loss, anemia (ranging from mild to severe), and fever.[234,235] One horse receiving treatment for an extended period also developed polyuria and polydipsia.[235] All clinical signs abated following cessation of therapy.

Amphotericin B causes distal tubular epithelial cell damage and renal ischemia. Tubular cell damage results from amphotericin B combining with sterols on the cholesterol-rich lysosomal membranes of the distal tubular cells. Direct injury to the cell membrane allows for increased cell permeability, leakage of cytosol, and cell death. Excessive potassium, bicarbonate, and water are lost into the urine, and hydrogen ions gain

entrance back into the epithelial cells. Hypokalemia and metabolic acidosis can develop and lead to death if renal disease is progressive.[236]

Renal vasoconstriction also plays a role in toxicity of amphotericin B. Significant afferent arteriolar vasoconstriction occurs by mechanisms not fully elucidated, but activation of the tubuloglomerular feedback system has been suggested as a possible cause.[231]

Diagnosis of toxicity is based largely on increasing concentration of BUN and abnormal urine findings of cylindruria, hematuria, and proteinuria. In human patients, an increase in BUN concentration greater than 40 to 50 mg/dl is cause for reduced dosage or temporary discontinuance of the drug.[237]

Treatment of toxicosis involves fluid diuresis and reduced dosage or temporary discontinuance of the drug until the BUN concentration reaches a normal value. Because nephrotoxicity caused by this drug is reversible, cessation of treatment results in return of renal function to almost normal levels. Additional therapies one may use include the concomitant use of mannitol and oral sodium loading in an attempt to decrease the occurrence of azotemia.[231]

20.6.2.3 Sulfonamides

The sulfonamides are a group of antimicrobial agents widely used in equine practice to treat gram-positive and gram-negative bacterial infections. Historically, they have been incriminated as a cause of renal dysfunction but now probably rarely are involved in nephrotoxicity.

The sulfonamides are poorly soluble in water but highly soluble in alkaline solutions. They are excreted via the kidney. The nephrotoxic potential of sulfonamides results from their precipitation into crystals in renal tubules, leading to obstruction and anuria or tubular epithelial necrosis. Crystal formation increases with increasing urine acidity and ultimately leads to acute renal failure.[15,231] Animals that are hypovolemic or have reduced renal function from other causes may be at greater risk of developing nephrotoxicity than other animals.

Diagnosis is based largely on signs of acute renal failure along with a history of drug use and presence of sulfonamide crystalluria. Treatment aims at fluid diuresis and concurrent urine alkalinization.[231]

The potentiated sulfonamides (trimethoprim-sulfonamide combinations) have much greater urine solubility and have not been incriminated as a cause of nephrotoxicity in horses. No documented adverse effects of these products in horses exist, but anecdotal reports have associated their use with acute onset diarrhea.[238] Drug withdrawal is usually sufficient therapy in such cases. In Great Britain, the concomitant use of intravenously administered trimethoprim-sulfadoxine with detomidine or halothane has been associated with severe cardiac dysrhythmias and sudden death in several horses.[239,240] One should avoid the simultaneous use of these products in horses if possible.

20.6.2.4 Vitamins D_2 and D_3

Horses are capable of meeting their requirement for vitamin D if they are exposed to sunlight or have access to sun-cured forages. Although dietary requirements for the horse have not been established, a maximum safe level of 44 IU/kg body mass per day has been proposed for long-term feeding (>60 days).[241] Most cases of

vitamin D intoxication are iatrogenic, resulting from overzealous use of vitamin supplements or from improperly formulated vitamin D–supplemented feeds. Ingestion of *Cestrum diurnum* (wild jasmine) also can result in vitamin D toxicosis because this plant contains a metabolically active glycoside of 1,25-dihydroxycholecalciferol (see Wild Jasmine *[Cestrum diurnum]*).

Vitamins D_2 (ergocalciferol) and D_3 (cholecalciferol) are potentially toxic, but vitamin D_3 is much more
active and results in more severe lesions with wider tissue distribution than does an equivalent dose of 1504
vitamin D_2.[242,243] Other variables that may affect toxicosis include duration of treatment and route of 1505
administration. High concentrations of dietary calcium also might enhance the effects of excessive amounts of vitamin D. The effect of vitamin D supplementation is cumulative, and signs of toxicity may occur weeks after supplementation has begun.

The clinical signs associated with vitamin D toxicosis are associated with impairment of the renal, cardiovascular, or musculoskeletal systems. Signs may include depression, anorexia, weakness, polyuria and polydipsia, cardiac murmurs and tachycardia, limb stiffness with impaired mobility, and recumbency. Calcification of tendons, ligaments, and other soft tissue structures may be palpable on physical examination.[242] Ultrasonographic examination of these structures also may demonstrate abnormal mineralization within the tissues.

The toxicity of excessive amounts of vitamin D_3 results from extensive dystrophic mineralization rather than from any inherent toxicity of vitamin D itself. Soft tissue sites most frequently affected include the kidneys, the endocardium and walls of large blood vessels, and tendons and ligaments.[242,243]

Laboratory findings associated with toxicosis can vary with the organ system affected, but generally include pronounced and persistent hyperphosphatemia and hypercalcemia, although the latter can vary daily. Serum calcium concentration may remain within a normal range in some horses. Other laboratory evidence of chronic renal failure may become evident with progression of toxicosis. One can make a definitive diagnosis by measuring serum concentrations of vitamins D_2 and D_3 and 1,25-dihydroxycholecalciferol.[243]

Treatment of vitamin D intoxication should include removal of all exogenous sources of vitamin D. A cation chelator such as sodium phytate may be helpful in reducing intestinal absorption of calcium, but the efficacy of this product has not been determined. One should use symptomatic therapy for renal insufficiency and failure, if necessary. Recovery may take months in less severely affected horses,[243] but treatment is usually unrewarding if excessive mineralization has occurred.

20.6.2.5 Menadione Sodium Bisulfite (Vitamin K_3)

Vitamin K_3 is a reported cause of acute renal failure in horses, but the product has been withdrawn from the United States market. When the product was given at the manufacturer's recommended dosage of 2.2 to 11 mg/kg intravenously or intramuscularly, signs of toxicity became evident within 6 to 48 hours following injection in affected horses.[244] Clinical signs included depression, anorexia, colic, hematuria, and stranguria. Azotemia, electrolyte abnormalities, proteinuria, and isosthenuria also were apparent. Pathologic lesions noted at necropsy were those of acute tubular necrosis. Interstitial fibrosis and chronic renal failure were also present in one horse.[244] Treatment of affected horses is symptomatic for acute or chronic renal failure.

20.6.3 MISCELLANEOUS AGENTS

20.6.3.1 Cadmium

Intoxication with cadmium rarely is reported in horses but has been seen in animals raised near smelting operations.[196] Environmental contamination of soil and forage by cadmium and zinc was the cause of excessive intake.

Affected horses exhibited signs of unthriftiness, lameness, and swollen joints. Some of these signs were attributed to excess zinc in the diet, but the horses also had pronounced osteoporosis and nephrocalcinosis, which along with proteinuria are typical findings of cadmium toxicosis in human beings.[196]

Serum concentrations of zinc and potassium were elevated in these horses, and the serum magnesium concentration was low in one foal. Sodium, calcium, chloride, and bicarbonate concentrations were also decreased. Extensive nephrocalcinosis was characterized by multifocal loss of cortical tubules, which were replaced by dense deposits of calcium phosphate crystals.

Cadmium induces change in proximal renal tubular cells by an unknown mechanism. However, increased numbers of lysosomes and mitochondrial swelling in the proximal tubular cells are early changes. Proteinuria is usually the first abnormality noted in human beings and laboratory animals. With continued chronic exposure, fibrosis and atrophy resulting from interstitial nephritis may ensue, leading to chronic renal failure.[245]

In human beings, treatment is essentially supportive, with elimination of exposure to cadmium being imperative. Research data suggest a possible beneficial role of zinc, vitamin B complex, and nickel preparations, but their clinical efficacy is unproven.[245]

20.6.3.2 Hemoglobin and Myoglobin

The endogenous substances hemoglobin and myoglobin can be a cause of acute renal failure in horses when they are present in serum in excessive amounts. Myoglobin nephrosis may occur following severe muscle damage such as extensive crushing or bruising injuries, large burns, heatstroke, or exertional rhabdomyolysis (tying-up). Excessive hemoglobinemia usually results from extensive intravascular hemolysis caused by acute hemolytic anemia, incompatible blood transfusions, or intravenous administration of certain medications, that is, hypotonic fluids and concentrated DMSO solutions. Possible causes of acute hemolytic anemia include *Babesia caballi* or *B. equi* infection, neonatal isoerythrolysis, phenothiazine toxicosis, onion 1505
(*Allium* species) toxicosis, and ingestion of withered red maple *(Acer rubrum)* leaves. Severe intravascular 1506
hemolysis also may occur with fulminant hepatic failure and with immune-mediated hemolytic anemia caused by antierythrocyte antibodies. Equine infectious anemia may cause intravascular hemolysis, but hemoglobinuria and pigment nephrosis rarely are associated with this disease.[222]

20.6.3.2.1 Clinical Signs

Clinical signs of pigment nephropathy are those of acute renal failure along with grossly discolored urine. Urine of affected horses is usually tinged red to brown, which can vary in intensity. Additionally, horses

with myoglobinuria would be expected to exhibit some degree of muscle soreness or inflammation or have a history of muscle trauma.

20.6.3.2.2

Pathophysiology

Excessive amounts of hemoglobin or myoglobin presented to the kidney result in tubular nephrosis by yet undetermined mechanisms. Hemoglobin casts are usually present within the renal tubules of affected horses and may induce ischemic injury to the tubular cells. Hemoglobin nephropathy also has been suggested to be caused by red blood cell stromal elements rather than by hemoglobin itself.[246] More recent studies in rats suggest that iron may play a significant role in the development of pigment nephropathy caused by hemoglobin or myoglobin. Iron liberated from the hemoglobin or myoglobin molecule can promote formation of oxygen free radicals, which then initiate lipid peroxidation and other reactions, leading to renal injury.[247] In these studies, hemoglobin-induced renal injury was attenuated greatly by deferoxamine, an iron chelator that binds ferric iron. Additional factors that may contribute to the development of pigment nephropathy include hypovolemia or dehydration, circulatory failure, endotoxemia, acidosis, and hypoxia.

20.6.3.2.3

Diagnosis and Treatment

The appearance of blood or hemoglobin in urine is not specific for pigment nephropathy, so other signs and laboratory findings are important for diagnosis. Hematuria, hemoglobinuria, and myoglobinuria result in a positive orthotoluidine reaction for occult blood on multitest dipsticks. However, hematuria is evident by the presence of intact red blood cells on microscopic examination of urine sediment, and anemia may be present if hematuria is of sufficient magnitude. With hemolysis the serum is usually discolored pink, and one can verify hemoglobinemia by routine laboratory methods. Anemia also may be present if hemolysis is severe. Elevated serum CK concentration is characteristic of rhabdomyolysis, and affected horses usually exhibit some degree of muscle soreness or have a history of muscle trauma. Myoglobin is poorly bound to plasma proteins and is filtered rapidly through the glomerulus. As a result the serum remains a normal color. Definitive tests for myoglobin in urine include electrophoresis or immunoassay techniques.[222,248]

Treatment of pigment nephropathy is essentially supportive and consistent with that of acute renal failure from other causes. One should identify any predisposing cause of excessive myoglobin or hemoglobin and remove it if possible. Compatible blood transfusions may be necessary in horses with severe hemolytic anemia, and one should treat horses with exertional rhabdomyolysis or other muscle injury accordingly.

20.6.3.3

Mercury

Mercury exists in a variety of organic and inorganic forms. Both forms can be toxic to horses, but more recently reported cases involve acute toxicity resulting from inorganic mercury-containing blistering agents topically applied to skin.[152,249] Ingestion of feed or seed grain contaminated with organic mercurial seed preservatives has been a source of contamination in previous years.

Acute and chronic forms of toxicosis can occur in horses. The acute toxic dose of inorganic mercury in adults is 5 to 10 g.[15] Experimentally, chronic toxicity from inorganic mercury has been produced by ingestion of

mercuric chloride 0.8 mg/kg/day over 14 weeks.[250] Chronic organic mercury toxicosis also has been produced experimentally by feeding methylmercury at 0.4 mg/kg/day for 10 weeks.[251]

20.6.3.3.1

Clinical Signs

Signs associated with toxicity of the various mercurial compounds can differ, but they all include some degree of renal dysfunction. Acute toxicity resulting from inorganic mercury can cause signs of acute renal failure, including oliguria and depression, and signs of gastrointestinal irritation. Ulcerative stomatitis, excessive salivation, colic, and diarrhea are common findings associated with gastrointestinal tract disturbances.[152,249] Chronic intoxication with inorganic mercury can result in signs of oral ulceration, reduced appetite and weight loss, alopecia, progressive respiratory difficulty, gradually increasing urine production, and terminal azotemia.[250] Signs reported with chronic organic mercury toxicity include development of neurologic dysfunction characterized by proprioceptive deficits, exudative dermatitis, reluctance to move, reduced appetite and weight loss, dullness, and renal changes exhibited by a steadily increasing BUN concentration and glucosuria.[251]

20.6.3.3.2

Pathophysiology

Inorganic mercury compounds are absorbed from the lungs and gastrointestinal tract and are absorbed poorly through the skin. Following ingestion and absorption, accumulation in the liver and particularly the kidney occurs. Some forms of organic mercury are degraded in the body to inorganic forms, which then also accumulate in the kidney before excretion.[15]

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Inorganic mercury is concentrated to high levels within the proximal renal tubular cells. Metallothionein, a low-molecular-weight metal-binding protein is synthesized within 48 hours following exposure to heavy metals. This protein binds mercuric ions within the endoplasmic reticulum of the tubular epithelial cells and then slowly releases mercury. This slow release of sequestered mercury can cause continuing damage to tubular cells after the source of mercury is removed.[252] Hence the development of mercury nephropathy appears to be a function of the amount of protein-bound mercury concentrated in the renal tubules. Bound mercury can persist in the kidneys for several weeks following exposure.[250] Acute toxicity results in massive tubular necrosis and acute renal failure, and chronic exposure may cause renal interstitial fibrosis leading to chronic renal failure.

Methylmercury can be biotransformed in the body to inorganic mercury, but methylmercury also accumulates in the brain to a much greater extent than do other forms of mercury.[15] The exact mechanism whereby methyl- and other alkylmercurials damage the nervous system is not understood.[251]

At the cellular level, mercury combines with sulfhydryl groups within cells. As a result, sulfhydryl enzyme systems essential to cellular metabolism and respiration are inhibited, resulting in cell death.

20.6.3.3.3

Diagnosis and Treatment

One should suspect mercury intoxication when horses show compatible clinical signs and have a history of exposure. Laboratory abnormalities are similar to those of other causes of acute or chronic renal failure and irritative gastrointestinal diseases. Definitive diagnosis usually is based on measurement of mercury

concentrations in kidney and liver.[15] One also may submit stomach and intestine samples for analysis in more acute cases.

Treatment of mercury intoxication initially involves removal of the source. In acute toxicity, evacuation of the bowel with a mild laxative may be helpful. The oral administration of 500 g of activated charcoal might help block absorption of mercury, but its efficacy has not been demonstrated. Dimercaprol (used to inactivate circulating mercury) can be given at a dosage of 3 mg/kg intramuscularly every 4 hours for the first 2 days, 4 times on the third day, and twice daily for the next 10 days until recovery is complete.[152] One also should follow other principles of therapy for acute or chronic renal failure. Treatment of chronic mercury intoxication is usually unrewarding.

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21 CHAPTER 21 VETERINARY EPIDEMIOLOGY

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This book is not meant to be another epidemiology textbook, but rather a compilation of concepts applicable to veterinary epidemiology as it pertains to equine internal medicine and equine veterinary practice. A number of such textbooks already have been written, but none have reported application to the equine species. For those who want more in-depth epidemiology, the following are textbooks to which the authors have referred in the last several years and to develop this chapter:

Martin, S., Meek, A., Willeberg, P.: *Veterinary Epidemiology*, Ames, 1987, Iowa State University Press.

Petrie, A., Watson, P.: *Statistics for Veterinary and Animal Science*, Malden, Mass., 1999, Blackwell Science.

Rothman, K., Greenland, S.: *Modern Epidemiology*, ed. 2, Philadelphia, 1998, Lippincott Williams& Wilkins.

Sackett, D., Haynes, R., Guyatt, G., et al.: *Clinical Epidemiology: a Basic Science for Clinical Medicine*, Boston, 1985, Little, Brown and Company.

Schwabe, C.W.: *Epidemiology in Veterinary Practice*, Philadelphia, 1977, Lea & Febiger.

Susser, M.: *Causal Thinking in the Health Sciences: Concepts and Strategies of Epidemiology*, New York, 1973, Oxford University Press.

Thrusfield, M.: *Veterinary Epidemiology*, ed. 2, Malden, Mass., 1995, Blackwell Science.

Torrence, M.: *Understanding Epidemiology*, St. Louis, 1997, Mosby.

An understanding of the principles of epidemiology is invaluable to internists and veterinary practitioners for understanding outbreak investigation, diagnostic testing, study design, principles of statistical analysis, and interpretation of scientific and medical literature. Epidemiology provides the background to enable clinicians to understand better what they do intuitively daily and to recognize the importance of evidence-based medicine.

Epidemiologic principles were not applied routinely to investigation of disease outbreaks until the mid-1800s when John Snow, a physician in England, determined that cases of cholera in certain areas of London occurred in a geographic pattern defined by drinking water sources. He hypothesized that waste from two companies was the main source of the epidemic. To confirm his suspicions, he removed the pump handle from one water pump with a resultant dramatic reduction in morbidity and mortality from cholera in that area of London (“The Broad Street Pump”). Another investigator, William Farr, looked at the cholera outbreak statistically and determined an effect of elevation on disease.[1] These investigations are considered the beginning of the modern science of epidemiology.

The first thing to do is to establish a definition of epidemiology. Noted epidemiologist Wayne Martin defines *epidemiology* as “the study of the frequency, distribution, and determinants of health and disease in populations.”[2] Martin notes that this is the analog of the pathogenesis of disease in individuals. Veterinarians, whether in private practice or academia, are called on daily to act as epidemiologists. When a practitioner is asked to investigate a disease outbreak, regardless of the species involved, that practitioner is going to look for common signs and common management factors by groups in an attemptto understand the outbreak and determine a cause. Epidemiology attempts to incorporate science into outbreak investigation by calculating an odds ratio, relative risk, or some other measurement to quantify the effect ofthe disease and to find factors that increase or decrease the risk 1513
of disease. The practitioner uses these risk factors as guidelines to develop a prevention plan for the owner of the 1514
facility. The purpose of this chapter is to introduce the reader to epidemiologic concepts one may use in practice to help solve clinical problems and understand better the current veterinary literature.

21.1 Epidemiologic Concepts

21.1.1 DIAGNOSTIC TESTING

The cornerstone of epidemiology is diagnostic testing. To determine the prevalence or incidence of disease, an accurate diagnosis is essential. Several basic principles of diagnostic testing are necessary for understanding how to use these tests properly, whether one is applying diagnostic tests to individual animals or is screening large numbers of animals. Diagnostic testing originally was based on postmortem findings in which changes in tissues and evidence of bacteria, viruses or parasites led clinicians to a definitive diagnosis. However, development of antemortem tests to diagnose and successfully treat individual animals has been necessary. In addition, the screening of groups of animals to detect disease and design preventive programs has become increasingly important in veterinary medicine. Diagnostic tests that directly assess the presence of infectious organisms vary greatly in sensitivity and specificity. Even more problematic is the use of surrogate tests that assess indirect evidence of infection, such as presence of antibody responses. These indirect diagnostic tests are only as good as the gold standard to which they are compared.

One must calculate and validate test sensitivity and specificity by using the test in an appropriate population in which the true disease status of the population is known. Animals that are truly negative (nondiseased) test negative, and animals that are truly diseased test positive using the gold standard. Unfortunately, no tests are perfect, and false-negative (FN) and false-positive (FP) results occur with any test. The sensitivity of a test is defined as the proportion of truly diseased animalsthat test positive (TP) (TP/TP + FN) (Table 21-1). The specificity of a test is defined as the proportion of truly healthy animals that test negative (TN) (TN/TN + FP).

TABLE 21-1 Sensitivity and Specificity of a Diagnostic Test Are Determined by Comparing the Test to a Gold Standard

	GOLD STANDARD DISEASE +	GOLD STANDARD DISEASE –	
Test +	True positive	False positive	Total test +
Test –	False negative	True negative	Total test –
	Total diseased	Total nondiseased	Total population

Diagnostic test results may be quantitative or qualitative. Qualitative test results are reported as positive, suspect, or negative. Examples of qualitative tests include Western blot for *Sarcocystis neurona* antibody or immunofluorescent antibody testing for *Neospora caninum*. Quantitative test results are available for enzyme-linked immunosorbent assay (ELISA) tests and many others. The ELISA tests are based on an optical density reading, and determination of positive tests versus negative tests is based on a cutoff value of optical density. The sensitivity and specificity of an ELISA test is determined by the cutoff used by the laboratory to which the samples are submitted.

As stated before, to establish a sensitivity or specificity for a diagnostic test, the test must be compared with a gold standard diagnosis. The population used to validate these parameters also must be a population in which the prevalence of that particular disease or infection is known. Unfortunately, validation processes are not always reported in the peer-reviewed literature. An exception to this is the validation of the Directigen Flu A assay during epidemics of influenza virus infections at a Thoroughbred racetrack.[3] This assay was developed for rapid identification of influenza virus infections and may be used by veterinary practitioners in the field. The sensitivity was reported as 33% to 45%, and the specificity was 95% to 98% depending on the standard used.[3] In other words, samples from horses with influenza virus infections yield positive Directigen Flu A test results 33% to 45% of the time. Therefore during an outbreak, one must sample several horses to determine the cause of the outbreak, perhaps for control purposes.

The prevalence of a disease in a population has an influence on the predictive value of a diagnostic test. The positive predictive value (PPV) of a diagnostic test is the probability that an animal testing positive is truly diseased (TP/TP + FP) (see Table 21-1). The negative predictive value (NPV) of a diagnostic test is the probability that an animal testing negative is truly healthy (TN/TN + FN). As prevalence of a disease increases in a population, PPV increases and NPV decreases. Conversely, as prevalence decreases, PPV decreases and NPV increases. Predictive values essentially provide the clinician with a level of confidence that the animal is truly diseased or truly healthy. This depends on whether the clinician is attempting to rule in or rule out disease in the animal(s), also called the SPin and SNout rules as explained by Sackett, Haynes, Guyatt, et al. If the cost of a false-positive test is high, then one should use the test with the highest specificity to rule in disease. This test reduces the number of false positives and therefore increases the PPV of the test. In contrast, if the cost of a false-negative result is high, one should use the test with the highest sensitivity, reducing the number of false- 1514
negative tests and increasing the NPV, leading to accurate rule-out of disease.[4] Possible reasons for false- 1515
positive results with a given test vary with the test format but may include group cross-reactions, presence of nonspecific inhibitors in serum and nonspecific agglutinins. Group cross-reactions occur commonly. For example, antibody tests for tuberculosis frequently yield false-positive results because of antigenic relatedness of *Mycobacterium* spp. Animals are likely to be exposed to related, nonpathogenic organisms that are ubiquitous in the environment. When testing for influenza viruses using hemagglutination tests, nonspecific inhibitorsmay mimic the effects of antibody. Similarly, nonspecific agglutinins may mimic the effects of antibodies.[5]

Possible explanations for false-negative test results include natural or induced tolerance to an infectious agent, improper timing of sample collection, improper test, presence of nonspecific inhibitors, antibiotic-induced immunoglobulin suppression, incomplete blocking antibody, or tests that are insensitive.[5] Induced or natural tolerance may explain why some horses do not develop antibodies to *S. neurona* in their cerebral spinal fluid.[6] Improper timing may explain false-negative serum neutralization test results from horses acutely infected with West Nile virus.[7] Subsequent samples from such horses would be positive on the same test because sufficient time would have elapsed for development of detectable antibody responses. Selection of a serum neutralization

test for African swine fever in pigs would be inappropriate because pigs do not produce detectable levels of neutralizing antibodies. Nonspecific inhibitors are a frequent problem when hemolyzed blood samples are submitted.[5] An example of false negatives resulting from using an insensitive test is use of polymerase chain reaction of cerebral spinal fluid for diagnosis of equine protozoal myeloencephalitis (EPM).[8,9]

21.1.1.1 Qualitative Tests

The effect of prevalence on predictive values of a diagnostic test is illustrated using EPM as an example. A sensitivity and specificity of 89% each is used for the Western blot analysis as reported by Granstrom and colleagues.[10,11]

In a population of neurologically normal horses, where prevalence of EPM is low, PPV of the test (probability that a Western blot–positive horse is truly diseased) is actually less than 7% (Table 21-2). Therefore testing normal horses, for example, during prepurchase examinations, is contraindicated because this test is not a good screening test. Diagnostic tests are most useful where prevalence of disease is between 40% and 60%.[4] In a population of horses with spinal ataxia and normal radiographs of the cervical spine, the prevalence of EPM is approximately 50%. In this population of horses the Western blot has a PPV and NPV of approximately 90%; that is, an approximately 90% chance exists that the horse truly has EPM if the Western blot is positive and an approximately 90% chance exists that the horse does not have EPM if the test is negative (Table 21-3). In a population of horses with asymmetric neurologic signs, the prevalence of EPM is approximately 90%. In this population the PPV of the Western immunoblot test for antibodies to *S. neurona* is approximately 99% and the NPV is approximately 45% (Table 21-4). As the prevalence increased from 1% to 50% to 90% in the populations depicted in Figures 21-1 to 21-3, PPV increased from 7% to 89% to 99% and NPV decreased from 100% to 89% to 45%. In the case in which the horse has the classic clinical signs of EPM, many clinicians presently are confirming exposure to the organism and administering treatment without performing a cerebral spinal fluid collection. Instead, they are using response to therapy as a confirmation of the disease (diagnostic test). Two recent reports have used response to therapy as a diagnostic modality for EPM; however, one should be careful in interpretation.[12,13]

TABLE 21-2 Sensitivity, Specificity, and Predictive Values of Western Blot Analysis for Antibody to *Sarcocystis neurona* in Neurologically Normal Horses When the Prevalence of Disease Is Less Than 1%[12]

	DISEASE +	DISEASE −	TOTAL	PREDICTIVE VALUE*
Western blot +	9	109	118	PPV = 9/118 = 7.6%
Western blot –	1	881	882	NPV = 881/882 = 100%
Total	10	990	1000	

* *PPV,* Positive predictive value; *NPV,* negative predictive value.

TABLE 21-3 Sensitivity, Specificity, and Predictive Values of Western Blot Analysis for Antibody to *Sarcocystis neurona* in the Neurologically Abnormal Horse When the Prevalence of Disease Is Approximately 50%

	DISEASE +	DISEASE −	TOTAL	PREDICTIVE VALUE*
Western blot +	445	55	500	PPV = $^{445}/_{500}$ = 89%
Western blot –	55	445	500	NPV = $^{445}/_{500}$ = 89%
Total	500	500	1000	

* *PPV,* Positive predictive value; *NPV,* negative predictive value.

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TABLE 21-4 Sensitivity, Specificity, and Predictive Values of Western Blot Analysis for Antibody to *Sarcocystis neurona* in Horses With Asymmetric Neurologic Signs and Significant Muscle Atrophy*

	DISEASE +	DISEASE −	TOTAL	PREDICTIVE VALUE†
Western blot +	801	11	812	PPV = 801/812 = 99%
Western blot –	99	89	188	NPV = 89/188 = 47.3%
Total	900	100	1000	

* In this population the prevalence of equine protozoal myeloen-cephalitis is approximately 90%.

† *PPV,* Positive predictive value; *NPV,* negative predictive value.

One may improve the accuracy of diagnostic testing using series or parallel testing. Series testing attempts to prove that an animal is truly diseased by increasing the specificity and consequently PPV. Animals that test positive on an initial screening test are retested using a different diagnostic test. The prevalence of truly diseased animals in the population undergoing the second test is higher than in the population undergoing the first test. For example, consider a population of horses with clinical signs of hepatic disease and increased serum alkaline phosphatase and bilirubin concentrations. If serumγ-glutamyl transferase and bile acid concentrations were determined only on those animals, one would find more abnormal results than if one tested all horses (regardless of clinical signs). In contrast, parallel testing attempts to prove that an animal is truly healthy or to increase the sensitivity and NPV. One example of parallel testing is the recommendation for five negative fecal cultures to demonstrate that a horse is not shedding *Salmonella* spp.

21.1.1.2

Quantitative Tests

If data obtained from test results are reported on a continuous scale, determination of cutoff points for positive and negative results and adjustment of those cutoff points have a tremendous effect on sensitivity,

specificity, false negatives, and false positives. In any population of animals some individuals are classifiedas healthy and others are classified as diseased. These distinctions may not always be clear. For example, when one examines an antibody response in populations, some antibody-negative animals may be in early stages of disease but have not yet seroconverted and are classified incorrectly as healthy. Other animals may be misclassified because of laboratory error or a failure in the validation process. When one uses a continuous scale for the test result, such as ELISA tests, one must establish a cutoff point that will aid in the most accurate classification of healthy and diseased animals.

Figure 21-1 Graphic representation of healthy and diseased populations when the results are reported on a continuous scale. Scale increases along the *x*-axis.

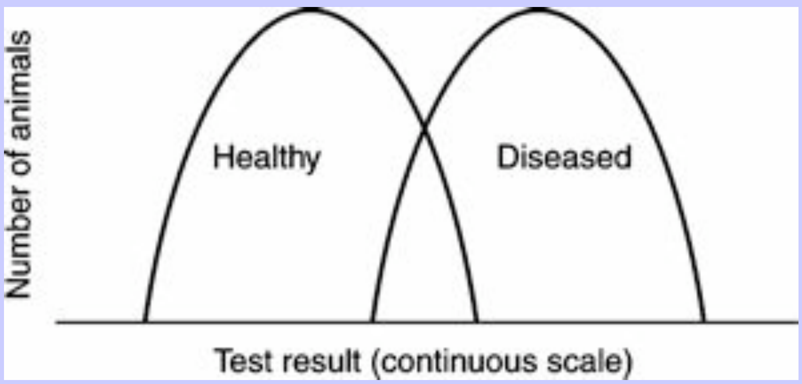
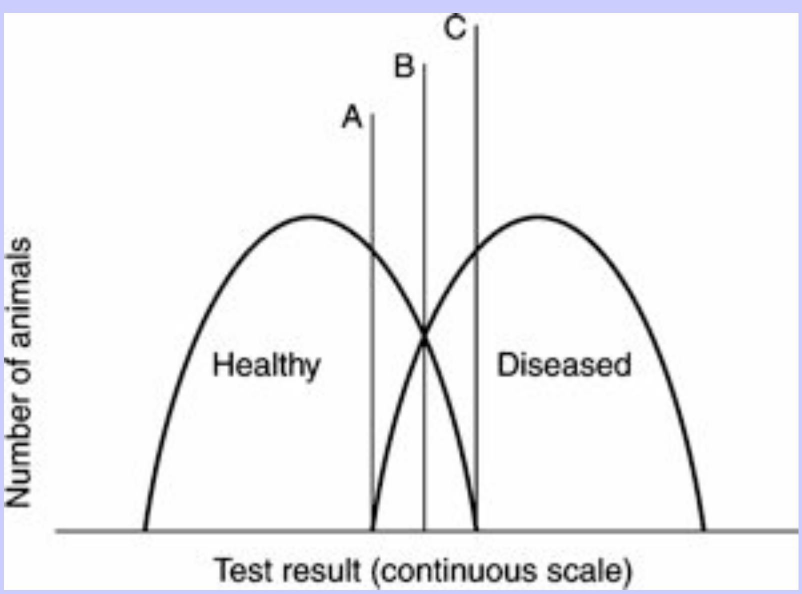


Figure 21-2 Graphic representation of test results on a continuous scale using three cutoff points: A, B, and C.



Figures 21-1 to 21-5 illustrate the changes that occur in sensitivity, specificity, false positives and false negatives when different cutoff points are used for a diagnostic test. Test results are reported on a continuous scale with increasing values depicted on the *x*-axis (see Figures 21-1 and 21-4). If the cutoff point is set at point A, the resulting test is highly sensitive (100%) and no false negatives occurred. However, specificity is low, and a large number of false-positive test results occurred (see Figure 21-2). This test would be useful as a screening test for a disease when the cost of a false negative is high.

Figure 21-3 Graphic representation of test results on a continuous scale using the cutoff at point A.

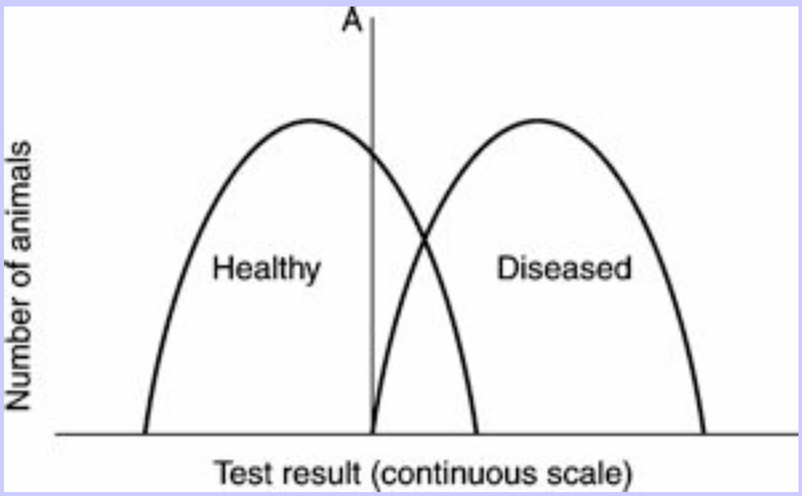


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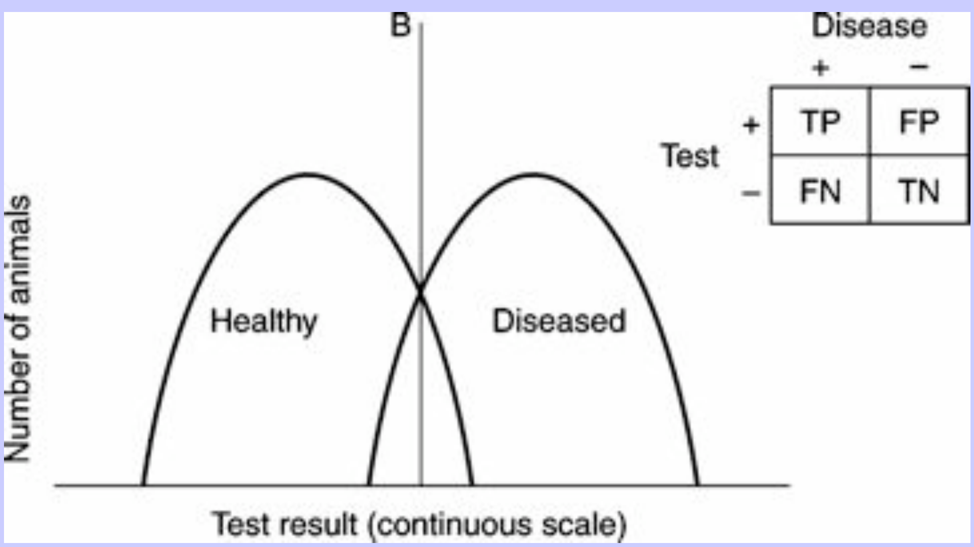
Figure 21-4 Graphic representation of test results on a continuous scale using the cutoff point B.



If the cutoff is set at point B, the test would be equally sensitive and specific, and the number of false positives and false negatives would be equal. The usefulness of a particular test with these characteristics depends on the disease being studied. If the cutoff point is set at point C, the sensitivity is low, but specificity is high (100%) (see Figure 21-5). This test would be useful if the cost of a false positive is high. When tests are recorded on a continuous scale, specificity of a test increases as sensitivity decreases and vice versa. This confirms the importance of understanding how a test was developed, conditions under which it was studied, and the precision and validity of the test.

The sensitivity and specificity of a test generally are considered to be fixed for a given test. However, predictive values differ greatly depending on the prevalence of disease in the population tested. For example, one may elect not to test for a condition where the prevalence of the disease is low because interpreting a positive test result may be difficult. However, one may find it important to receive negative test results, and therefore in that instance the value of a negative confirms one's belief of nondiseased. As the prevalence of disease increases, so does the PPV. Conversely, as the prevalence decreases, the NPV increases. Figure 21-6 illustrates the relationship between prevalence and predictive values.

Figure 21-5 Graphic representation of test results on a continuous scale using the cutoff point C.

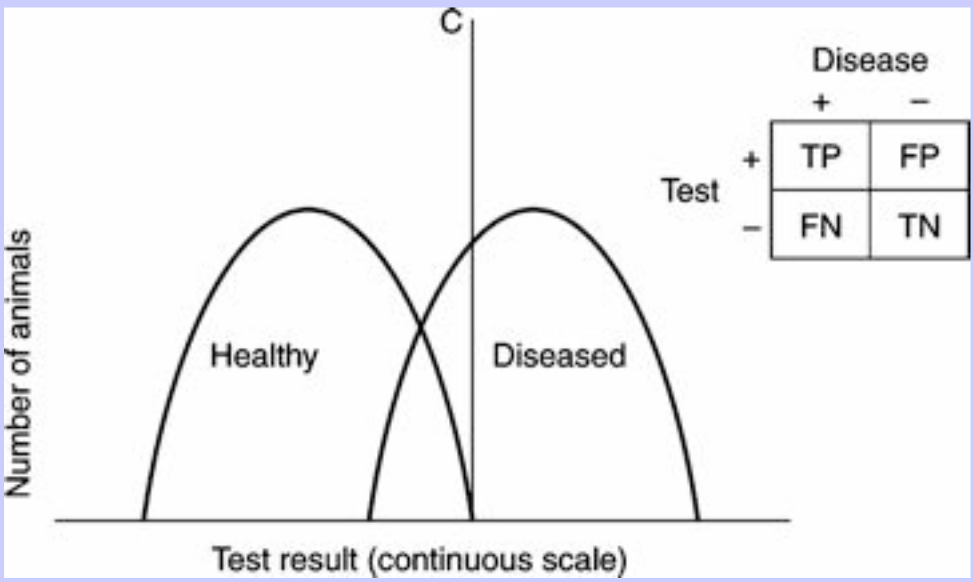
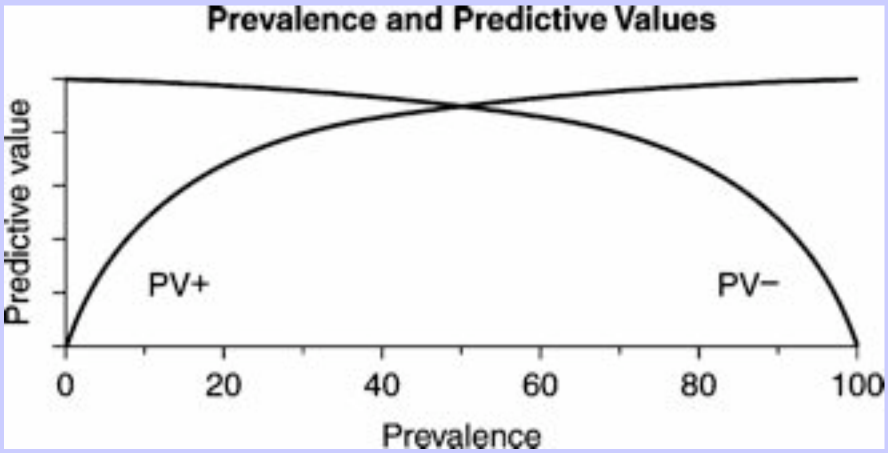


Figure 21-6 Graphic representation of the change in positive predictive value and negative predictive value as a concomitant change occurs in the prevalence of disease.



21.1.2 DATA COLLECTION

To measure the frequency and distribution of disease, one must collect data. Data are qualitative or quantitative. Qualitative data are often categoric; for example, breed of horse or gender. Categoric data may be nominal or ordinal. Nominal data assign a number to each data point, where each number represents a specific property of the data. For example, one may code gender as female = 1 and male = 2. Ordinal data use a specific term to describe members of a group that are related. For example, one might grade clinical disease as normal, mild, moderate, and severe. Quantitative data may be discrete or continuous. Discrete measurements must have a specified set of numbers such as whole numbers, often referred to as counts. Continuous data are measured data that may have any value within a defined range (interval or ratio). Examples of continuous data include prevalence, incidence, body weight, temperature, and antibody titer.

Terms used to define data one collects when studying a disease or syndrome include accuracy, refinement, precision, reliability, and validity. Accuracy is an indication of the extent to which the data conforms to the truth. The more refined the data are that describe the diseaseis referred to as refinement. *Precision* sometimes is used synonymously for refinement but more properly refers to the consistency of the measurements made in the study. Reliability usually refers to the repeatability of a measure when applied several times to the same animals. Validity suggests that the diagnostic test measures what it is purported to measure. These concepts are analogous to target shooting, in which validity refers to hitting the bull's-eye and reliability to the proximity of all shots on the intended target (the bull's-eye). 1517

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The objective of a study is to be able to collect unbiased data and report valid results. Bias is defined as a systematic error in the design, conduct, or analysis ofa study that leads to invalid results. Major biases onemust consider include confounding, interviewer bias, measurement bias, and selection bias. Confounding is the effect of an extraneous variable that may wholly or partially account for a spurious association between variables or can mask a real association. An example of a confounding variable is age, where exposure to an infectious organism is likely to be more common in older animals than young ones. Interviewer bias may result from

personal opinions of the interviewer altering the presentationof queries or statements to the experimental subjects. Measurement bias results from inaccurate measurements of disease, which may result in misclassification of animals into diseased or nondiseased groups. If the misclassification is differential (different for the diseased versusthe nondiseased), the bias will be severe. However, if the misclassification is nondifferential (equal between the groups), it will result in a reduction in the risk estimates. Lastly, selection bias results when study subjects are selected with different characteristics than subjects that are not selected. One can control many potential sources of bias in the experimental design. Randomization of study subjects is a primary method for reducing bias, along with matching and analysis. Unfortunately, matching on a particular characteristic results in an inability to examine the matching variable for a possible association with the outcome.

21.1.3 DESCRIPTIVE DATA

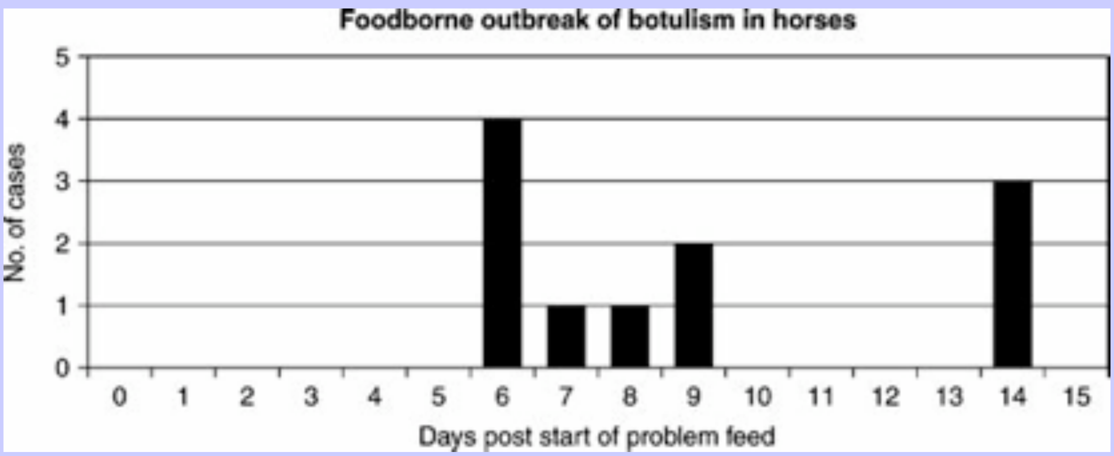
One may describe populations as contiguous or segregated. Examples of contiguous populations are wild horse herds on Bureau of Land Management land, the feral cat population in the United States, and wildlife populations. Enumeration of these populations and the occurrence of disease in these populations is difficult. In contrast, herds or flocks (segregated populations) are discrete groups that lend themselves to enumeration. Segregated populations may be closed or open, depending on the management style. Closed populations are less susceptible to introduction of infectious disease compared with open populations. Most horse populations are open because of frequent movement for showing, racing, or breeding. This movement makes for easy transfer of disease from population to population.

One measures disease in a number of different ways depending on the population and the basic factors known. For instance, prevalence is a measurement of the number of diseased animals at any given point in time without distinction between new and old cases. To calculate prevalence, the number of animals sampled on a particular day is the denominator and the number diseased or infected animals is the numerator. Prevalence is a proportion with a value between 0 and 1. One also may express prevalence as a number of the population at risk, for instance, per 10,000 animals. Prevalence estimates based on detection of antibody in populations are reasonably common. For example, a number of studies have examined the prevalence of serum antibodies to *Sarcocystis neurona* in horses.[14–18] Knowledge of prevalence rates for specific diseases are important for accurate interpretation of diagnostic tests, as discussed previously. One may calculate incidence only if the population at risk at the beginning of the study and the number of new casesthat occur over a specified period of time are known. One also may express incidence as a number of affected animals in a given population at risk, or as one study reported, in horse-years.[19] A recent study reported by the U.S. Department of Agriculture on EPM in the U.S. horse population determined the incidence of disease was 14 cases per 10,000 horses per year.[20] Morbidity data and mortality data often are expressed based on the time and place of occurrence of the disease. These expressions are referred to as temporal occurrence of disease, when timing of the disease is studied, or spatial occurrence of disease, when location of diseased animals is studied. Use of temporal and spatial cluster analysis is important in understanding pathogenesis of a disease and may provide important information to aid in prevention or development of disease.[21] Mortality data is similar to incidence data except the outcome is death rather than new cases. Case fatality rate is the number of deaths divided by the number of diseased animals. The complement of the fatality rate is survival; survival analysis is used commonly to assess the probability that an animal in a defined population will survive given a disease or other risk factors over a specified period of time.[22]

Descriptive measures such as rates, ratios, and proportions are often important tools for disease analysis. In a ratio the numerator is not a part of the denominator. A proportion is a ratio in which the numerator includes

animals that are in the denominator. Examples of proportions in epidemiology include prevalence, incidence, case fatality rate, and survival. Rates are a measure of a change in the numerator compared with the denominator, where time is incorporated into the denominator. These are used most commonly in epidemiology with incidence rates. One may express rates and proportions as crude, specific, or adjusted. For crude rates, onedoes not consider the structure of the population and possible confounding factors. One calculates specific rates for specific categories of host characteristics suchas age, breed, gender, or method of husbandry, providing more useful information than crude rates. To adjust for confounding variables and to compare rates in 1518
different populations, one may report adjusted rates. A common variable used to control or adjust for 1519
confounding is age.

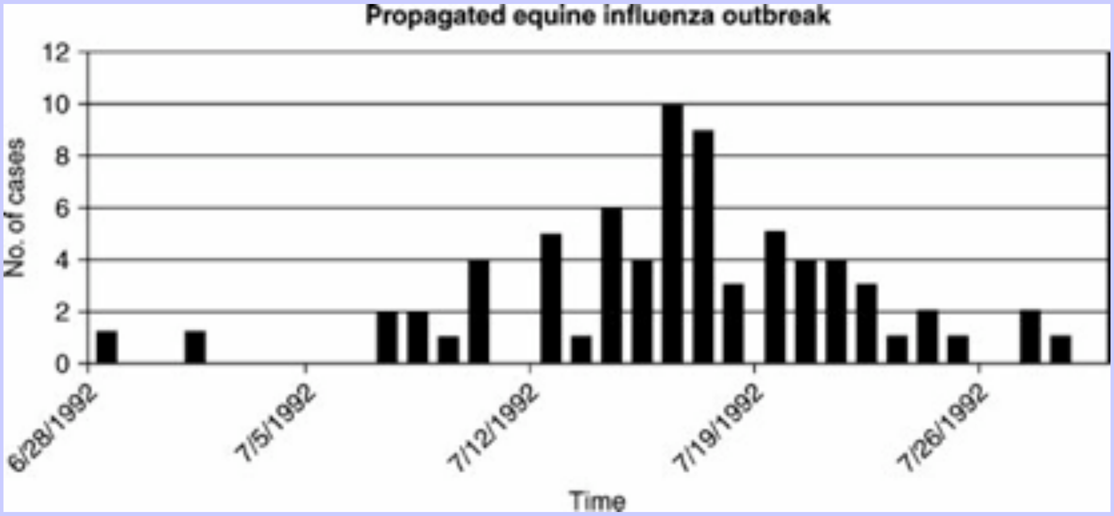
Figure 21-7 A graph depicting a common source outbreak of botulism in horses in England.



Another method of displaying descriptive data is through the use of tables, bar charts, and time trend graphs. Tables are used commonly to display numerical data. One may use bar charts for discrete data, and one may display continuous data in a similar manner using histograms.

One may use time trend graphs to plot epidemic curves. The two basic types of epidemics are common source and propagated epidemics. Point source or common source epidemics usually occur with some type of food poisoning such as occurred in horses affected with botulism after ingestion of contaminated oaten chaff.[23] The oaten chaff was distributed over many farms and fed at different intervals. Figure 21-7 illustrates the number of cases from four different premises feeding the same chaff. A propagated epidemic results from an index case with subsequent (secondary) cases developing in clusters. These secondary clusters occur after an interval determined by the incubation period of the infection. An excellent example of propagated epidemics in horses is the outbreak of influenza at a western Canadian racetrack (Figure 21-8).[24]

Figure 21-8 A propagated outbreak of equine influenza at a western Canadian racetrack.



21.1.4 DISEASE CAUSATION

Originally, early infectious disease researchers believed that each disease was associated with a single causative agent. Robert Koch proposed the one agent–one disease concept, and it remains useful in specific situations such as anthrax, rabies, and many others. However, application of Koch's postulates proved difficult in situations in which the causative agent was a necessary but not sufficient factor to cause disease. In those cases, induction of disease might depend on diverse variables such as the presence of co-factors or the susceptibility of the host.[25] Evan's postulates are more applicable to a broader range of situations[5]:

1. Proportion of animals with the disease should be higher in those exposed to the causal agent than in those not exposed. 1519
2. Exposure to the supposed cause should be present more commonly in the diseased compared with the nondiseased when all other factors remain constant. 1520
3. The number of new cases of disease should be significantly higher in those exposed compared with the nonexposed, as shown in prospective studies.
4. Temporally, the disease should follow exposure to the supposed cause with a distribution of incubation periods on a bell-shaped curve.
5. A spectrum of host responses, from mild to severe, should follow exposure to the supposed cause along a logical biologic gradient.
6. A measurable host response should appear regularly following exposure to the supposed cause and should not occur in animals not exposed.

7. Experimental reproduction of the disease should occur with greater frequency in animals appropriately exposed compared with those not exposed.

8. Elimination of the cause or modification of the cause should decrease the frequency of occurrence of the disease.

9. Prevention or modification of the host response should decrease or eliminate the disease that normally occurs on exposure.

10. All relationships and associations should be biologically and epidemiologically credible.

These postulates as put forward by Evans require that the association between a hypothesized causal factor and a disease be statistically significant; therefore the postulates require comparison between groups of animals.[5]

Two types of variables involved in the examination of disease causation are the explanatory variable (independent) and the response variable (dependent). The response variable is the variable affected by the explanatory variable. These variables may have statistical or nonstatistical associations. Nonstatistical associations are those that occur because of chance and that therefore cannot be considered causal. Not all statistical associations are causal. For example, an explanatory variable may be associated statistically with two response variables and be causal in both, but the two response variables also may be statistically associated and not be causal. Explanatory variables also may be causal directly or indirectly. Indirect causal associations occur with an intervening variable. An example is acquisition of rabies directly by human beings who enter a bat cave or indirectly through the bite of a rabid fox that inhabits bat caves. In this case the intervening variable is the fox acquiring the rabies because of cohabitation with the bats.[5]

21.1.5 CAUSAL MODELS

One may view indirect and direct causes in two causal models. Causal model 1 classifies causes into sufficient and necessary causes. Sufficient cause occurs if the cause produces an effect. In most cases several component causes make up a sufficient cause, consistent with a multifactorial model of disease as a function of host, agent, and environment (Figure 21-9). An example of this triad of disease is EPM of horses, in which *S. neurona* is the agent (Figure 21-10). Environmental factorsthat influence disease expression include stress such as transport of the host and a farm with woods on the property.

Figure 21-9 Graphic representation of the triad of disease: agent, host, and environment.

Many sufficient causes for a disease potentially exist. If a component cause is in every sufficient cause, that component is called a *necessary cause* (Figure 21-11). Figure 21-12 includes sufficient and necessary causesfor EPM. Equine protozoal myeloencephalitis has seven component causes, three sufficient causes, and one necessary cause, the parasites *S. neurona* and/or *Neospora hughesi* (see Figure 21-12). A disease may have many sufficient causes that include multiple component causes. Depending on the disease, it may have many sufficient causes, but not a necessary cause. An example of this is acute diarrhea of horses. The causes of diarrhea are many. However, if the diagnosis is salmonellosis, then the necessary cause is *Salmonella* spp.

In causal model 2, direct and indirect causes may occur in a chain of events leading to disease (a path model) (Figure 21-13). If multiple relationships occur with many factors acting at the same level and in more than one level, the result is a web of causation (Figure 21-14).

Figure 21-10 Equine protozoal myeloencephalitis as an example of the triad of disease.[26]

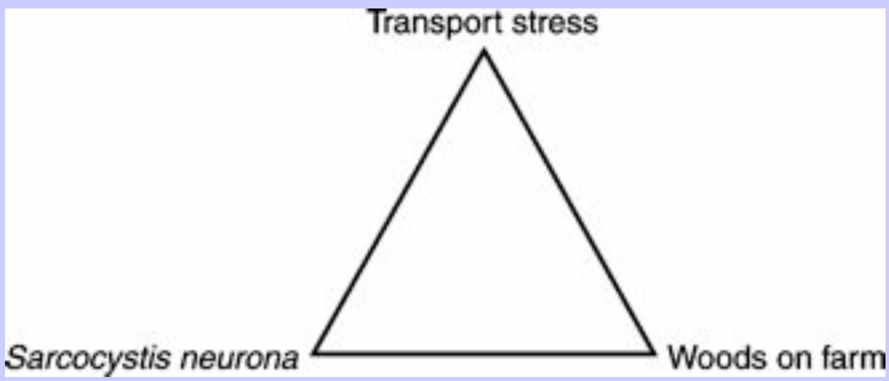


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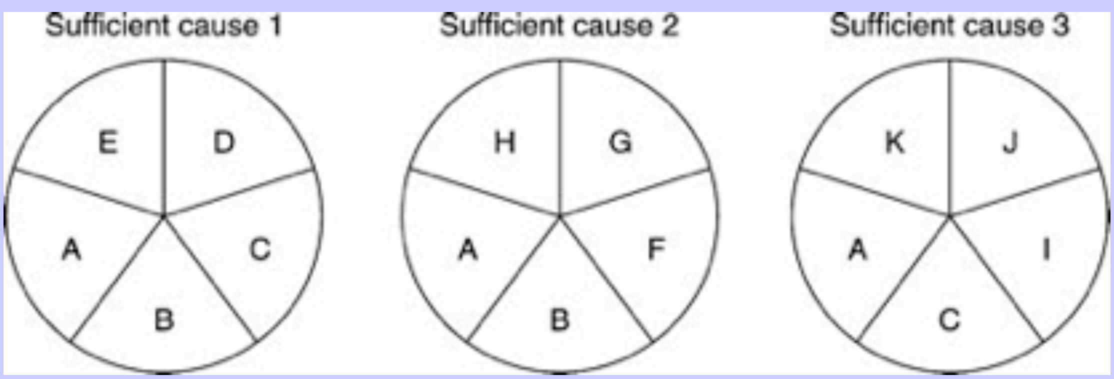
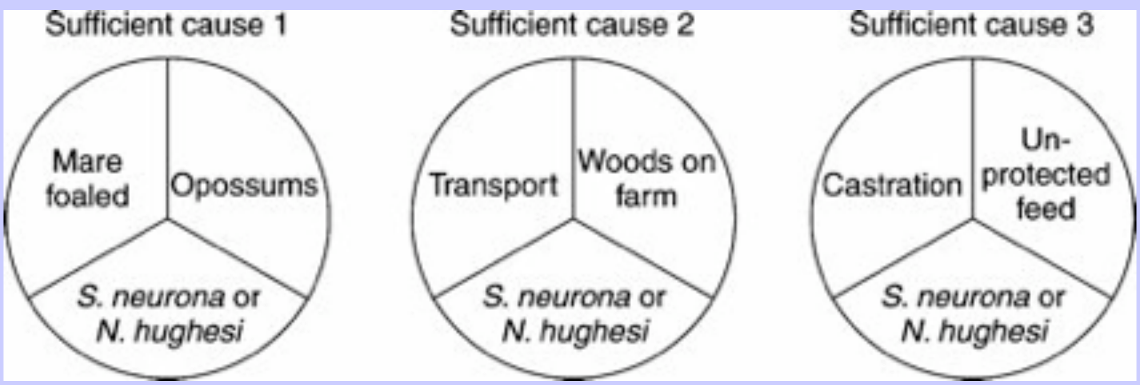
Figure 21-11 An example of sufficient causes of disease with 1 necessary cause *(A)*, 11 component causes, and 3 sufficient causes.[5,27]



21.2 Causal Hypothesis Generation

After collecting data, one generates descriptive statistics in relation to the time, place, and population under study. On completing the descriptive data, one may develop causal hypotheses. One generates hypotheses by one or more of four methods: method of difference, method of agreement, method of concomitant variation, and method of analogy. Method of difference refers to differences in disease frequency in two populations. Observation of a factor present in one population and not in the other suggests the factor may be causal. For example, in two groups of horses (exercised and unexercised) challenged with influenza virus, respiratory clinical scores were worse in the exercised group.[26] Therefore exercise may be deemed causal for the difference in clinical scores. Method of agreement refers to a factor present in several different populations in which a disease is also present, suggesting the factor may be causal. For example, several studies report that dietary factors may increase the likelihood of colic, suggesting these factors may be causal.[27–29] In the method of concomitant variation, the frequency or strength of a factor varies continuously with the frequency of the diseasein different situations. In one study, compared with horses that did not receive concentrate in their diet, as the quantity of concentrate increased, the risk for colic increased from 2.4 to 4.8 to 6.3 times.[29] An analogous situation is the increase in frequency of lung cancer in human beings with an increase in the number of cigarettes smoked daily. The method of analogy compares the pattern of disease being studied to the pattern ofa disease already understood. In a recent study of *Cryptosporidium* spp. infection in horses, the organism was suggested to become endemic on certain horse farms, similar to the observation on certain cattle farms in previous reports. Using the method of analogy to gather evidence of causation can be misleading; therefore the method is used most commonly to establish probabilities of causation.

Figure 21-12 Figures depicting three sufficient causes for equine protozoal myeloencephalitis.[26]



Sir Austin Bradford Hill proposed several criteria that one should consider before considering an associationof a factor and a disease causal.[30] These criteria include strength, consistency, specificity, temporality, biologic gradient, plausibility, coherence, experiment, and an analogy of the association. *Strength of association* refers to the increase in risk in one group compared with that of another group. For example, a tenfold increase in risk provides better evidence than a fivefold increase. One should not discount light increases in risk totally, however, because they may be important. Poor strength of the association may be caused by problems with numbers studied or study design and may need further examination. Hill's second criterion was *consistency of association*. Results 1521

of separate, similar studies should be consistent. Preferably these studies should be done at different places in 1522
different times and with different study designs (e.g., studies done retrospectively and prospectively). For example, the highest risk for EPM is in the fall of the year. This observation was corroborated by two separate studies.[20,31] Another example of consistency is in separate observations that dietary factors increase the risk for equine colic.[27–29] *Specificity of association* was the third criterion considered by Hill. This criterion may be difficult to meet in some circumstances because of multifactorial causes of some diseases. One example is the increased risk of EPM in horses if opossums are seen on the premises.[31] This finding was corroborated by a study by the National Animal Health Monitoring System of the U.S. Department of Agriculture.[20] The next criterion of importance is *temporality*, or exposure to the factor before disease. For example, horses must be exposed to *Sarcocystis neurona* or *N. hughesi* before development of neurologic signs of EPM. Demonstration of a *biologic gradient* or dose-response curve is strong evidence of causation. Compared with horses diagnosed with mild clinical signs of EPM, horses with moderate neurologic signs are 3.2 times more likely to die, and horses with severe clinical signs are 9.9 times more likely to die.[32] The biologic gradient is severity of neurologic signs. Whether biologic *plausibility* can be a steadfast criterion for association and causation is open for debate. A causal factor should be plausible; however, if the causal factor is new science never before reported or studied, it should not be refuted at least until it is repeated. Causal factors should cohere with known facts of the natural history and biology of the disease. *Experiment* is considered strong evidence for causation. If disease does not occur when a factor is removed, the evidence is strong that the factor is causal.[30] Hill's ninth criterion is that of *analogy*, in which studies that find similar conclusions offer some credence for causation. Hill's criteria for causal association are not meant as absolutes but should help guide conclusions as to cause and effect.

Figure 21-13 Path diagram for equine protozoal myeloencephalitis.[26]

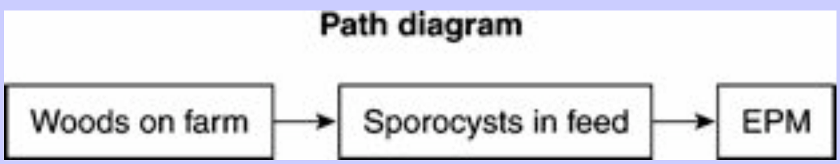
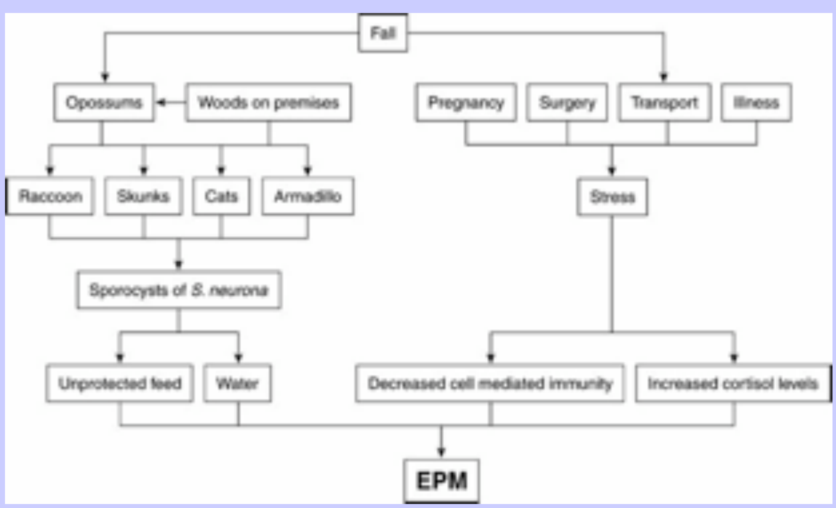


Figure 21-14 Web of causation for equine protozoal myeloencephalitis.[26]



21.3 Measures of Association

21.3.1 HYPOTHESIS TESTING

One can approach association in two ways to establish causation: comparing means between two populationsor comparing proportions. Levels of significance are set based on probabilities that the differences between sample means or categoric variables would occur based on chance. Conventionally, the level of significance or α-value is set at P <0.05, meaning that the probability a finding would occur because of chance is less than 5%. The investigator may set a more conservative α-value such as 1% or 0.1%.

Statistical tests are established based on the hypothesis that no difference exists between the sample being
studied and the reference population, hence the *null hypothesis*. If the test of significance is less than the α- 1522
value established a priori, then the null hypothesis is rejected and the alternative hypothesis is accepted. 1523
Accepting the alternative hypothesis suggests that the sample population is different from the reference population. Two types of error are possible, type I and type II (Table 21-5). The type I error occurs when the null hypothesis is rejected when it is in fact true (i.e., detecting a difference when one is not present). Type I error is the same as the level of significance or alpha (α). Type II error (β) occurs when the null hypothesis is accepted when it is in fact false (i.e., failure to detect a difference when one exists). As the sample size of a study increases, α and β become smaller. In general, α and β are related inversely: as one increases, the other decreases. Type II error is related to the power of a study: 1 – β = Power. *Power* is defined as the probability of detecting a true difference (i.e., the probability of rejecting the null hypothesis when it is false). One should set α and β when designing the study, keeping in mind that each value has a direct influence on theother.

TABLE 21-5 The Four Possible Outcomes of a Study Depending on Whether Difference Is True and Whether the Study Finds a Significant Difference

		SITUATION IN THE REAL WORLD	
		No difference, H_0 true*	Difference, H_a true
Study results	No difference, H_0 true	Correct result	Type I error
	Difference, H_a true	Type I error	Correct result

* H_0, Null hypothesis; H_a, alternative hypothesis.

21.3.2 RELATIVE MEASURES OF ASSOCIATION

Relative risk is the ratio of the incidence of disease inthe exposed population to the incidence of disease in the unexposed population (Tables 21-6 and 21-7). If the relative risk is greater than 1, the association is predisposing; if the relative risk is equal to 1, no association exists; and if the relative risk is less than 1, the association is protective. One should use relative risk only in cohort studies in which the individuals are observed throughout the period of the study. Table 21-7 gives the formula for calculation of a relative risk. One can calculate the confidence interval for the relative risk estimate to help define the precision of the estimate, along with a test of significance. Confidence intervals that include 1 indicate no difference in the relative risk.

A variety of statistical software programs are available to perform these calculations; therefore explanation of the mathematic calculations of these values are not included in this chapter. Readers are referred to published references for illustrations of the use of relative risk in veterinary medical epidemiologic studies.[19,33]

TABLE 21-6 Evaluating Associations Between Exposures or Risk Factors and Disease

	DISEASE +	DISEASE −	TOTAL
Exposure +	a	b	Exposed: a + b
Exposure –	c	c	Unexposed: c + d
Total	Diseased: a + c	Nondiseased: b + d	Population: a + b + c + d

21.3.3 ODDS RATIO

The odds ratio is the ratio of the probability of anevent occurring to the probability of it not occurring (see Table 21-7). In a cohort study the odds ratio is the odds of disease in the exposed group versus the odds of disease in the unexposed group. In a case-control study the odds ratio is the odds of exposure in the diseased versus the odds of exposure in the nondiseased individuals. In cross-sectional studies, one may calculate a prevalence odds ratio using the same formula as in Table 21-7. As with the relative risk, one also may calculate a *P* value and confidence interval. Many examples of the use of odds ratios are in the veterinary literature.

21.3.4 ATTRIBUTABLE RISK

The attributable risk (risk difference or attributable rate) is the difference between the incidence of disease in the exposed population versus incidence in the unexposed population (see Table 21-7). In other words, the attributable risk is the extent to which the incidence of disease would be reduced had the individuals not been exposed to the factor. The attributable risk includes the baseline risk of the disease and therefore gives the 1523
magnitude of the effect of the causal factor in the population. Therefore the attributable risk is a better indicator 1524
of the effect of a preventive campaign when the factor is removed than is the relative risk. One must intrepret the attributable risk carefully because it measures the strength of association and demonstrates causality. Therefore demonstration of causation of a factor in the disease is important as well. A report on the adverse effects of antibiotic therapy in pneumonic foals includes an example of attributable risk calculations.[34]

TABLE 21-7 The Most Common Formulae Used to Calculate Measures of Association[*]

FORMULA NAME	FORMULA
Relative risk (RR)	RR = (a/a + b)/(c/c + d)
Odds ratio (OR)	OR = ad/bc
Attributable risk (AR)	AR = (a/a + b) – (c/c + d)
Population attributable risk (PAR)	PAR = (a + c)/n – (c/c + d)
Etiologic fraction (EF)	AF = (RR – 1)/RR
Population etiologic fraction (PAF)	PAF = [(RR – 1)/RR] × *f* (*f* = a/(a + c), the proportion of diseased individuals exposed.)

* The formulae are based on Table 21-6.

21.3.5 POPULATION ATTRIBUTABLE RISK

The population attributable risk is the difference between the total disease in the population and the disease in the unexposed individuals (see Table 21-7). This measurement indicates the amount of disease in the total population attributed to a particular risk factor. One can calculate this measurement only when one knows the disease morbidity for the total population. These population attributable risks have been calculated for cases of influenza at 3 outbreaks at a Western Canadian racetrack.[35]

21.3.6 ETIOLOGIC FRACTION (EXPOSED)

The etiologic fraction is the proportion of the incidence of disease in exposed animals attributable to exposure to a particular risk factor (see Table 21-7).

21.3.7 POPULATION ETIOLOGIC FRACTION

The population etiologic fraction is the proportion of the incidence of disease in the population attributable to exposure to the risk factor being studied. Therefore elimination of the risk factor should result in elimination of this proportion (see Table 21-7).

21.4 Study Design

Most research is designed as an experimental or an observational study. The one fundamental difference between the two is that in experimental studies the researcher can control the conditions of the experiment, whereas observational studies include no interventionby the researcher. Observational studies are basically observations and analyses of an event as it happened (prospectively) or after the fact (retrospectively).

21.4.1 EXPERIMENTAL STUDIES

One main advantage of experimental studies is the opportunity to randomize animals into treatment groups. Every animal has the same probability of being in the treatment or control group. Randomization of study subjects is one primary method to control for bias in a study. Randomization is also advantageous because one may study one variable at a time, allowing for more consistency because one can attribute the results entirelyto the exposure or treatment being studied. One of the most common types of experimental studies in veterinary medicine is the clinical trial.[26,36–38]

21.4.2 OBSERVATIONAL STUDIES

Many diseases cannot be studied in experiments because of considerations of ethics, time, or expense. An alternative is to study disease and exposure in the populations in which they are occurring by observation and analyses. Prospective or retrospective observational studies examine the effect of an exposure or the cause of a disease. These studies require a group that is exposed versus a group that is not, or a group that has the disease and a group with no disease. Advantages to observational studies include speed, ease of completion, and less expense. Because randomization is not possible, one uses other methods to attempt to control for extraneous factors.

Types of observational studies include natural, ecologic, case series or case reports, cross-sectional or surveys, prospective or cohort, and case-control or retrospective studies. *Natural experiments*, which rarely are documented, occur when an event in nature provides an opportunity to follow an exposure and its effect on animals, human beings, and the environment. An excellent example is the cholera epidemic in London in the 1850s. John Snow was able to determine that the causative agent was transmitted through the water. Ecologic studies examine exposure or an occupation and its effect on disease in communities, geographic regions, or during particular time periods. Because one does not collect the individual data, one can make no conclusions regarding the individual. Extrapolations made from the community to the individual are termed *ecologic fallacies*. This type of information is used to generate cancer registriesand state or federal databases. These studies frequently provide data in support of hypotheses for future analytic studies. *Case reports* or *case series* are descriptions of disease, whether a single clinical case or a series ofcases. These reports provide information on which to base epidemiologic studies. Numerous case reports and case series have been published in the veterinary literature.

Cross-sectional studies or *surveys* are observational studies that provide a snapshot of an exposure or disease at a particular point in time. These studies are easy to perform, often at minimal cost. One may conduct surveys using several methods: telephone interviews, face-to-face interviews, mailed or written questionnaires, self-administered questions, or by computer. The design, administration, and sampling strategies for questionnaires is critical. Sampling strategies include random, stratified, clustered, or multistage. Random sampling occurs when every animal in the population studied has the same opportunity to be included. Stratified sampling occurs when animals are sampled based on a particular characteristic, for example, age. Cluster sampling refers 1524
to sampling of groups of animals such as herds, pens, or barns. In some studies, multistage sampling occurs as 1525
the individual horse is sampled at one stage, the herd in the next stage, and then a county or a state in a third stage. Some examples of this type of survey study in veterinary medicine are those to determine exposure rates to *S. neurona*, the primary cause of EPM.[14–16] These survey studies originated from one county of a state,[14] a specific region,[15] and a specific state.[16]

Longitudinal studies include *prospective* or *cohort studies* and *case-control* or *retrospective studies*. Longitudinal studies follow exposures forward or backward in time and are designed to study risk factors and disease based on prestated hypotheses. Longitudinal studies are separated in design based on the factor being studied, exposure, or disease. Prospective or cohort studies are considered ideal. An exposure is known to have occurred in one study group, and the other group is known notto have been exposed. In cohort studies, one follows groups over time to determine if they develop disease. These studies may be time-consuming and expensive. A major advantage to cohort studies is the ability to establish incidence of disease with relative risk or attributable risk. In addition, one establishes temporality, assisting in confirmation of causation. One major disadvantage of a cohort study is the length of time necessary to confirm or deny development of disease in each group. During the time of study, individuals animals may be removed from the study or lost to follow-up. Tracking those individuals may be costly. Investigators may perform historical cohort studies if they can establish the true exposure status and development of disease.[19,29,33,39]

Lastly, *case-control* or *retrospective studies* can be completed quickly with limited expense. Initially, one must select cases based on disease, not exposure. One studies these cases retrospectively for exposures or risk factors of interest. One must select cases and controls carefully, and the criteria for selection should be the same for both. Sometimes investigators choose to use more than one set of controls for each case to increase the power of the study. For example, a recent study of EPM used one control group of horses with other neurologic diseases and another control group including horses with diseases of other organ systems.[31] Unfortunately, because these studies examine prevalent cases, one may calculate only an estimate of risk using an odds ratio. Reliance on retrospective information is a major disadvantage of these studies, particularly if data in clinical records are incomplete.

21.5 Sample Design and Size

21.5.1 SAMPLING DESIGN

A variety of sampling designs are used in epidemiologic studies. The best design is the *simple random sampling*, in which every individual in a population has an equal opportunity to be sampled. The selection of one individual has no influence on the selection of another, hence they are independent. *Stratified random sampling*, which is used commonly in the study of wildlife populations, randomly samples various strata or subpopulations, combining results to obtain overall population estimates. The aim is to have the sampling units in each strata as similar as possible and the means of the strata to be as different as possible. *Cluster sampling* commonly is used to examine herds or groups of animals. The clusters or herds to be sampled are selected randomly, and all unitsin each cluster are sampled. Unfortunately, with this technique the parameter estimates tend to be less precise than with random samples. Statistical methods are available to adjust for cluster sampling by inflating the variance. Cluster sampling can be extended to multistage sampling. In *multistage sampling* clusters are selected and the units of the selected clusters are sampled randomly. *Systematic sampling* is random selection of the first sample in the population and subsequent choosing of individuals at specific intervals thereafter. For example, the first sample may be the thirty-sixth unit in the population. One would sample every thirty-sixth unit after the initial unit until all necessary samples were collected from the population.[16]

21.5.2 SAMPLE SIZE

One should choose sample size to optimize the opportunity to detect meaningful differences between groups of study subjects without wasting resources. Unfortunately, sample size determination depends on some knowledge of likely end results of the study before conducting the study. Reliance on strict statistical calculations to determine sample size often leads to recommendations for inclusion of unrealistic numbers of animals. Sample size should be of sufficient magnitude that the study will detect statistical differences reliably if they are present. This concept is stated in terms of the *power* of the study. Most studies strive for a statistical power of 80% or higher. Appropriate sample size also is determined by the level of significance or α-level, most commonly set at 0.05. For optimal sample size calculations the investigators also must designate a minimal difference between the two populations that the study is required to detect. The standard deviation of the observations in each group must be known or estimated. This information often is estimated based on results of previous studies of the investigators or results in published literature. Alternatively, one may perform a pilot study to obtain preliminary results that will provide an estimate of population variance. After one has determined power, significance, difference to be detected, and population variance, one may use Altman's nomogram to determine the optimal sample size.[40] A variety of statistical programs are available to assist in these calculations if Altman's nomogram is not available. 1525

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21.6 Basic Statistical Concepts

After collecting the raw data, one calculates descriptive statistics to determine measures of position (mean, median) and measures of spread (standard deviation, standard error, range) of the population. One graphs data (frequency distributions or histograms) to observe the distribution and compare it with the normal bell-shaped curve. One determines the sample mean and uses it to provide an estimate of the population mean. The larger the sample size, the closer to the true mean of the population the sample mean becomes. The median isthe value that half of the observations are below and the other half are above (the 50th percentile). One also may divide the data into quartiles (25th, 50th, and 75th percentiles).

After determining measures of position, one calculates measures of spread, which include the range, variance, and the standard deviation of the sample. These measures are estimates of the population variance and standard deviation. Another measure of spread sometimes usedis the semiinterquartile range, the 25th to the 75th percentiles. One determines distribution of the sample mean by calculating the standard error of the mean (SEM), which is the square root of the variance of the sample means. One estimates the standard error of the sample means by using the standard deviation divided by the square root of n, the number of samples. This calculation provides an estimate of the precision of the sample mean as an estimate of the population mean. The smaller the standard error, the greater the precision. Often one calculates a confidence interval to provide a range in which the true population mean will lie, most commonly a 95% confidence interval. The formula to calculate a 95% confidence interval is as follows: mean ± 1.96 × SEM. The lower and upper values of the confidence interval are termed the *confidence limits*. One may calculate confidence intervals for numerous estimates, but formulae for calculations may change. The reader should consult epidemiology texts for these formulae.

21.6.1 STATISTICAL DISTRIBUTIONS

Before detailed statistical analysis, one must examine the data to determine whether they are distributed normally. This normal or gaussian distribution is a bell-shaped curve in which 68% of the observations should lie within 1 standard deviation of the mean and 95% should fall within 2 standard deviations. This distribution

may be appropriate for many biologic variables but may not be appropriate for ordinal or nominal data, unless the sample size is large. The distribution determines the types of statistical tests one may apply. When only two outcomes are possible where discrete data are involved, the data has a binomial distribution. For example, horse gender at birth is male or female. Another expression for the outcome of a binomial distribution could be success or failure. One applies a Poisson distribution to counts that occur randomly in space and time. Therefore the distribution describes spatial and temporal distribution of disease, an example of which could be the distribution of blood cells in a hemocytometer. Sometimes these distributions may be skewed one way or the other, or the shape of the curve may be too thin or too wide. In those circumstances, one may transform data using logarithms or some other mathematic transformation, a process done in veterinary medical studies dealing with measurements such as antibody titers. Where the sample size is large, binomial and Poisson distributions approximate normal distributions.

21.6.2 STATISTICAL TESTS

Statistical tests are chosen based on the type of samples collected. Independence of the samples is important in determining the type of test to use. If the samples are not independent (for example, repeated samples from the same animals), then one must use statistical tests to account for these repeated observations. Statistical tests may be called parametric or nonparametric. *Parametric tests* are used for analysis of normally distributed data when the variables are on the continuous scale (interval or ratio data). Some tests may require equal variance for the two populations being studied. Parametric tests are based on the use of the mean, a parameter of the normal distribution. An example of such a test for comparison of continuous data from two populations is the Student's *t*-test. One uses *nonparametric tests* with nominal, ordinal, interval, and ratio data in which the distribution of the data is not normal but the data do assume symmetry. Nonparametric tests are less robust than parametric tests and require larger sample sizes to detect differences. Examples of nonparametric tests include the Wilcoxon signed rank test, Wilcoxon-Mann-Whitney *U* test, Kruskal-Wallis one-way analysis of variance, and Spearman rank-order correlation coefficient.

When one compares proportions or nominal data, as occurs with many observational studies, one uses the chi-square test of association. The chi-square is performed routinely using a 2 × 2 contingency table, but one may use tables with many rows and columns. If sample size is small and some cells in the 2 × 2 contingency table equal less than 5, one should use a Fisher exact test. When samples are related or one is examining matched samples, one may calculate the chi-square using the McNemar change test of discordant pairs.

Another test used commonly to compare two variables is a correlation. In this measure of association, graphic 1526
presentation of data places the independent variable on the *x*-axis and the dependent variable on the *y*-axis. If 1527
the combination of pairs leads to a line from the bottom left of the graph to the top right, the association is positive. Conversely, if the line generated is from the top left to the bottom right, the association is negative. If no association exists, the data will be scattered all over the graph. One may use this procedure in normally distributed data, whether continuous or discrete. One can calculate a sample correlation coefficient *(r)* with most statistical software. If data is not normally distributed, nonparametric statistical tests such as Spearman's rank correlation are recommended.

21.6.3 MULTIVARIABLE ANALYSIS

One uses multivariate or multivariable analysis to examine the relationship between many independent variables and a dependent variable. One should decide to use a particular method before initiating the study. This decision comes after one has formulated the hypotheses, which are predicated on the type of data

generatedand the objectives to be accomplished. Statistical methods used to perform this type of analysis include survival analysis, linear regression, and logistic regression.

Multiple linear regression is recommended when more than one explanatory or independent variable is regressed on a continuous predictor or outcome or dependent variable. The primary assumption is that the dependent variable is distributed normally. Another assumption is that the independent variables are measured without error but are not necessarily required to be continuous. The primary reason to use multivariate regression isto examine the effect of one explanatory variable on the dependent variable while controlling for the effect of the other explanatory variables in the equation. The number of explanatory variables used in an analysis should not be excessively large. As a rule of thumb, the sample size should be at least 10 times the number of explanatory variables.

When the dependent or outcome variable has a binary outcome (yes or no), multiple logistic regression is recommended. Because the outcome is 0 or 1, one cannot interpret any values that are not exactly 0 or 1. Therefore one considers the probability that the dependent value takes a value of 0 or 1 and transforms this probability using logistic transformation to overcome this inability to interpret values other than 0 or 1. Hence the name logistic regression. Because of the logistic transformation, the model produces the odds of the outcome with a 1 unit increase in the explanatory variable whileall other explanatory variables are held constant. The logistic coefficients are determined by an iterative method called maximum likelihood. Examination of one explanatory variable on the outcome is explained while controlling for the effects of the other explanatory variables.

Survival analysis examines the length of time to a critical event such as death and often is used to evaluate the effect of a treatment. Special techniques are warranted because of censored data, such as animals being lost to follow-up or they never experience failure and are alive at the end of the trial. If investigators wish to examine the effect of several variables at the same time as the survival analysis, a Cox proportional hazards model, similar to logistic regression, is recommended.

In many analyses, one makes repeated measurements on the same animals over a predetermined length of time (repeated measures). In this case, the measurements are not independent and this has a tendency to decreasethe variability of the data. Most statistical programshave methods in which one can adjust the variance to take into account the lack of independence of the samples collected. This problem is common when groups of animals under the same management schemes are sampled as a unit.

21.6.4 KAPPA STATISTICS

One may compare two or more diagnostic tests or clinical examinations to measure their agreement beyond that caused by chance. One can do this only using categoric data and kappa statistics. One uses a 2 × 2 table in which the kappa (κ) is actually a measure of the discordant pairs, that is, the two cells in which the tests do not agree. The formula is as follows:

$$K = \frac{\text{Observed agreement} - \text{Chance agreement}}{\text{Maximum agreement (1)} - \text{Chance agreement}}$$

The meaning of this calculation has come into question, and ranges for the measure vary. However, an example would include the following: $\kappa < 0.20$ = poor agreement; $\kappa = 0.21$ to 0.40 is fair; $\kappa = 0.41$ to 0.60 is moderate; $\kappa = 0.61$ to 0.80 is substantial; and $\kappa > 0.81$ is good.[40] One may use statistical programs to perform these calculations.

21.7 REFERENCES

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22 CHAPTER 22 RECOGNIZING AND TREATING PAIN IN HORSES

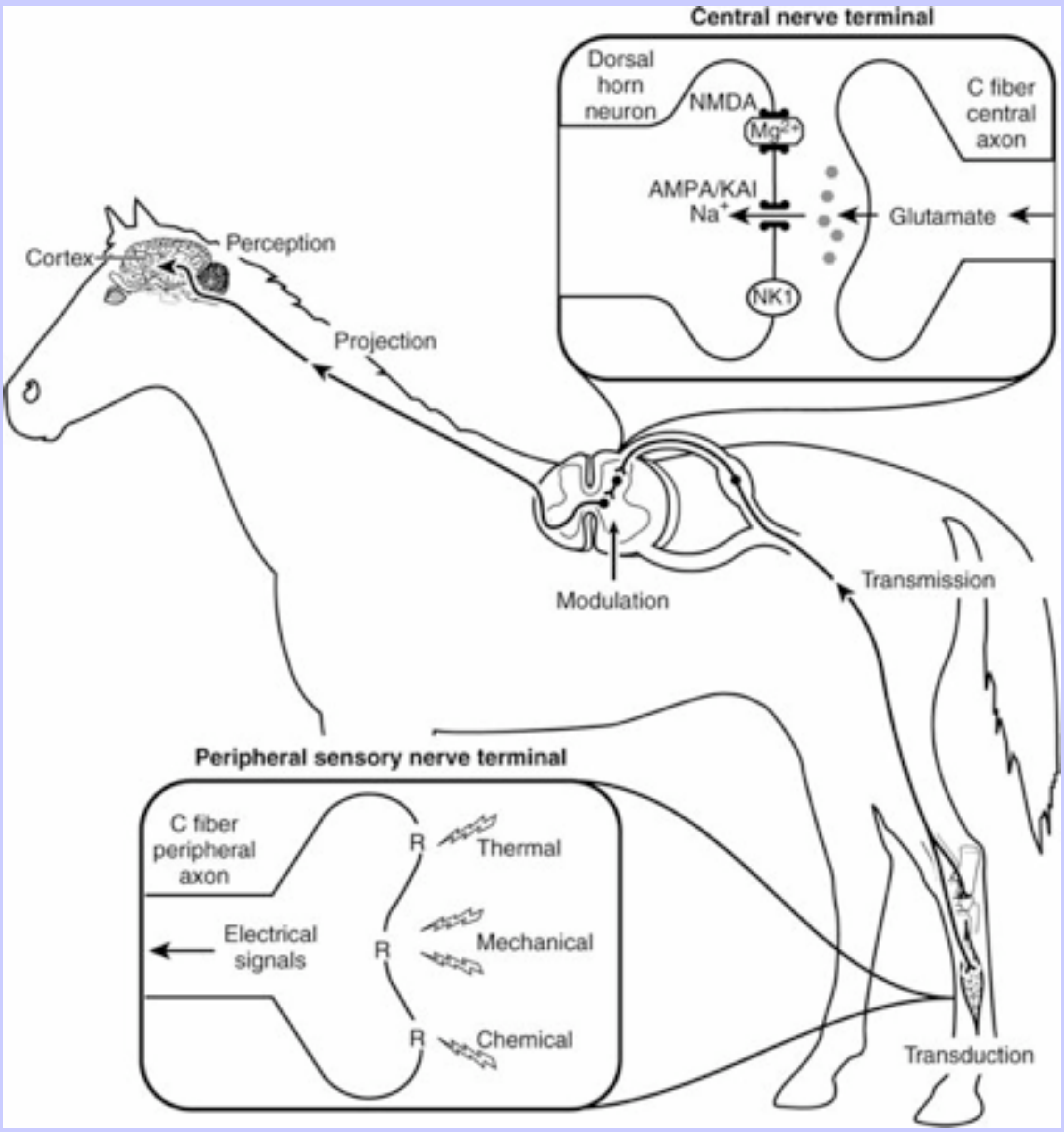
William Muir

The recognition and treatment of pain in horses, although commonplace, is evolving as evidencedby the wide variations in the attitudes, opinions, and methods used to control pain by equine practitioners.[1] Traditionally, however, pain has been used as a diagnostic tool for determining the source and potential cause (e.g., laceration, articular damage, laminitis, abscess, and colic) of pain rather than as an independent pathophysiologic process. What is intuitive to anyone that has experienced pain, particularly severe pain, is that pain can produce profound behavioral, neuroendocrine, metabolic, and immunologic effects. Chronic pain produces poor performance, inappetence, weight loss, and immunologic compromise predisposing to a generalized “sickness syndrome,” which often contributes to the natural or predetermined death of the horse.[2] Increased understanding of the neuroanatomic and molecular mechanisms responsible for pain and the discovery that untreated pain produces changes in the central nervous system (CNS) that contribute to the development of chronic pain have focused attention on the development ofmore effective and safer analgesic therapies.[3–5] One should consider pain as an independent rather than dependent variable, particularly in horses in which the cause ofpain is uncertain (idiopathic pain), pain is difficult to treat (e.g., myopathy or uveitis), or in which the causeof pain is impossible to eliminate (e.g., laminitis or osteoarthritis). The veterinary profession should consider pain as the fourth clinical sign (heart rate, respiratory rate, temperature, pain) requiring evaluation, documentation, and treatment. This attitude will help inspire the development of practical and clinically relevant diagnostic, evaluative, and therapeutic procedures for horses. This chapter presents a simplified review of the major pain pathways and the physiologic and pathophysiologic mechanisms responsible for pain and suggests clinically useful methods for evaluating and treating pain in horses.

22.1 Pain

Pain in human beings is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.”[6] This definition encompasses those neurophysiologic processes that warn and protect the horse from actual or potential tissue damage and help to prevent further injury and promote healing. Clinically pain involves two multifaceted components, nociception and perception.[4] Both facets are integrated to provide the immediate recognition and elimination (if possible) of the painful stimulus. Nociception is the detection of a noxious mechanical, thermal, or chemical stimulus and by itselfis not pain, because the transformation of noxious stimuli to electric impulses (transduction), their transfer to the spinal cord (transmission), and eventual projection to the brain does not ensure that the electric impulseswill be recognized (perceived), for example, as during anesthesia (Figure 22-1). Perception is the recognition that nociception has occurred or continues to occur and triggers responses that protect the horse from further insult and help to maintain homeostasis. Pain perception or the actual registration of a noxious stimulus depends on external (environmental) and internal (patient-related) issues and is responsible for the secondary behavioral 1529 1530 (vigilance, immobilization, fear), metabolic (hyperglycemia), neuroendocrine (cortisol, catecholamines), autonomic (heart rate, respiratory rate, pupillary), and immunologic responses that frequently are used to evaluate the severity of pain (pain score), determine stress, and ultimately aid in evaluating the quality of life of the horse.

Figure 22-1 Pain nociception and perception. Painful thermal, mechanical, and chemical stimuli are tranduced to electric potentials (action potentials) that are transmitted to the spinal cord, where they are modulated and then projected to the brain (perception). The major excitatory neurotransmitter in the spine is glutamate, which normally activates α-amino-3-hydroxy-5-isoxazole proprionic acid *(AMPA)* and kainate *(KAI)* receptors. *NMDA,* N-methyl-D-aspartate; *NK1,* neurokinin.



22.1.1 PHYSIOLOGIC AND PATHOLOGIC PAIN

Pain is caused by the activation of high-threshold (high stimulus intensity) nociceptors (pain receptors) located at the distal ends of unmyelinated (C) or poorly myelinated (Aδ) nerve fibers[2,4] (see Figure 22-1). The free nerve endings of these peripheral afferent nerve fibers encode noxious stimuli depending on the modality, intensity, duration, and location of the stimulus. The intensity of the stimulus required to produce painful sensations is considerably more than that required to produce nonpainful sensations (touch) and is the most important factor determining the severity of pain.[2] Pain that does not cause tissue damage is referred to as physiologic or nociceptive pain and serves to warn the horse of potential tissue damage. Pathologic pain occurs when tissue or nerve damage has occurred and frequently is categorized as inflammatory or neuropathic and occasionally as idiopathic.[5] The temporal aspects of pathologic pain are dynamic and are characterized by a 1530
reduction in the intensity of the stimulus required to initiate pain (hypersensitivity) and the production of pain 1531
by a stimulus that does not normally provoke pain (allodynia).[7] Inflammatory and neuropathic pain often are characterized by an exaggerated response (hyperalgesia) when a noxious stimulus is applied to the injured area. Peripheral sensitization is caused by the local production and release of chemicals (prostaglandins, leukotrienes, neuropeptides, nerve growth factors) that sensitize peripheral nerve fibers and activate additional pain receptors ("silent" receptors) to stimuli that normally would never produce pain.[4,8] Some of the hypersensitivity at the site of injury and all of the hypersensitivity that occurs outside the site of injury (secondary hyperalgsia) results from central sensitization.[7–11] The primary difference between peripheral and central sensitization is that the former activates and sensitizes nociceptors at the terminal ends of Aδand C fibers to low-intensity stimuli, whereas the latter changes sensory processing in the spinal cord resultingin pain from stimuli initiated in normal low-threshold sensory (touch) receptors.[5,8] Visceral pain (e.g., gut, liver, spleen, kidney, or bladder), unlike somatic pain, is transmitted by parasympathetic (principally vagal) and sympathetic splanchnic afferent nerve fibers.[12,13] The autonomic fibers involved in transmitting noxious inputs from visceral organs including the distal colon rectum and bladder are diffuse and extensively overlap. This difference from somatic transmission (transmission by parasympathetic and sympathetic nerves) means that much of the pain originating from viscera frequently involves an obvious autonomic component (tachycardia, tachypnea, mydriasis) and may be difficult to localize.[12] Generalized or diffuse inflammation, ischemia, and mesenteric stretching or intestinal dilation (e.g., of the stomach or cecum) can produce severe, unrelenting pain. Inflammatory diseases can also activate silent nociceptors in the gut or bladder, resulting in mechanosensitivity to otherwise innocuous smooth muscle contractile (normal peristaltic) activity.

22.1.2 PERIPHERAL SENSITIZATION

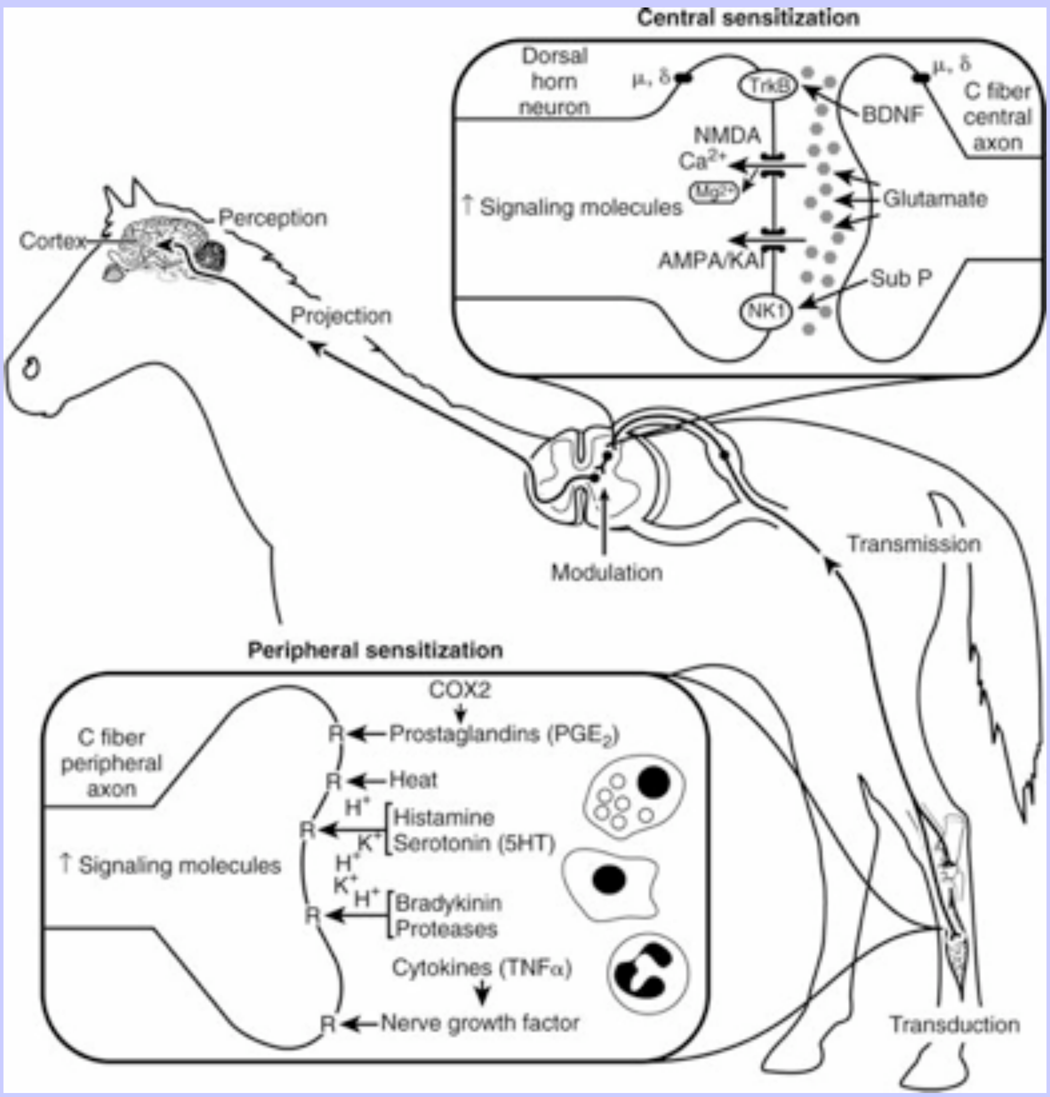
Peripheral sensitization is produced by the activationof enzymatic and reparative processes initiated by tissue damage and inflammation at the site of injury. The production or dissemination of the by-products of damaged tissues, inflammatory cells (lymphocytes, neutrophils, mast cells, macrophages), and autonomic nerves excite and increase the sensitivity (lower the activation threshold) of peripheral nociceptors[4,8] (Figure 22-2). Tissue damage results in the production of prostaglandins, bradykinin, and neurotrophic growth factors and the local release and spread of adenosine, adenosine triphosphate, and ions (H^+, K^+). Leukocytes and macrophages release cytokines (interleukin-1 [IL-1], IL-6, tumor necrosis factor α). Primary afferent sensory nerve fibers release neuropeptides (substance P, calcitonin gene-related peptide), causing mast cells to degranulate, promoting local vasodilation and plasma extravasation, further amplifying the inflammatory response. Collectively, these substances combine to produce a "sensitizing soup" that lowers nociceptor threshold and

activates silent nociceptors, thereby amplifying the pain response.[8] Peripheral sensitization is responsible for primary hyperalgesia or pain that is initiated by innocuous stimuli applied within the area of tissue injury.

22.1.3 CENTRAL SENSITIZATION

Central sensitization results from the cumulative effects (temporal summation) of repetitive and sustained summation of nociceptive inputs on central (spinal cord) neurogenic pathways.[4,7,8] Normally temporary noxious stimuli generate fast excitatory potentials within the CNS that are short lived but indicate the onset, duration, intensity, and location of the painful stimulus. This fast excitatory transmission is transmitted by $A\delta$ and C fibers and is mediated by glutamate acting on α-amino-3-hydroxy-5-isoxazole proprionic acid and kainate receptors within the dorsal horn of the spinal cord. Sustained input from damaged tissue or injured nerves releases glutamate and additional neuropeptides (substance P, neurokinin A), which activate *N*-methyl-D-aspartate and tachykinin receptors, resulting in sensitization (windup) of neurons in the dorsal horn of the spinal cord[8] (see Figure 22-2). Windup, central sensitization, and biochemical changes in the CNS represent a continuum of the pain process that generally is initiated by an acute painful process or chronic untreated pain. Central sensitization can last for hours to days and is believed to be responsible for increases in the receptive field and hypersensitivity to noxious stimuli from outside the area of primary tissue damage (secondary hyperalgesia).[8,10] Changes in the spinal cord segmental inhibitory input also can contribute to dorsal horn hyperexcitability, resulting in the perception of pain from otherwise innocuous stimuli carried by $A\beta$ (touch) nerve fibers.[8] Central sensitization is fundamentally different from peripheral sensitization in that it enables low-intensity stimuli to produce pain because of changes in sensory processingin the spinal cord. The projection of electric impulses, initiated by a painful stimulus from the spinal cord to the brain, can modify memory and may be the reason that severe or untreated acute or chronic pain can resultin permanent behavioral changes.[9] The development of central sensitization has several clinically relevant implications, including a change in the therapeutic focus from peripheral (local anesthetics, nonsteroidal antiinflammatory drugs [NSAIDs]) to central sites (opioids, α_2-agonists), the selection of potent centrally acting analgesic drugs, and the introduction of 1531
strategies that help limit or prevent the development of central sensitization (preemptive and multimodal).[5] 1532
Once hyperexcitability (central sensitization) is established, one must administer larger doses of analgesics for longer periods of time for therapy to be effective. Said another way, one should treat pain as early as possible and preemptively when possible.

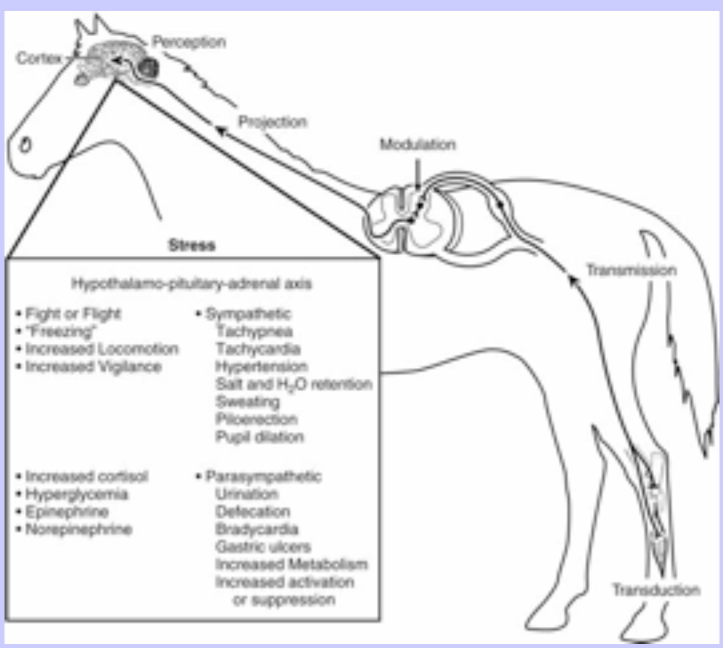
Figure 22-2 Peripheral and central sensitization. Peripheral sensitization caused by tissue or nerve injury lowers the threshold of nociceptors and activates “silent” nociceptors, leading to primary hyperalgesia. Central sensitization caused by the temporal summation of nociceptive imput activates *N*-methyl-$_D$-aspartate *(NMDA)* receptors in the spinal cord, resulting in secondary hyperalgesia. *TrkB*, Tyrosine kinase B; *AMPA*, α-amino-3-hydroxy-5-isoxazole proprionic acid; *KAI*, kainate; *NK1*, neurokinin; *Sub P*, substance P; *COX-2*, cyclooxygenase 2; *5-HT*, 5-hydroxytryptamine; *TNF-α*, tumor necrosis factor α.



22.2 Stress and Distress

Pain associated with disease, accidental trauma, or surgical trauma or originating from unknown causes can result in activation of the sympathetic nervous system, secretion of glucocorticoids (primarily cortisol), hypermetabolism, sodium and water retention, and altered carbohydrate and protein metabolism. The term given to this response is *stress*, which encompasses the biologic processes whereby the horse attempts to maintain homeostasis and cope with threats to its well-being.[14] Acute and chronic pain are capable of producing a significant stress response in horses. The stress response is an adaptive pattern of behavioral, neural, endocrine, immune, hematologic, and metabolic changes directed toward the restoration of homeostasis (Figure 22-3).
During threatening circumstances, the stress response prepares the horse for an emergency reaction that fosters 1532
survival in circumstances of immediate threats (fight or flight). Most stress in horses is short lived because of the 1533
removal or short duration of exposure to the stressor. Surgery and anesthesia are a common source of stress in horses that can be modified by the appropriate choice and use of preanesthetic and anesthetic drugs.[15] Suffering occurs when horses are forced to endure the infliction or imposition of physical pain and the feeling of impending destruction or harm (fear).[16] Severe or chronic stress can become maladaptive, producing distress and the triggering of self-sustaining cascades of neural and endocrine responses that upset the physiologic homeostasis of the animal and negatively affect biologic functions critical to the well-being ofthe animal. Stress and distress directly affect the well-being of the horse, produce immune incompetence, and can lead to the gradual deterioration of the patient, resulting in death.[17] At a minimum, all animals should be granted the “five freedoms”: freedom from hunger and malnutrition, freedom from discomfort, freedom from disease, freedom from injury, and freedom from pain (Box 22-1).

Figure 22-3 Pain produces stress that activates the hypothalamus-pituitary-adrenal axis, resulting in varied behavioral, neuroendocrine, metabolic, and immune responses.



22.2.1

BOX 22-1 INDICATORS OF PAIN, STRESS, AND WELL-BEING

Attitude

Activity

Appearance

Appetite

Facial expression

Behavior

Posture

Response to handling

Willingness to perform work

22.2.2

NEUROENDOCRINE AND AUTONOMIC STRESS

Auditory, visual, and somatosensory afferent sensory information are transmitted to the thalamus or directly to the amygdala in the brain. The noxious stimuli that produce acute and chronic pain induce stress that activates the hypothalamus-pituitary-adrenal axis.[14] This afferent information stimulates the secretion of corticotropin-releasing factor, vasoactive intestinal peptide, and adrenocorticotropic hormone (ACTH), to name a few. Corticotropin-releasing factor acts synergisticallywith vasopressin to stimulate the production of ACTH and β-endorphin, thereby enhancing cell survival and producing analgesic effects, respectively. Corticotropin-releasing factor also stimulates the adrenomedullary release of ACTH and catecholamines. Corticotropin-releasing factor is an excitatory neurotransmitter in the locus ceruleus that increases norepinephrine release and excitatory behaviors. Adrenocorticotropic hormone stimulates the adrenal cortex to secrete cortisol, corticosterone, aldosterone, and weak androgenic substances and also stimulates increased glucocorticoid production and the adrenomedullary secretion of catecholamines. Cortisol stimulates gluconeogenesis, increases proteolysis and lipolysis, facilitates catecholamine effects, and produces antiinflammatory actions. Importantly, many of these same factors (corticotropin-releasing factor, ACTH, and corticosterone) are significant modulators of learning and memory processes.[18] Increases in CNS sympathetic output increase respiratory rate, heart rate, and arterial blood pressure and produce sweating, piloerection, and pupillary dilation. Catecholamines are released from the adrenal medulla into the systemic circulation, amplifying these effects. Increased concentrations of circulating catecholamines augment glycogenolysis, increase gluconeogenesis, inhibit insulin release, and promote insulin resistance. Skeletal muscle blood flow generally increases out of proportion to increases in blood flow to other organ systems, preparing the animal for fight or flight. Epinephrine causes glycogenolysis, gluconeogenesis, and inhibition of insulin release, peripheral insulin resistance, and lipolysis. Thyroid hormones stimulate carbohydrate metabolism and heat production and increase and sensitize β-adrenergic receptors in the heart, thereby sensitizing it to the effects of circulating catecholamines.

22.2.3 METABOLISM

The neurohumoral changes produced by stress increase the secretion of catabolic hormones, promoting the production of food substrates from the breakdown of carbohydrates, fats, and protein.[17] Hyperglycemia is produced and may persist because of the production of glucagon and relative lack of insulin, although insulin levels may periodically increase. Cortisol, catecholamines, and growth hormone stimulate lipolytic activity, resulting in an increase in circulating glycerol and free fatty acids. Protein catabolism is a common occurrence and a major concern following severe trauma or extensive surgical procedures. Cortisol increases protein catabolism, resulting in the release of amino acids.

22.2.4 IMMUNE SYSTEM

The immune system functions as a diffusely distributed sense organ communicating injury-related information and the severity of stress to the brain. Pain therefore can modulate the immune response.[19] The key elementsfor determining the immune response to pain are its intensity and duration. The messengers of the immune system are cytokines (IL-1, IL-6, tumor necrosis factor α). Interleukin-1 and IL-6 induce the release of acute phase (inflammatory) reactants, cause fever, and initiate prostaglandin production (PGE_2). Severe stress fromany cause can trigger the acute phase response.[14] The main feature of the acute phase response is the release of proteins from the liver that act as inflammatory mediators and scavengers in tissue repair. These proteins includeC-reactive protein, fibrinogen, macroglobulin, and antiproteinases. Interleukin-1 and IL-6 can stimulate the secretion of ACTH from the pituitary gland and the subsequent release of cortisol. Tumor necrosis factor α produces signs of shock, including hypotension, hemoconcentration, hyperglycemia, hyperkalemia, and nonrespiratory acidosis, and activates the complement cascade. Excessive production of these proteins can contribute to systemic inflammatory response syndrome. The peripheral white blood cell count generally reflects a stress leukogram typified by an elevated number of immature polymorphonuclear leukocytes (left shift) and reduced numbers of lymphoctes. Chronic pain produces sustained increases in circulating concentrations of cortisol, epinephrine, norepinephrine, and glucagon and suppresses the humoral and cellular immune response.[17] The systemic release of endogenous opioids (endorphin and enkephalin) may contribute to immunosuppresion.

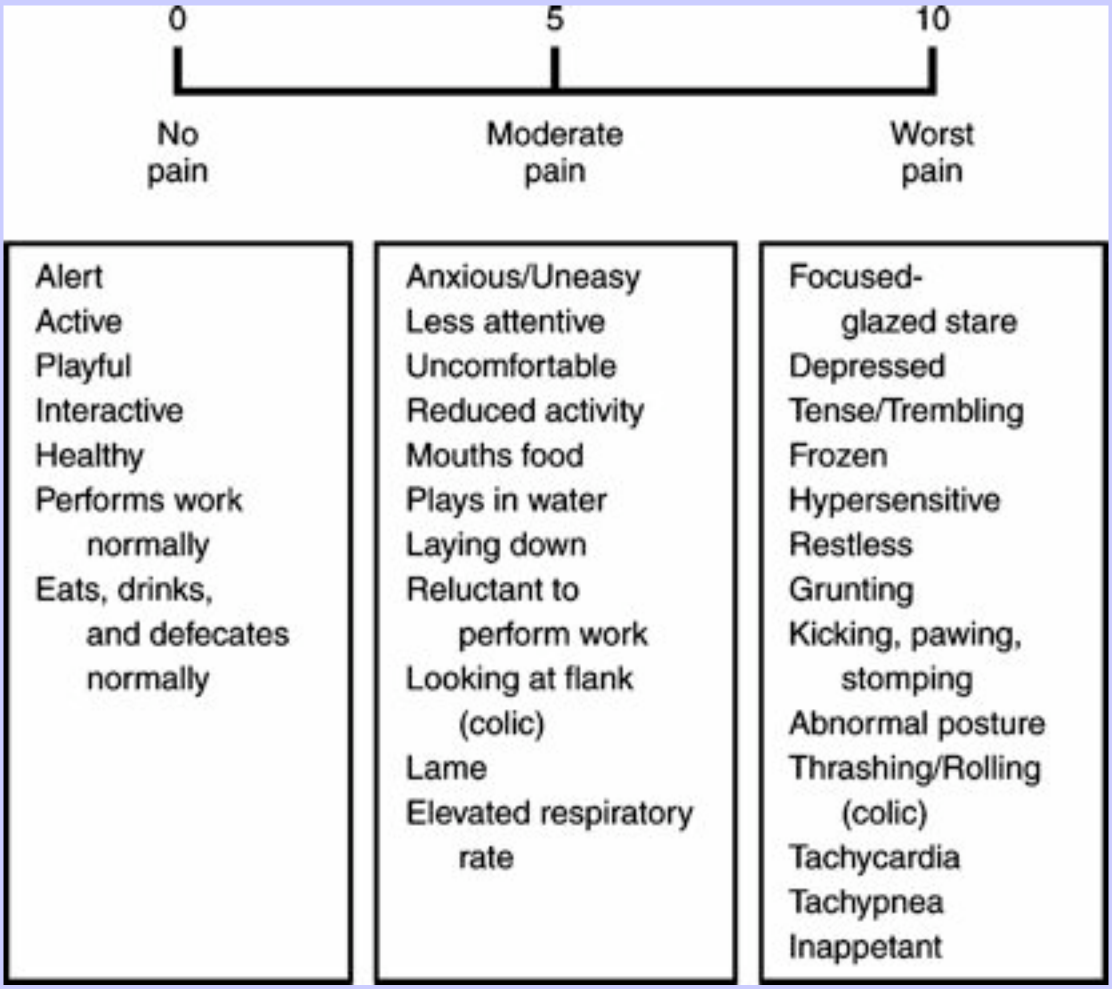
22.2.5 BEHAVIOR AND MORPHOLOGY

Pain is notorious for producing changes in behavior, attitude, performance, and memory. Pain activates the locus ceruleus, limbic regions (hypothalamus, hippocampus, amygdala), and cerebral cortex, which are involved in the adaptive responses to stress.[17] Pain-induced increases of corticotropin-releasing factor in the hypothalamus, amygdala, and locus ceruleus, for example, produce an increased startle response, anxiety, and fear. Corticotropin-releasing factor therefore serves as an excitatory neruotransmitter in the locus ceruleus, resulting in release of cortical norepinephrine, dopamine, and 5-hydroxytryptamine with resultant behavioral hyperresponsiveness, hyperarousal, vigilence, and agitation. Prolonged stress impairs the desire and ability of the horse to performand learn. Chronic stress caused by pain producestypical phenotypic changes that include a failure to thrive, poor hair coat, weight loss, and an accelerationof aging. 1534 1535

22.3 Evaluation of Pain

Although truly rational therapeutic approaches cannot be developed until the majority, if not all, of the specific mechanisms responsible for the physiologic and pathologic causes of pain are known, the use of simple yet detailed pain scoring systems can help categorize the intensity, duration, and location of pain, thereby suggesting potential treatments. Pain scoring systems, however, are of little value in suggesting therapy until the evaluator has a general understanding of the physiologic and pathophysiologic processes involved in the productionof pain.[7] This implies an understanding of the reasonsfor tissue hypersensitivity, peripheral sensitization, primary and secondary hyperalgesia, central sensitization, allodynia, spontaneous and referred pain, and the differences between somatic and visceral pain. All of these aforementioned changes modify the gain of the pain system leading to permanent alterations in the neurochemical regulation of the CNS and resultant phenotypic (physical characteristics) changes.[8] One of the most challenging problems in the clinical study of pain has beenthe development of a universally accepted and applied method of classification. The absence of a universally applied, clinically relevant pain scoring system has made it difficult to categorize patients, prescribe therapy, evaluate treatment, and compare research trials. Pain classification systems based on anatomy, etiology, body system, severity, duration, behavior, or cause are descriptive but do not identify the mechanism(s) responsible for pain and therefore do not provide the type of information required for determining effective therapy. One classification system proposes to diagnose pain based on the relationship between clinical signs, tissue type, disease, and the patient's response to predefined neurobiologic criteria.[20] This approach suggests that pain should be classified as inflammatory, neuropathic, or both and that the presence of peripheral sensitization, central sensitization, allodynia, and hyposensitivity to pain be used to subcategorize patients further and guide therapeutic decisions. Application of this approach along with anatomic, etiologic, and time- and behavior-based schemes will lead to the development of more informative diagnostic methods. When used with a visual analog scale, numerical, or categoric rating system, the aforementioned pain classification system should improve the diagnosis and the treatment of pain in horses (Figure 22-4).

Figure 22-4 The visual analog scale is a simple, practical method for evaluating the severity of pain and the efficacy of therapy.

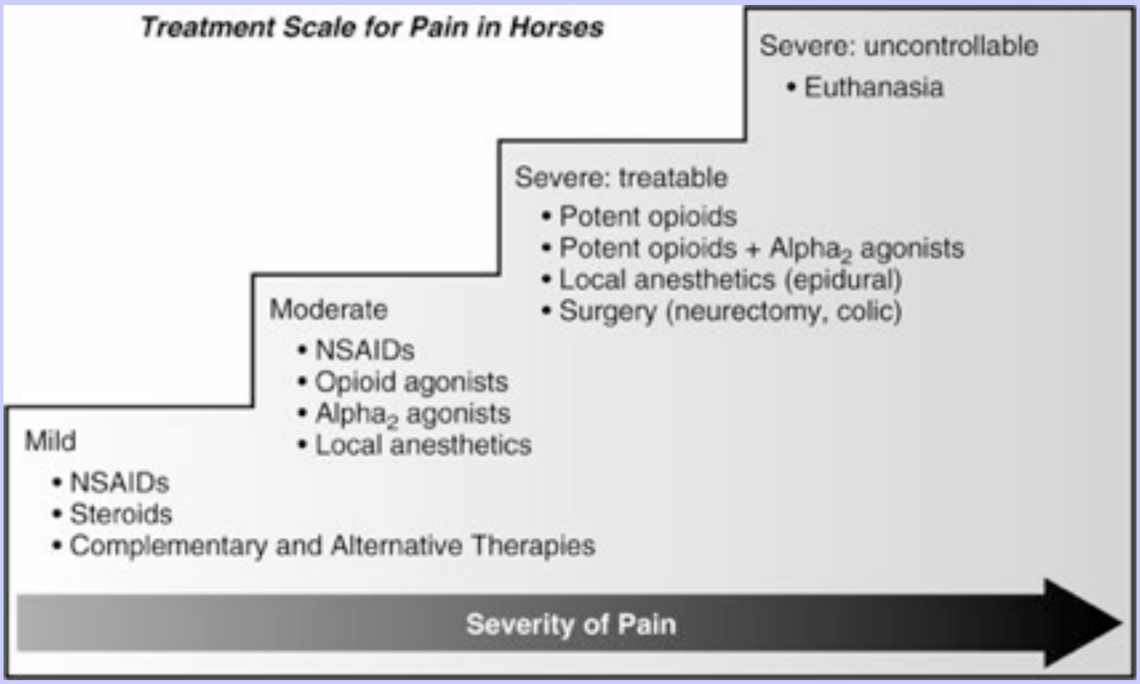


22.4 Drugs Used To Treat Pain

A vast array of clinical therapies claim to be efficaciousin the treatment of pain (inflammatory, neuropathic) in horses. Most are anecdotal or reflect individual attitudes.[1] Many therapies have been evaluated in experimental 1535
pain models (balloon colic, heat lamp, hoof nail) using otherwise normal horses, but few therapies have been 1536
subjected to blinded, randomized, controlled clinical trials in horses with spontaneous disease.[21–25] Regardless, a great deal of anecdotal data suggest that many of the same drugs used to treat pain in human beings, dogs, and cats are effective for treating pain in horses. Similarly, alternative therapies including acupuncture, chiropractic, and nutraceuticals can provide relief from acute and chronic pain in horses.[26–31] Pharmacologic approaches to the treatment of pain in horses derive their greatest benefits when drugs are selected for specific purposes (inflammation, nerve injury), scaled to the severity of pain, and used in combination (multimodal therapy) or preemptively, before the pain-inducing event(Figure 22-5). Most drugs used to treat pain fall into one of four

broad categories, which include the steroidal antiinflammatory drugs and NSAIDs, opioids, α_2-agonists, and local anesthetics. To this list can be added an expanding list of adjunct medications that produce antiinflammatory, anticonvulsant, antidepressant, or calming (acepromazine) effects[28] (Table 22-1).

Figure 22-5 A drug treatment pain scale for horses. *NSAIDs*, Nonsteroidal antiinflammatory drugs.



22.4.1 ANTIINFLAMMATORY DRUGS

Antiinflammatory drugs are comparatively weak analgesics but are effective for the most causes of mild to moderate pain particularly if the cause is inflammatory.[32–38] Glucocorticosteroids produce antiinflammatory effects by inhibiting phospholipase A_2 and the breakdown of membrane phospholipids to arachadonic acid and subsequently to prostaglandins and leukotrienes (Figure 22-6).[33] Prostaglandins and leukotrienes exaggerate the inflammatory response and are key factors in the productionof peripheral sensitization. Prostaglandins (PGE_2, thromboxane A_2) activate prostaglandin receptors throughout the body, producing pain, inflammation, and fever. Prostaglandins also are responsible for producing a protective gastric barrier to intralumenal acidity, sustaining normal gastric secretory activity, and maintaining normal gut motility. Prostaglandins regulate renal blood flow and maintain normal renal tubular function. Cyclooxygenase (COX) is an enzyme that metabolizes arachidonic acid to prostaglandins. At least two types of COX (COX-1, COX-2) exist. Cyclooxygenase 1—also termed *housekeeping COX*—is constitutive and helps maintain normal cell and tissue function. Cyclooxygenase 2 has housekeeping functions in the CNS, kidney, eye, and reproductive organs but is also inducible. Cyclooxygenase 2 is produced by inflammatory cellsand is upregulated greatly following tissue injury. Most NSAIDs produce their antiinflammatory and analgesic effects by differentially inhibiting COX-1 and COX-2.[33] Inhibition of COX-1 is believed to be responsible forthe NSAID toxicity (altered gastrointestinal motility and

ulceration). The availability of newer NSAIDs that are more selective for COX-2 versus COX-1 (COX-2; deracoxib) offers the potential to provide COX-1–sparing effects, minimizing the toxic effects on the gut.[37] Gastrointestinal, renal, or liver toxicity and the potentialfor delayed clotting are important issues that are more common with chronic drug administration.[36,39] One also must consider the potential for individual NSAIDs to alter platelet function and produce blood dyscrasias in sick horses or those with hemorrhage. Toxicity is more common in very young or old, dehydrated, or immunocompromized horses or in horses with preexisting cardiovascular, renal, or liver disease. 1536

TABLE 22-1 Drugs Used to Treat Pain in Horses

DRUG	INTRAVENOUS DOSE (mg/kg)	DOSING INTERVAL
ANTIINFLAMMATORY DRUGS		
Corticosteroids		
Hydrocortisone sodium succinate	1.0–4.0	—
Dexamethasone isonicotinate	0.015–0.050	—
Methylpednisolone	0.1–0.5	—
Prednisolone	0.25–1.0	—
Nonsteroidal		
Antiinflammatory Drugs		
Phenylbutazone	2.2–4.4	s.i.d. to b.i.d.
Flunixin	1.1	s.i.d. to b.i.d.
Ketoprofen	2.2	s.i.d. to b.i.d.
Carprofen	0.5	s.i.d. to b.i.d.
OPIOIDS		
Butorphanol	0.01–0.04	—
Buprenorphine	0.01–0.04	—
Morphine	0.05–0.1	—
Meperidine	0.2–1.0	—
Fentanyl	0.01–0.1	—
α_2-AGONISTS		
Xylazine	0.5–1.0	—
Detomidine	0.03–0.04	—
Medetomidine	0.01–0.02	—
Romifidine	0.04–0.08	—
NEUROLEPTANALGESICS		
Acepromazine	0.05–1.0	—
Butorphanol	0.05–0.1	
Acepromazine	0.05–1.0	—
Buprenorphine	0.005–0.01	
Acepromazine	0.02–0.05	—
Xylazine	0.2–0.5	

Xylazine	0.5–1.0	—
Butorphanol	0.01–0.05	
Xylazine	0.5–1.0	—
Morphine	0.1–0.5	

22.4.2

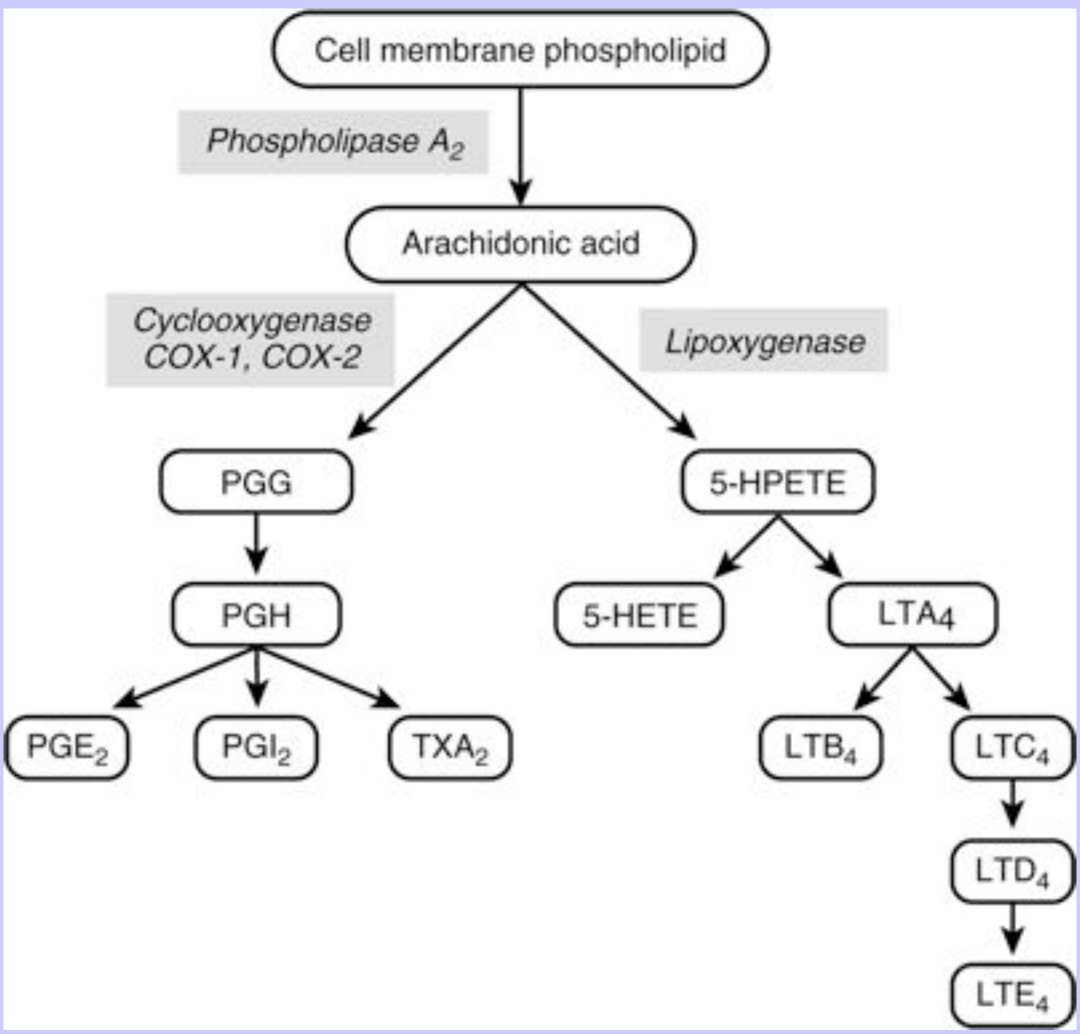
OPIOIDS

Opioids generally are considered to be the most effective of all analgesic medications but vary widely in their analgesic potency and clinical efficacy.[40–44] Opioid agonist-antagonists and partial agonists produce μ-receptor or morphinelike effects and are in general less toxic but less potent than morphine.[44] Opioid antagonists are devoid of agonist activity and are used clinically to antagonize or reverse opioid effects. Morphine is considered the prototypic opioid to which all other opioids are compared and occupies a particularly unique position among opioids in horses because of its long duration of action.[45,46] Opioids produce their effects by activating opioid receptors(μ, κ, δ, σ).[47] Most opioids produce their beneficial (analgesic, euphoric) pharmacologic effects by activating central and peripheral opioid μ-receptors, although evidence indicates that drugs with opioid κ-receptor activity may be particularly effective in treating gastrointestinal pain.[43,46] Analgesia along with minimal sedation places opioids, particularly butorphanol, in an ideal position to treat mild to moderate visceral pain.[40,44,48,49] These beneficial properties, however, are overshadowed by the requirement for special licensing and record keeping; the occasional development of ileus, constipation, and colic; and the potential for all opioids to produce excitement (increased locomotor activity), disorientation, and ataxia in horses. Opioids delay gastric emptying and prolonging intestinal transit time.[43] Increases in intestinal smooth muscle tone are caused by centrally mediated increases in vagal tone and activation of opioid receptors throughout the gastrointestinal tract. These effects are followed by a decrease in propulsive peristaltic activity and absorption of water from the intestinal tract, predisposing the patient to constipation, a condition that becomes more prominent when opioid administration lasts several days. Large or repeated dosages of opioids can inhibit the urinary voiding reflex and increase external urethral sphincter tone, resulting in urine retention. Urine retention can be an important postoperative consideration in some horses. Recommended dosages of opioids can produce sympathetic activation (sweating, mydriasis), nervousness, increased locomotor activity, agitation, and dysphoria in some horses.[47,50] The latter effects (excitement, disorientation, ataxia) are particularly troublesome because they can occur at clinically relevant dosages. Opioids, especially morhine, produce profound analgesic effects when combined with α_2-agonists (xylazine, detomidine).[51] The combination of opioids and α_2-agonists (morphine, butorphanol, and xylazine or detomidine) to produce so-called neuroleptanalgesia has been used preemptively to perform standing medical and surgical procedures that otherwise would require general anesthesia.[51–53] Fentanyl is a particularly potent but expensive opioid μ-receptor agonist with a short duration of action, making its clinical use impractical unless administered by constant rate infusion to painful horses (severe colic, laminitis).[54] Alternatively, a fentanyl transdermal drug delivery system (patch) is available, but clinical experience is limited and the patches may need to be replaced every 36 to 48 hours in horses to maintain analgesia.

As suggested previously, different opioids produce varied pharmacologic activity based on their ability to combine with and differentially activate the various opioid receptors. The prevalence and location of the various receptor subtypes determines the pharmacologic properties of the opioids. Practitioners know little concerning opioid receptor distribution, density, and selectivity in horses compared with other species. 1537
Furthermore, the potential of opioids to produce CNS agitation, increased locomotor acitivty, and excitatory 1538

effects when used alone or with inhalant anesthesia may negate their potential usefulness as analgesics.[47,50,55] Finally, few are the comparative studies or large scale randomized, controlled, and blinded multicenter clinical trials evaluating the efficacy of opioids in horses with naturally occurring disease, making their clinical use a process based on published experimental trial and error.[41,48]

Figure 22-6 Tissue or nerve injury damages cell membranes, leading to the production of arachidonic acid, prostaglandins *(PG)*, and leukotrienes *(LT)* via the activity of phospholipase A_2, cylcooxygenase, or lipoxygenase enzymes, respectively. These enzymatic pathways are important for producing inflammation and pain. They can be inhibited by corticosteroids, nonsteroidal antiinflammatory drugs, and lipoxygenase inhibitors. *5-HPETE*, 5-Hydroperoxyeicosatetraenoic acid; *TXA_2*, thromboxane A_2.



22.4.3 α_2-AGONISTS

α_2-Agonists (xylazine, detomidine, medetomidine, romifidine) produce sedation, muscle relaxation, and analgesia by activating various α_2-receptors in the CNS and peripherally.[56] Analgesia is confounded, however, by mild to significant sedation that can result in profound stupor, ataxia, and reluctance to move, although thismay be beneficial in horses suffering from colic.[34,48,57] Regardless, α_2-agonists are particularly effective therapy for the acute treatment of moderate to severe pain (colic) and can be administered by infusion to treat acute injuries or intraoperatively to reduce the requirement for inhalant anesthesia.[41,56] α_2-Agonists are particularly effective when administered with an opioid (butorphanol, morphine) or acepromazine.[51,53,58] The effects of α_2-agonists can be antagonized by the α_2-antagonists yohimbine, tolazoline, and atipamezole. Sedation is attributed to activation of the CNS α_2-A– and D–receptors in areas of the brain that are responsible for awareness, arousal, and vigilance. Activation of α_2-receptors in the brain and spinal cord decreases the release of excitatory neurotransmitters and interferes with sensory processing and transmission.[56] Individual drug effects vary based on chemical structure, α_2 versus α_1 selectivity, metabolism, and elimination. Xylazine is considered the prototypic α_2-agonist and demonstrates a comparatively low α_2-receptor selectivity and short half-life. Xylazine also produces local anesthetic effects, presumably because of its structural similarity to lidocaine.

Clinically the administration of α_2-agonists generally produces profound sedation, bradycardia, respiratory depression, and occasionally unexpected or aggressive behavior. Sinus bradycardia and bradyarrhythmias are common following the administration of α_2-agoniststo horses and are typified by the development of sinus arrhythmias and first- or second-degree atrioventricular block. Decreases in heart rate and atrioventricular block 1538
are caused by the combined effects of decreases in CNS sympathetic output and increases in vagal tone.[59] 1539
Atropine or glycopyrrolate can help to prevent bradycardia and atrioventricular block but generally are not required. Arterial blood pressure initially increases transientlyand then decreases from baseline values. Arterial hypertension is initiated by stimulation of peripheral vascularα_1- and α_2-receptors and resultant increases in baroreceptor activity. The subsequent decrease in arterial blood pressure is caused by a decreases in CNS sympathetic output and heart rate.[56,60] Cardiac output decreases almost immediately in parallel with decreases in heart rate.[59] Respiratory rate and tidal volume decreasewith CNS depression. These effects contribute to the development of respiratory acidosis and hypoxemia insome horses, although this rarely is a clinical problem. Relaxation of the muscles of the nostrils, pharynx, and larynx can result in respiratory stridor, snoring, irregular breathing patterns, inspiratory dyspnea, and upper airway obstruction in some horses, particularly those with upper airway diseases.

α_2-Agonists produce immediate and pronounced decreases in gastrointestinal tone and motility that can be pronounced and last for 8 to 10 hours.[61–63] Gastrointestinal stasis is caused by stimulation of α_2-receptors in the gut and increases in serum gastrin concentration. The decrease in gut motility is dose dependent but is believed to be responsible for postoperative ileus, gas accumulation, and the occasional development of colic in some horses and requires the administration of anα_2-antagonist (yohimbine).[64] α_2-Agonists promote diuresis in horses by increasing blood pressure and producing direct actions on the renal tubules to decrease renal tubular salt and water absorption. Labor can be delayed or prolonged because of sedative and muscle relaxant effects.

22.4.4 LOCAL ANESTHETICS

Local anesthetics (lidocaine, mepivacaine) frequently are used to produce a loss of sensation by administering them at specific sites (topical, local) or on nerves (regional), and they also have been administered epidurally and systemically to decrease the need for additional analgesics or to reduce the requirement for inhalant anesthesia[43] (Table 22-2). The systemic infusion of lidocaine decreases the requirement for inhalant (isoflurane, sevoflurane) or injectable (ketamine, propofol) anesthetics and potentiates the analgesic actions of opioids or α_2-agonists.[65,66] Analgesia results from blockade of sodium ion channels, thereby preventing the initiation and conduction of electric activity (action potentials) in small-diameter (C, Aδ) sensory nerve fibers. Larger (clinical) dosages of local anesthetics also block electric activity in large fibers (Aβ), thereby producing loss of motor function and temporary motor paralysis. Most local anesthetic drugs produce mild CNS depression (anesthetic sparing), antiarrhythmic, antishock, and gastrointestinal promotility effects.[43,67,68] Mild sedation occurs because of membrane-stabilizing effects, a generalized decrease in neuronal activity, and a centrally mediated decrease in sympathetic tone. Local anesthetics have the potential to produce a loss of motor function (paralysis) that can become problematic in some surgical patients (rectal-vaginal fistula repair), leading to untoward behavioral responses. Local anesthetics produce minimal cardiovascular or respiratory effects in otherwise normal, healthy horses but can decrease cardiac output, arterial blood pressure, and heart rate when administered intravenously because of decreases in CNS sympathetic output, myocardial contractile force, and venous return.[65,66] These effects are more prominent in stressed or sick animals that depend on sympathetic nervous system activity for maintaining homeostasis. Although local anesthetics are considered to have antiarrhythmic effects, they can produce sinus bradycardia and bradyarrhythmias and hypotension when administered rapidly intravenously. Significant differences exist among the various local anesthetics regarding these effects and their metabolism, elimination, and potential to produce CNS toxicity (disorientation, ataxia, seizures).[68] Horses are comparatively sensitive to the neurotoxic side effects of local anesthetics and bolus 1539

1540 dosages exceeding 2 mg/kg usually produce CNS stimulation typified by nervousness, excitement, agitation, disorientation, nystagmus, and seizures. These effects can result in death from respiratory paralysis. Local anesthetic drugs have the potential to produce respiratory paralysis when administered by epidural or spinal (subarachnoidal) routes. The migration of the local anesthetic cranially to the sixth cervical nerve roots can paralyze the diaphragm, resulting in hypoventilation and apnea.

TABLE 22-2 Caudal Epidural Analgesia in the Horse

DRUG	DOSE (mg/kg)	POINT OF ADMINISTRATION	DURATION OF ANALGESIA
LOCAL ANESTHETICS			
Mepivacaine HCl	0.20	S3-S4, S4-S5	1–1$^{1}/_{2}$ hours
Lidocaine HCl	0.16–0.22	Coccygeal 1–2	30–60 minutes
Lidocaine HCl	0.28–0.37	S3-S4, S4-S5	1$^{1}/_{2}$-3 hours
α_2-**AGONISTS**			
Xylazine	0.03–0.35	Coccygeal 1–2	3–5 hours
Detomidine HCl	0.06	S4-S5	2–3 hours
Romifidine	80 g/kg		2–3 hours
OPIOID			
Morphine	0.05–0.10	Coccygeal 2	8–16 hours
COMBINATIONS			
Lidocaine	0.22	Coccygeal 2	5$^{1}/_{2}$ hours
Xylazine	0.17		
Morphine	0.20	S1-L6	>6 hours
Detomidine	0.03		
Ketamine	0.5	S1-L6	12–18 hours
Morphine	0.1		
Ketamine	0.5	S1-L6	>2 hr
Xylazine	0.2		

22.4.5
NONTRADITIONAL ANALGESICS

New drugs are being developed continuously to treat acute and chronic pain. Anticonvlusants (gabapentin) and behavior-modifying drugs (chloripramine) have been administered to horses to produce adjunct analgesic effects, but their efficacy and safety are unknown. Alternative therapies including acupuncture, chiropractic, and nutraceuticals are used to treat pain in horsesas adjuncts to various drug regimens. Several of these therapies are considered effective pain therapy by clinical experts, but most have not been evaluated carefully. 26–31

22.5
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23 APPENDIX A APPLIED NUTRITION*

Donald R. Kapper

The mainstay of all diets for horses is the forage they consume. Understanding how the equine digestive tract functions optimally and how forage quality and quantity help maintain the health of the horse, reduce the incidence of colic, and keep the horses growing, reproducing, and performing to their genetic ability is what every veterinarian should know. With this knowledge veterinarians can help their clients select the best forage in their area and the appropriate grain mixture to complement it to meet all the nutrient needs of their horses. Once horsemen understand that horses do not have a requirement for cereal grains—for example, oats, barley, corn—but they do have an absolute requirement for forage, they understand better how to manage and feed their horses successfully. The only reason to feed a grain mixture or ration balancer to horses is to make up the difference between what nutrients are in their forage and what horses need to meet their daily nutrient requirements.

23.1 Equine Forage Quality

23.1.1 FEEDS

All horsemen add cereal grains or grain mixtures to increase the calories in the diet of their horses to meet their desired body condition. The feed manufacturer has the responsibility to explain for which physiologic status their feed mixture is prepared, what type of forage their grain mixture is formulated to complement, and what is the minimum amount of this grain mixture to feed per day to meet the nutritional requirements of the horse. These requirements vary with the age, size, growth rate, reproductive status, and performance level of the horse.[1,2] The owner or manager then is responsible for reading the information on the feed tag or bag and following the directions. If one feeds fewer pounds per day than the manufacture recommends as minimum in its feeding directions, one is feeding the horse a deficient diet.[3]

* With contributions from Gayle Ecker, Sarah L. Ralston, and James B. Rowe.

23.1.2 FORAGES: DETERMINING QUALITY

By definition, forages are the aerial parts of the plant commonly fed to livestock. Their history, like pasture, predates that of human beings. Forages supply various levels of nutrients depending on their type (species) and maturity at harvest. Today horses consume forages fresh, as pasture; dried, as baled or cubed hay; or preserved in silos or plastic bags, as haylage. Horses have limited ability to use poor-quality forages, so owners and trainers must understand how to determine quality and different types of forages. Because the maturity of the plant affects the digestibility of the fiber and the availability of the protein, calories, major minerals, trace minerals, and vitamins, feeding immature forages to horses that need the highest amount of nutrients per day is vital. Such horses are sucklings, weanlings and yearlings, nursing mares, and horses in moderate to intense training. The first visual appraisal of all types of forage includes (1) the length of the seed head in grasses (boot stage) and (2) the percent of blossoms in bloom in legumes (bud stage). Ideally, grasses should have seed heads less than one inch long, and legumes should have less than 10% of the buds in blossom. Because all plants prepare to blossom, the acid detergent fiber (ADF), which measures crude fiber (cellulose plus insoluble lignin) and soluble lignin, and neutral detergent fiber (NDF), which measures the ADF plus hemicellulose, increase so 1543
the stem has enough strength to hold up the seed heads or blossoms. Unfortunately, as the fiber portion 1544
increases, all other nutrients decrease and become less digestible and available to the horse.

The Hay Market Task Force of the American Forage and Grassland Council has published a quality grading standard to help determine the quality of different forages based on the maturity of the plants when harvested.

Table 1 explains ADF and NDF levels during different levels of maturity in forages and grades them accordingly with a scoring system of Prime and 1 through 5, with a corresponding relative feed value number. The description, in parentheses, beside each forage grade is the author's terminology.

Forage with a relative feed value (RFV) score of 102 or less will have a negative effect on the ability of the horse to meet its nutrient needs from this quality of forage. The lower feed value is due to the thickened layer of hemicellulose surrounding the cell walls that prevents the fermentation process from breaking them down to make the nutrients available to the horse. As the plant matures, the nondigestible fiber increases, affecting palatability negatively and slowing the rate of passage through the gut. These lower-quality forages cause the horse not to be able to maintain the desired body condition on forage alone, and one must add cereal grain or grain mixtures to the diet. A RFV score between 103 and 150 will have a positive effect on the nutrients in the plant being available to the horse. A RFV score greater than 151 will increase the absorption of nutrients in the small intestine because of the lower lignin content. A portion of the proteins contained in this high-quality forage can be absorbed as amino acids in the small intestine, depending on its RFV. Thus when feeding a forage with a RFV score of 103 or higher, one needs to feed less cereal grain or grain mixtures per day to maintain desired body condition. When fed forage with a RFV score between 103 and 150, mature horses can consume from 2.0% to 3.0% of their body weight in forage dry matter per day.

TABLE 1 Relative Feed Value of Grass, Mixed, and Legume Forages

	ANALYSIS (DRY MATTER BASIS)†			
QUALITY STANDARD*	**% ADF‡**	**% NDF**	**DMI,§ % OF BODY WEIGHT**	**RFV**
Prime (Prime)	<30	<40	>3.0	>151
1 (Premium)	31–35	41–46	3.0–2.6	150–125
2 (Good)	36–40	47–53	2.5–2.3	124–103
3 (Fair)	41–42	54–60	2.2–2.0	102–87
4 (Poor)	43–45	61–65	1.9–1.8	86–75
5 (Reject)	>46	>66	<1.8	<74

* Quality grading standard assigned by Hay Market Task Force of the American Forage and Grassland Council.

† Analysis associated with each standard.

‡ *ADF,* Acid detergent fiber; *NDF,* neutral detergent fiber; *RFV,* relative feed value.

§ Dry matter intake (DMI) as percentage of body weight. This measure is for mature horses only. Young, growing horses consume lesser amounts of this forage.

TABLE 2 Protein and Calorie Levels in Forages Based on Type and Maturity[*]

	GRASS			GRASS/LEGUME MIXED			LEGUME		
QUALITY STANDARD	% PROTEIN	% LYSINE[†]	Mcal DE/lb[‡]	% PROTEIN	% LYSINE	Mcal DE/lb	% PROTEIN	% LYSINE	Mcal DE/lb
Prime (Prime)	>11	>0.38	>1.05	>16	>0.68	>1.10	>21	>1.07	>1.17
1 (Premium)	8–10	0.31	0.95	13–15	0.60	1.03	18–20	0.97	1.10
2 (Good)	7–8	0.26	0.86	11–13	0.51	0.93	16–18	0.87	1.00
3 (Fair)	5–7	0.21	0.80	9–11	0.43	0.86	14–16	0.77	0.94
4 (Poor)	4–5	0.16	0.77	7–9	0.34	0.82	12–14	0.66	0.89
5 (Reject)	<4	<0.14	<0.73	<7	<0.30	<0.78	<12	0.56	<0.83

* These percentages are based on forages analyzed by Holmes Laboratory from 1980 to 2002 and are on a dry matter basis.

† Percentage of lysine in crude proteins: grass, 3.4%; mixed (50/50), 4.25%; legume, 5.1%.

‡ *DE,* Digestible energy.

Table 2 explains the relationship between maturity of the plant, or grading system, and the amount of protein, lysine, and calories available. Crude protein is not included in the RFV equation because it is not correlated highly with forage digestibility or intake. However, the more mature the plant, the lower the percent of protein, lysine, and calories per pound.

Table 2 demonstrates why purchasing hay based only on percent protein is not recommended. A 12% crude protein analysis could come from Prime grass, 2 (good) mixed, or 4 (poor) legume forages. The calories and availability of all nutrients are less per pound in the more mature forages. Just because hay contains a legume such as alfalfa or clover does not mean it is always better quality. Maturity of the plant when harvested (RFV) determines the palatability, digestibility, and availability of the nutrients and the true value of each type of forage, whether it is grass, mixed, or legume. 1544

The timing of harvest is the most important consideration when trying to produce top-quality feed. If forage is 1545 cut after maturation, one can do nothing to change the quality of that forage. All forage crops decline in feeding value as they mature. In the cool growing seasons, once the buds appear, forages lose 0.2% per day in protein and about 0.4% per day in digestibility.[4] During the hotter growing seasons, the protein and digestibility (RFV) decrease even faster. Therefore the time allotted for forages to go from Prime to 5 in the cool seasons of spring and autumn takes 4 to 5 weeks. In the hot summer months in the northern states or the entire growing season in the southern states, complete maturation takes less than 3 weeks. This faster maturing is related directly to ambient temperature.

TABLE 3 Mineral Ranges Based on Type of Forage When Relative Feed Value Is Between 103 and 150

NUTRIENTS	GRASS FORAGE ANALYSIS	MIXED FORAGE ANALYSIS	LEGUME (ALFALFA) FORAGE ANALYSIS
Calcium	0.25%-0.80%	0.80%-1.20%	1.20%-1.80%
Copper	2–10 ppm	4–10 ppm	4–10 ppm
Iron	60–200 ppm	60–200 ppm	60–200 ppm
Magnesium	0.15%-0.25%	0.20%-0.30%	0.20%-0.35%
Manganese	40–70 ppm	40–60 ppm	40–50 ppm
Molybdenum	1–2 ppm	2–4 ppm	3–6 ppm
Phosphorus	0.20%-0.30%	0.25%-0.35%	0.25%-0.35%
Potassium	0.80%-1.50%	1.50%-3.00%	2.00%-3.50%
Sulfur	0.15%-0.30%	0.20%-0.35%	0.25%-0.35%
Zinc	12–26 ppm	14–26 ppm	14–28 ppm

Table 3 shows the expected major mineral and trace mineral ranges by forage type when the RFV is between 103 and 150. These mineral ranges are from actual analysis completed by Holmes Laboratory, Millersburg, Ohio, from 1980 through 2001, and are based on type of forage and maturity when harvested.[5] If the RFV is greater than 150 or less than 103, the nutrient analysis is expected to change accordingly. The following also affect the mineral levels in the plant: type (species) of forage, maturity at harvest, type of soil where they are grown, amount of fertilizer applied, amount of rainfall, and ambient temperature during the growing season.

In general, grass forages are lower in crude protein, the amino acid lysine, calcium, and potassium. One must take these values into consideration when evaluating and recommending forages and feeding programs for horses. These nutrients are more critical in diets of horses under stress conditions, such as growing, reproducing, and training.

23.1.3 GRASSES

Grass species generally are divided into two groups: cool season and warm season. They are shallow rooted and therefore not drought tolerant. Grass species germinate and grow best in soils with a pH of 6.5, when the subsoil pH is less than 6.0. All grasses are classified as monocotyledon, and lysine makes up 3.4% of the crude protein.[6]

The cool season grasses include Kentucky Bluegrass, Orchard grass, Timothy, Tall Fescue, Smooth Bromegrass, Reed Canary grass, Ryegrass and cereal grasses. These grasses are grown predominantly in the northern half of the United States. These species are of greatest nutritional value as pasture in the spring and fall when the temperature is lower and the rainfall is higher.[7]

The warm season grasses include Bermuda grass, Bahia grass, Prairie grass, Bluestem, Sorghum, and Wheatgrass. These grasses are grown in the southern half of the United States and are more drought tolerant.[8] They have the potential to produce good hay and pasture growth during the warm and dry midsummer months. Warm season grasses produce well on soils with low moisture holding capacity, low pH, and low phosphorus levels.

23.1.3.1 Cool Season Grasses

23.1.3.1.1 Kentucky Bluegrass

Kentucky bluegrass is a long-lived perennial grass especially well suited to pastures. Kentucky bluegrass forms a dense, tough sod under favorable conditions, providing good footing for grazing animals. Kentucky bluegrass is one of the most forgiving grasses, able to tolerate and persist under a wide range of soil conditions and mismanagement; however, it responds well to good management. Kentucky bluegrass grows best under cool and moist conditions and usually becomes semidormant during the summer. Planting good-quality seed results in a more productive pasture than the naturally volunteering bluegrass.

23.1.3.1.2 Orchard Grass

Orchard grass is a versatile perennial bunch-type grass (no rhizomes) that establishes rapidly and is suitable for hay and pasture. Orchard grass is probably the most productive cool season grass, especially under good fertility management. Orchard grass has rapid regrowth, produces well under intensive cutting or grazing, and attains more summer growth than most of the other cool season grasses. Orchard grass tolerates drought better than several other grasses and grows best in deep, well-drained, loamy soils. 1545
Orchard grass is especially well suited for mixtures with tall legumes such as alfalfa and red clover. The 1546
rapid decline in palatability and quality with maturity is a limitation with this grass. Timely harvest is essential for obtaining good-quality forage. Improved varieties of orchard grass, with high yield potential and improved resistance to leaf diseases, are available. For seeding orchard grass/legume mixtures, one should select varieties that mature at about the same time. The later maturing varieties are best suited for growing with alfalfa, because they match the maturity development of alfalfa and are easier to manage for timely harvest to obtain good-quality forage.

23.1.3.1.3 Timothy

Timothy is a hardy perennial bunchgrass that grows best in cool climates. It generally grows better in the northern states because its regrowth is limited by its intolerance of hot and dry conditions. The shallow root system of timothy makes it unsuitable for droughty soils. Timothy produces most of its annual yield in the first crop. Timothy is used primarily for hay and is especially popular for horses, requires well-drained soils, and is less competitive with legumes than most other cool season grasses. Because frequent cutting easily weakens timothy, a sufficient recovery period is necessary for accumulation of energy reserves for regrowth.

23.1.3.1.4

Tall Fescue

Tall fescue is a deep-rooted, long-lived, sod-forming grass that spreads by short rhizomes. Tall fescue is suitable for hay and pasture because it is widely adapted and persists on acidic, wet soils of shale origin. The grass is drought resistant and survives under low fertility conditions and abusive management. Tall fescue is the best grass for areas with heavy livestock and excessive traffic. Most of the tall fescue in older, permanent pastures contains a fungus (endophyte) growing in the plant. The endophyte is associated with poor palatability and broodmare performance.[9] This endophytic fungus, *Acremonium coenophialum*, in the pastures and the hay affects the reproductive performance of the broodmare and the growth performance of young horses. One should include deep-rooted legumes with tall fescue if the grass is to be used for pasture. Legumes improve animal performance, increase forage production, and dilute the toxic effects of the endophyte when it is present. Newer endophyte-free varieties are recommended for pastures and hay. When buying seed, one should make sure the tag states that the seed is endophyte free. Because endophyte-free varieties are less stress tolerant than the endophyte-infected varieties, they require more careful management.

23.1.3.1.5

Smooth Bromegrass

Smooth bromegrass is a leafy, sod-forming perennial grass best suited for hay and early spring pasture. Smooth bromegrass spreads by underground rhizomes and through seed dispersal and is best adapted to well-drained silt-loam or clay-loam soils. Smooth bromegrass is a good companion with cool season legumes, matures later than orchard grass in the spring, and makes less summer growth or tons per acre. Smooth bromegrass is winter hardy and because of its deep root system survives periods of drought. Smooth bromegrass produces excellent-quality forage, especially if harvested in the early seed head (boot) stage.

23.1.3.1.6

Perennial Ryegrass

Perennial ryegrass is a bunchgrass suitable for hay or pasture. Perennial ryegrass produces excellent-quality and palatable forage, is a vigorous establisher, has a long growing season, and is high yielding under good fertility when moisture is not lacking. Because perennial ryegrass is less winter hardy than other grasses, it is best seeded in combination with other grasses and legumes. Perennial ryegrass does not do well in the central and southern states because it is not heat or drought tolerant; however, perennial ryegrass is used commonly in those regions to provide winter grazing. Perennial ryegrass can be grown on occasionally wet soils. Perennial ryegrass is less competitive with legumes than orchard grass and is usually later to mature than orchard grass. Perennial ryegrass can withstand frequent cutting or grazing but is more difficult to cut with a sickle bar mower and is slower to dry than other grasses, causing it to mold more easily.

23.1.3.1.7

Cereal Grasses

Cereal grasses are those from common cereal grain crops: oats, barley, wheat, and rye. They are cut while the crops are still green, usually in the dough stage, and the grain remains part of the hay.[10] When cut at the appropriate stage of maturity, cereal grasses can be a satisfactory forage for horses. Field observations indicate that horses may prefer small grain hays in the order of oats, barley, wheat, and rye.[11] As these

cereal grain crops mature, their quality and digestibility decrease rapidly and they become tough and fibrous as the ADF and NDF increase.[12]

23.1.3.2 Warm Season Grasses

23.1.3.2.1 Bermuda Grass

Bermuda grass is popular in the southern United States. Coastal Bermuda grass more often is grown for hay because it grows taller than common Bermuda, which is generally too short for good hay yields. The nutrient content is similar to timothy, if cut at the same stage of maturity. To assure as high a RFV as possible, Bermuda grass should be well fertilized and cut at a 15-inch height for the first cutting hay and every 21 to 28 days thereafter.

23.1.3.2.2 Bahia Grass

Bahia grass is grown over much of the southern coastal plain, primarily for grass pasture and secondarily for hay.[8] Therefore hay often is made from surplus pasture growth, which results in overly mature hay with a RFV of less than 86. However, Bahia grass cut in the prebud stage has a RFV of greater than 103.

23.1.3.2.3 Prairie Grass

Prairie grass (Matua) is a tall-growing perennial bunchgrass introduced from New Zealand. Prairie grass is
used mainly in the midwestern and western United States. Prairie grass is adapted to well-drained soils 1546
with medium to high fertility and a pH of 6.0 or higher. Prairie grass is a type of bromegrass but differs 1547
from smooth bromegrass in that it does not spread by rhizomes and produces seed heads throughout the growing season. The RFV of prairie grass can equal cool season grasses, if cut in the boot stage of maturity.

23.1.3.2.4 Bluestem

Bluestem grows throughout the Central Plains of the United States. The most common types used for hay are big and little bluestem. Both make highly palatable horse hays of acceptable quality, but they mature rapidly. Therefore the RFV drops quickly if they are not cut in the preboot stage.

23.1.3.2.5 Sorghum

Sorghum families of grass include Sudan grass, Johnsongrass, and Sorghum/Sudan hybrids. Johnsongrass is a tall, rapidly growing grass often regarded as a weed because of its rapid maturity rate, leading to coarse, unpalatable, and low RFV hay.[8] Sudan grass hay if cut too early can contain toxic levels of prussic acid and should be tested before feeding. A report by J. C. Reager (Texas Veterinary Medicine Diagnostic Laboratory, unpublished data, 1987) indicates that Sudan grass hay may cause the same types of problems described for horses consuming Sudan grass pasture.[1] Careful management and harvesting are necessary to avoid these problems, and one should take extra precaution if one intends to feed any of the sorghum family to horses.

23.1.3.2.6 Wheatgrass

Wheatgrass normally is grown for pasture in the Northern Plains states. Wheatgrass is hardy and if cut in the boot stage can produce good-quality forage with a RFV greater than 103. However, as this grass matures, its quality and digestibility decrease rapidly.

23.1.4 LEGUMES

Legume species include alfalfa, clovers, lespedeza, and bird's-foot trefoil. All legumes require a deep, well-drained, fertile soil with a near neutral pH of 7.0 and a subsoil pH of 6.0 for germination, greatest production, and persistence. All legumes are classified as dicotyledon, and the lysine makes up 5.1% of the crude protein.[6]

23.1.4.1 Alfalfa

Alfalfa has become the predominant legume grown in the United States. Alfalfa is leafy and fine stemmed, produces high yields, is resistant to many pests and diseases, and is high in digestibility and palatability when harvested with a RFV of greater than 103. Alfalfa has good seedling vigor and excellent drought tolerance and produces good summer growth. However, alfalfa is prone to damage by the alfalfa weevil and potato leafhopper. To capitalize on the potential of alfalfa, one should select high-yielding alfalfa varieties with resistance to problem diseases in the growing area. Blister beetles sometimes enter alfalfa fields and can end up in the harvested product. Cantharidin toxicosis from the ingestion of the blister beetles was first reported in horses in 1978. The larva of the blister beetle only consumes grasshopper eggs, so the areas with the best control of the grasshopper have less chance of their forages containing the beetle. The adult blister beetle is attracted to the flower of the alfalfa plant, so it becomes critical to cut and harvest alfalfa in the prebud stage, before the blossom develops, or to remove the crimper on the hay bind so the beetle can escape.[13]

23.1.4.2 Red Clover

Red clover is a short-lived perennial legume grown for hay, pasture, and green manure crop. Red clover is better adapted than alfalfa to soil that is poorly drained and slightly acidic; however, greatest production occurs on well-drained soils with high water holding capacity and pH above 6.5. Red clover is one of the easiest legumes to establish but is not as productive as alfalfa in the summer. Most of the improved varieties are medium types and have good levels of disease resistance to northern and southern anthracnose and powdery mildew. These and other diseases can reduce stands quickly. If red clover is cut after the bloom stage of maturity, the hay has course, thick stems that are difficult to dry and may become moldy, dark, and dusty when baled. Red clover also can be infected by *Rhizoctonia leguminicola*, a fungus that produces an alkaloid called slaframine, resulting in the condition know as “slobbers.”[13] This fungus is found in the flower, so harvesting or topping the pastures in the bud stage can keep this from occurring.

23.1.4.3 White Clover

White clover is a low-growing, short-lived perennial legume well suited for pastures. White clover improves forage quality of grass pastures and reduces the need for nitrogen fertilizer. White clover has a shallow root system compared with other legumes and does not tolerate prolonged dry spells. Although well-drained soils improve production, white clover tolerates periods of poor drainage.

23.1.4.4 Alsike Clover

Alsike clover is a short-lived perennial legume that is tolerant of wet, acidic soils. Alsike clover grows in low-pH soils, which are too acidic for red clover and alfalfa, and survives in alkaline (high pH) soils. Alsike can withstand spring flooding for several weeks. A cool and moist environment is ideal for alsike clover growth; it has poor heat and drought tolerance. Mild to severe photosensitization reactions and hepatitis have been reported in horses consuming alsike clover.[14] The thick stem and leaf make alsike clover difficult to dry for hay, increasing its susceptibility to mold. Common molds such as *Penicillium* species can convert coumarin, a nontoxic substance, to dicumarol, which interferes with the activation of vitamin K, making it a potent anticoagulant. 1547

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23.1.4.5 Lespedeza

Lespedeza is a warm season legume and can be used for hay, pasture, and soil erosion control. Lespedeza is low yielding but can be grown on acidic and low-phosphorus soils; however, it responds well to lime and phosphorus fertilization. Lespedeza grows best on well-drained soils, is a dependable reseeder, and can persist in pastures for years if allowed to reseed each year.

23.1.4.6 Bird's-Foot Trefoil

Bird's-foot trefoil is grown in parts of the temperate zone but has lost favor with many producers because it is difficult to establish and weak stemmed and is slow to recover from gazing.[16]

23.1.5 SOIL FERTILITY

Management of soils for pastures and hay fields is a continuous process, often only considered by horsemen during establishment. Managing fertility for continued productivity and health of the plant is also important.[16] If the soil lacks major or trace minerals, the health and productivity of the plant is affected negatively. When horses consume plants, as pasture or hay, grown on poorly maintained soils, their health, skeletal development, reproductive status, and performance level also can be affected negatively.

If forages are grown and fertilizer is not added to replace the nutrients the plants have used, the nutritional value of the soil decreases. Over time, from “mining” of the soil, the quantity and quality of the forage grown on it also decreases. The decrease in forage quality and yields depend on the following:

- The initial nutrients in the soil, as indicated by soil test results
- The nutrients removed each year by the harvested crop (pasture or hay)
- The reserve nutrients of the soil
- The length of time from the last application of fertilizer

Forage crops traditionally have received less fertilizer than grain crops, yet high-yielding forage crops remove much more phosphorus and potash than grain crops. Adequate fertility is essential to maintain the forage stand and to prevent forage yields from tapering off as the stand ages.[4] Each ton of forage is estimated to remove 13

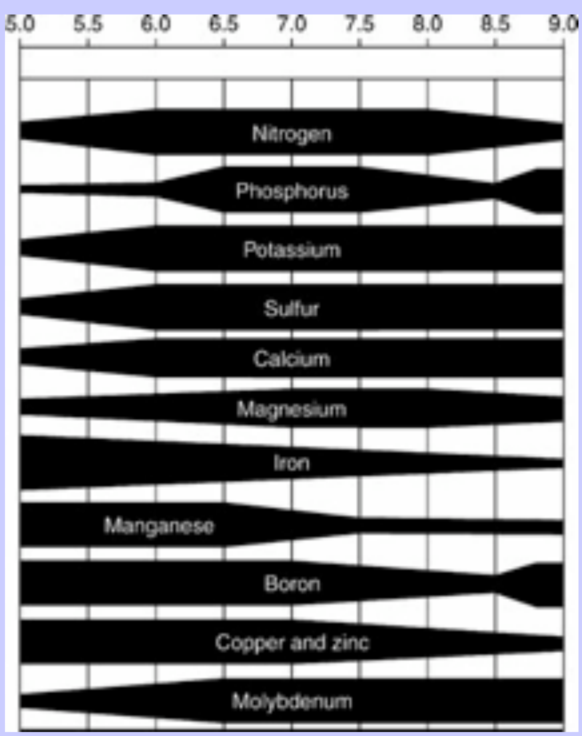
pounds of phosphate and 50 pounds of potash from the soil per year. Forage productivity varies from 1 ton to 8 tons per acre, depending on plant type, soil type, climate conditions, rainfall, and fertilizer applied. These nutrients must be replaced through commercial fertilizers or spreading manure to recycle the urine and manure of the horse[16]; otherwise, the health of the plant and then the health of the horse also will be affected negatively.

Soil pH has considerable influence on forage quality and plant growth. Soils may become more acidic naturally because of the leaching of basic cations, primarily calcium, magnesium, potassium, and sodium. Furthermore, when the plant takes up these basic cations from the soil, they are replaced with hydrogen, contributing further to acidification of the soil. Soil pH is of the utmost importance in plant nutrition, because it has an influence on the availability of many crop nutrients. The most common way to increase the soil pH is to apply lime. Application of limestone at least 6 months before seeding and splitting the yearly applications for spring and fall gives time for the lime to affect the soil pH.[4] One should consult an agronomist, fertilizer company, or local agriculture extension agent for assistance. Forage plants have optimal growth and nutrient absorption when the soil pH is between 6.0 and 7.0 (Figure 1).[16]

Nitrogen availability in the soil is associated with microbial activity. Microbes are involved in organic matter decomposition, which helps convert organic nitrogen into forms that are available to the plant. As soil pH decreases, the bacteria become less adapted, decreasing the mineralization of organic nitrogen compounds.

Phosphorus becomes less available as soil pH decreases. Insoluble iron and aluminum phosphates form, thus phosphorus becomes essentially unavailable to plants grown on soils below 5.5 pH.

Figure 1 The relative availability of nutrients essential for plant growth at different soil pH levels.



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Calcium and pH are directly related because the soil calcium concentration primarily determines pH. When the soil base saturation of calcium is high, 90% or more, the pH usually is high (6.5 or greater). When the base saturation of calcium is low, 50% or less, the pH usually is low (5.0 or less).

Potassium is particularly important for legumes, because it influences not only yield but also persistence. When legumes lack potassium, they are more likely to suffer winterkill. Two applications per year, rather than one, may be desirable on sandy soils that have a low exchange capacity. High applications of potassium lower the magnesium content of forages, which often has no affect on legume yield but may influence the mineral feeding program required.[4]

As with calcium, soil pH also influences magnesium, a basic ion. When the soil pH is low, magnesium becomes less available and has the potential to leach.

Sulfur and its availability are influenced greatly by the activities of microorganisms. A soil pH that favors forage growth generally favors microbial activity and sulfur availability. Sulfur has great potential to attach to many of the other minerals (calcium, magnesium, and potassium), which then leach out as sulfates.

Iron, manganese, zinc, and copper become increasingly available as soil pH drops. Soil pH levels of 7.0 or greater can result in low concentrations of these trace minerals in forages.

Aluminum availability increases when the pH drops below 5.0, such that it may become toxic to horses.[16]

23.1.6 FEEDING FORAGES

23.1.6.1 Fresh Pasture

Horses are herbivores by design and foragers by nature. They graze up to 18 hours per day on pasture, hay, or haylage.[17] Climates that allow forages to grow and horses to be pastured on them provide the ideal setting to raise horses. A well-managed pasture provides excellent-quality forage at a reasonable price, and if the types of forages can be blended together, one can maximize the number of grazing months available for horses and minimize the amount of hay and grain used for feed. An example is to blend cool season grasses to provide excellent spring and fall grazing and warm season grasses to provide summer grazing and to mix in a legume, which withstands grazing, to provide a higher quality of protein and calcium. The legumes also help reduce fertilizer cost because legumes have the capacity to use atmospheric nitrogen, reducing the need to apply it as commercial fertilizer. Legumes also supply a considerable amount of nitrogen to the grasses associated with them in the mixture, depending on the percentage of legume in the mixture.[4]

One should not feed fresh lawn clippings to horses. The possibility of (1) molds forming in the uneaten wet clippings and (2) high nitrates in lawn grasses that have been fertilized frequently can be detrimental to their health. One should *never* feed clippings from ornamental shrubs to horses. Most of the shrub clippings are or become toxic to horses as they wilt.

23.1.6.2 Dried Hay

If forages in a pasture field are not available for horses to graze, farm managers must provide dried hay, ad lib, in their exercise lot or box stall. Horses are continuous grazers because of their small stomach and the

fast rate of passage of forage through their intestinal tract. To maximize feed efficiency, increase nutrient absorption, decrease the incidence of colic, and improve the mental state of the horses, managers should provide hay for them to eat 24 hours per day. This is the same as allowing horses to graze on pasture 24 hours per day.

Harvesting forage and putting it into bales is advantageous to preserve it and enable it to be transported in a more dense form. The disadvantages are the labor to move the bales, storage requirements, and absorbing the losses incurred with baling and storage. The nutrients lost in storage are (1) protein, which occurs in the first 3 weeks after storing, and (2) the oxidizable vitamins A and E. Major and trace minerals, fiber level, and digestible calories per pound do not change in stored hay. Hay having more than 20% moisture is at risk from spoilage because of microorganisms metabolizing sugars in the hay and giving off heat. The final internal temperature reached in the curing hay is related to (1) percentage of moisture in the hay, (2) density of the bales and how tightly they are packed in the mow, and (3) the temperature and humidity of the outside air. The Browning reaction, caused by too high a temperature during the curing process, can be identified first by the odor of caramel candy, from the caramelization that occurs. The heat damaged protein can be analyzed for and subtracted from the crude protein in the hay when one balances the diet.[5] Flash fires also can result from the internal curing temperature becoming too high within 3 weeks after storing. Ninety percent of the oxidizable vitamins are lost during the drying and curing process, and their half-life after that is 60 days. For example, fresh grass pasture contains 330 IU or mg/lb of vitamin E on a dry matter basis.[18] After cutting, drying, and curing in storage for 3 weeks, the grass contains 33 IU/lb. For every 60 days in storage after that, the amount of vitamin E decreases by half. Therefore after 6 months the hay would contain approximately 4 IU/lb of vitamin E. This is one reason during the winter that one sees vitamin A and vitamin E deficiencies in horses fed hay that has been stored more than 6 months along with straight cereal grains.

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Table 4 provides the horse owner with a visual estimate for evaluating hay quality. Although not as reliable 1550
as forage testing, these guidelines allow sensory evaluation. Hay that is cut early, leafy, soft, green, and free of foreign material and has a pleasant aroma is high-quality and has a high RFV.[19]

TABLE 4 Scorecard for Visual Hay Quality Evaluation

VISUAL EVALUATION[*]		POSSIBLE SCORE	YOUR SCORE
I. Stage of Harvest			
Example: Timothy head length			
1. Prebud	1. Before blossom or heading	26–30	
2. Less than 1 inch	2. Early blossom or early heading	21–25	
3. 1 to 4 inches	3. Mid to late bloom head	16–20	
4. More than 4 inches	4. Seed stage (stemmy)	11–15	
II. Leafiness	1. Very leafy	26–30	
	2. Leafy	21–23	
	3. Slightly stemmy	16–20	
	4. Stemmy	0–6	
III. Color			
Note: The *outside* of a bale will bleach out from light, but this does not affect quality.	1. Natural green color of crop	13–15	
	2. Light green	10–12	
	3. Yellow to slightly brownish	7–9	
	4. Brown or black	0–6	
IV. Odor	1. Clean: crop odor	13–15	
	2. Dusty	10–12	
	3. Moldy: mousy or musty	7–9	
	4. Burned (caramel smell)	0–6	
V. Softness	1. Very soft and pliable	9–10	
	2. Soft	7–8	
	3. Slightly harsh	5–6	
	4. Harsh, brittle	0–4	
		Subtotal:	
VI. Penalties	1. Trash, weeds, dirt, etc.	Subtract 0–35	
QUALITY STANDARD[†]	**RFV**[†]	**VISUAL GRADING SCORES**[‡]	

Prime (Prime)	>151	95–100	Total:
1 (Premium)	150–125	85–94	
2 (Good)	124–103	75–84	
3 (Fair)	102–87	65–74	
4 (Poor)	86–75	55–64	
5 (Reject)	<74	Below 54	

* From Evaluating hay quality, Fact Sheet AGR-62, Quality hay production, Lexington, University of Kentucky.

† Quality standard and relative feed value (RFV) are assigned by Hay Market Task Force of the American Forage and Grassland Council.

‡ Visual grading scores.

Dried hay can be fed as long stemmed, as a square or round bale, or chopped and then cubed or pelleted. The form is irrelevant, once the horse becomes used to it. But the manager must feed these different forms by the pound. If cubes or pellets are fed, they too must be fed in similar quantity to the long-stemmed hay. However, the horses are able to consume the processed forages faster, so feeding them the cube or pellet 4 to 6 times per day is recommended. This reduces the incidence of boredom and prevents bad habits from developing, such as eating their bedding and feces, cribbing, wood chewing, weaving, and stall walking.

23.1.6.3 Preserved Haylage

In areas of the country where drying is difficult, harvesting forage at 50% dry matter and putting it into a plastic bag to ferment and stabilize has become popular. Fermentation has some advantages over storing the forage as hay that include (1) lower harvest losses and (2) less dependence on good drying conditions, thereby allowing the crop to be cut at the desired maturity. The disadvantages are that (1) if a rodent or machine punctures the bag, allowing oxygen inside the bag, the forage will mold or spoil and should not be fed and (2) the higher moisture level increases the cost of dry matter pounds shipped. When forage is first put into a plastic bag, conditions are aerobic (air is present). Aerobic bacteria produce heat as they breakdown 1550
carbohydrates and sugars into carbon dioxide and water and use up the trapped oxygen. When the air has 1551
been consumed, the haylage becomes anaerobic (free of oxygen) and this promotes the growth of anaerobic bacteria. These organisms convert the carbohydrates and sugars to organic acids that preserve the haylage. Within 3 weeks, the haylage reaches a stable pH of 4.5 to 5.0 and all bacterial and enzymatic activity stops. Once this stable pH has been reached, further breakdown of nutrients and spoilage stops, preserving the haylage for extended periods, provided that no oxygen enters.[4]

Preserved haylage has proved to be palatable to horses when harvested at optimal maturity and can provide excellent-quality forage. Unfortunately, if harvested late, the quality of the haylage is the same as dried hay cut at the same maturity level.

23.1.7 SUMMARY

All horse owners and managers must become more quality conscious of the forages they are feeding. Knowledge of forage quality and how it influences the growth, reproduction, and performance of their horses also can result in a more efficient and economical feeding program. Reliable analytic data is the foundation of a

balanced diet. Nutrients in forages such as protein and major and trace minerals can be measured accurately and their results entered into an equine computer ration–balancing program for ease of calculation.

However, the requirements of horses for ADF and NDF have not been established. Fiber levels in the total diet may have to be adjusted based on the veterinarian's and farm manager's experience and specific conditions found on the farm. When evaluating problem cases, nutritionists and veterinarians should not place much confidence in book values. They represent a good average but may not necessarily represent the specific forage being fed on that problem farm. When a diet appears adequate on paper but does not perform as expected, one should evaluate the data used to formulate the total diet. One still should consider equine nutrition an art and a science.

23.2 Cereal and Protein Grains: Explanation and Analysis

Concentrate is a broad term for all grains or mixture of grains used to manufacture feeds for the sole purpose of feeding it to horses. Concentrate is one grain mixed with another to improve the nutritive balance of the total and is intended to be diluted further to produce a grain mixture or a complete feed. Concentrates also are classified into two types: calorie-dense cereal grains from the grass family and high-protein grains from the legume and the grass families. All cereal grains, including oat, barley, corn, milo, wheat, rice, are grasses, and their lysine levels make up 2.9% to 3.8% of the crude protein. Cereal grains are considered low in quality amino acids, based on their lysine levels, and are higher in starch (carbohydrates) than all grass forages. Cereal grains also can be added to the diet of horses to provide extra calories to help maintain or gain body weight. The highest-quality protein grain is soybean, a legume with the lysine level making up 6.3% of its crude protein. The quality of amino acids is excellent, based on the lysine level.[2] Medium to high oil grains from the grass family include cottonseed, canola, linseed, and distiller's and brewer's grains and have a lower lysine level of 1.7% to 4.3% of the crude protein, and the quality of their amino acids is low based on the lysine levels. However, oil grains are higher in crude protein concentration than their grass or legume forage counterparts (Table 5). Lysine is the first limiting amino acid in horse diets today. To optimize growth and reproductive performance when feeding growing horses and pregnant or lactating mares, all amino acid concentrations, including lysine, must be present and in adequate amounts in their total diet. If a protein source other than soybean meal is used in the grain mixture, additional amino acids may be required. One adds protein grains or individual amino acids when they are needed to complement the forage part of the diet and to meet the nutrient needs of the horse. Usually one mixes a combination of cereal grains and protein grains with major minerals, trace minerals, and vitamins to balance the diet of horses. This grain mixture improves the nutritive balance or performance of the total and is intended to complement the forage part of the diet.[20] The percentage of each cereal and protein grain in a grain mixture depends on (1) the type and quality of forage it is formulated to complement, (2) how many pounds are to be fed per day per horse, and (3) the physiologic status of the horse, that is, idle, growing, reproducing, or performing.

23.2.1 CEREAL GRAINS

Many cereal grains are fed to horses throughout the world. The most abundant cereal grains in North America are oat, barley, corn, milo, and wheat. These grains vary in calories per pound and in percentage of crude protein, fat, and fiber. The largest variable is the quantity and type of starch structure found in each cereal grain. Each cereal grain varies based on percent fat, lysine, and the percentage of starch. Because all oils and proteins (amino acids) are found in the germ portion of the seed, the higher the protein and oils, the lower the starch content and weight per bushel. Therefore the higher the bushel weight of cereal grains, the lower the percentage of protein and oil in the analysis, and visa versa. All cereal grains are fed to provide a more concentrated source of calories in the diet of the horse. Compared to forage with a RFV below 86, cereal grains can provide 50% to 1551

100% more digestible energy per pound and in a much smaller volume. Cereal grains vary in size, weight per unit of volume, and nutrient content. For this reason, grains and grain mixtures should be fed by weight and not volume (i.e., by coffee cans or scoops).[15] Cereal grains, in order of ease of digestion, are oat, rice, wheat, barley, red milo, and corn. This order is based on the percentage of tertiary bonds in the starch structure, which slow the rate at which starch can be broken down by the enzyme amylase. 1552

TABLE 5 Comparison of the Range of Nutrient Contents of Cereal Grains, Grass Hays, High-Protein Grains, and Legume Hays

NUTRIENTS	CEREAL GRAINS[*]	GRASS HAY[†]	PROTEIN GRAINS[‡]	LEGUME HAYS[§]
Crude protein (%)	6.00–12.00	4.00–12.00	27.00–48.00	18.00–24.00
Cystine (%)0.07–0.14	0.04–0.14	0.32–0.68	0.24–0.34	
Lysine (%)0.20–0.42	0.14–0.42	0.90–3.30	0.92–1.22	
Methionine (%)	0.08–0.16	0.05–0.16	0.35–0.75	0.28–0.37
Threonine (%)	0.22–0.43	0.14–0.43	0.97–2.10	0.79–1.05
Fat (%) 1.80–4.60	1.00–3.00	1.00–18.00	2.50–3.50	
Digestible energy (Mcal/lb)	1.20–1.60	0.80–1.00	1.30–1.90	1.00–1.20
Acid detergent fiber	3.60–14.20	35.00–48.00	5.50–17.50	28.00–35.00
Calcium (%)0.01–0.10	0.25–0.80	0.17–0.35	1.20–1.80	
Copper (ppm)	4–8	4–10	20–25	6–10
Iron (ppm)31–73	80–200	35–319	100–200	
Magnesium (%)	0.11–0.14	0.15–0.25	0.30–0.60	0.20–0.35
Manganese (ppm)	5–36	40–70	21–49	40–50
Phosphorus (%)	0.25–0.35	0.20–0.30	0.50–1.10	0.25–0.35
Potassium (%)	0.32–0.44	0.80–1.50	1.20–2.10	2.00–3.50
Sulfur (%)0.11–0.21	0.15–0.30	0.26–1.20	0.25–0.35	
Zinc (ppm)17–25	16–26	60–73	20–28	

From Bath D, Dunbar J, King J et al: Byproducts and unusual feedstuffs, *Feedstuffs* 73(29):30–37, 2001 (reference issue); and Dale N: Ingredient analysis table: 2001 edition, *Feedstuffs* 73(29):28–29, 2001 (reference issue).

* Oat, barley, corn, milo, wheat, and rice.

† Timothy, orchard grass, Kentucky bluegrass, fescue, bromegrass, Bermuda, Bahia, prairie, and bluestem.

‡ Soybean, linseed, cottonseed, peanut meal, distiller's grains, and brewer's grains.

§ Alfalfa, clover, peanut, lespedeza, and bird's-foot trefoil.

Monitoring body condition and weight changes of individual horses is generally the preferred method to assess calorie balance, because differences in horse breeds, metabolic rates, body type, and temperaments tend to produce more variation among equines than all other livestock species. Carbohydrates, fats, proteins, and some fibers are all useful sources of energy. Horses can use fats/oils effectively and easily absorb fat at concentrations of up to 7% in the total diet or 15% in the grain mixture, which is approximately 0.8 kg of fat/oil per 500 kg of body mass per day. Even though horses have no gall bladder, they have a continuous flow of bile from the liver into the small intestine to emulsify the fats/oils in their diets. Everything that the horse consumes contains some fat/oils. For example, fresh forage and pasture contain greater than 5.0%, whereas dried, baled forages contain between 1.0% and 3.0%, based on the maturity when harvested. Adding fat to equine diets is an effective method of increasing caloric density for horses that have difficulty meeting calorie needs because of maladies of old age, such as poor teeth or malabsorption; for horses with high calorie demands because of intense work schedules[21]; for horses affected with equine polysaccharide storage myopathy; or for horses with glucose intolerance and hyperinsulinemia. Research suggesting that added dietary fat may reduce the heat production of performance horses working under hot and humid conditions[22] and enhance the oxidative capacity of muscle[23] continues to stimulate interest in this dietary supplement for horses.[21]

23.2.1.1 Oat

Oat has long been the favorite and preferred grain of horse owners. Oat, depending on its weight per bushel, contain between 9.0% and 12.0% crude protein, 0.26% and 0.46% lysine,[20] and 10.0% and 15.0% crude fiber. The heavier the oat, the lower the percentage of protein, lysine and fiber, because it has a higher percentage of starch. The starch content in heavier oat averages 38% and is amorphous structured, which means the carbon chains are in a straight line and digestive enzymes can break them down easily so the horse can absorb them in the small intestine. Therefore oat is the safest of all the unprocessed cereal grains to feed
to horses because more pounds are required per feeding to cause a digestive upset. The maximum safe 1552
amount to feed per feeding of oats is 5 lb/1000 lb body weight. Oat is purchased in the United States based 1553
on weight per bushel. Normally the price is based on 32 lb; however, a bushel can weigh as much as 46 lb. To grow a plump, white oat, the growing season must be cool and have low humidity. Thus oat grown in the northern part of the United States, Canada, or the Norwegian countries is the most sought after and the most expensive. Cold crimping or heat processed crimping of the oat does not make the starch easier to digest but makes it more available for the enzymes to break down because it cracks the outer hull. Crimping increases the cost 10% to 14% over whole oat. A good rule of thumb is that if one sees 10% or more of the oat passing through in the manure, one should purchase the crimped oat and feed 10% less per day.

Steam rolled oats and naked oats have a similar analysis because they have no oat hull. The steam rolled oats have had their hull removed before rolling, and the naked oat is grown without a protective covering (hull). They are basically the groat of the oat, which is where the digestible nutrients are found. They analyze at between 14% and 16% crude protein, 0.39% and 0.46% lysine,[20] and 2.0% and 3.5% crude fiber. The starch content averages 60% and is the easiest type of starch for the equine digestive system to break down (amorphous structure). The calories per pound are almost as high as corn but are much easier to digest. Thus oat is a common ingredient in foal feeds, for horses which have sensitive digestive systems, and the halter horse industry, where feeding 10% to 20% higher calories/day is necessary to maintain the desired 10% to 20% extra body condition.

23.2.1.2 Corn

Corn has taken over as the number one grain fed to horses in the United States because of its lower cost and higher calories per pound. Corn, depending on its weight per bushel, contains between 6.5% to 9.0% crude protein, 0.20% and 0.27% lysine,[20] and 2.0% and 3.0% crude fiber. The starch in corn averages 65% and is crystalline, which means the carbon chains are tightly bound tertiary bonds that hydrate more slowly than the amylopectin starches and are less vulnerable to enzymatic activity while passing through the equine digestive system.[24] Therefore one should feed corn most carefully because it is the easiest to overfeed and can cause digestive upsets in the horse. The maximum safe amount to feed per feeding of whole or cracked corn is 4 lb per 1000 lb body weight. Corn is purchased in the United States by how much it weighs per bushel. Normally the price is for 56 lb, but corn can vary from 50 to 60 lb per bushel. The same is true for corn as for all grains: the heavier the grain, the higher calories per pound, but the lower the percent of crude protein and fiber, and visa versa.

Cracking or cold rolling the corn does not change the starch structure; however, breaking the hull makes the starches more available for the enzymes to break down. Heat processing, which is the approved term of the Association of American Feed Control Officials (AAFCO) to use on a feed tag for "cooking," changes the starch structure and makes it easier to break down in the small intestine. The different internal temperature of the grain, when cooked, results in different responses to improved digestibility. The approximate values are these:

- Pelleting, at 180° F, improves feed efficiency of corn by 5% to 6%.
- Steam, at 212° F, improves feed efficiently by 10% to 12%.
- Extruding, at 260° F and under pressure, has the highest effect on digestibility and improves feed efficiently by 30%.

Extrusion or popped corn results in the starch being broken down to maltose, allowing it to be absorbed easily in the small intestine. For this reason, extruded horse feed may be recommended for horses with hindgut functioning problems, horses prone to colic, horses having had intestinal surgery, those rescued from starvation, and high-performance horses when more than half of their diet is grain and they are still loosing body condition. The manufacturing cost of pelleting and steam crimping increases the price 6% to 8%, and 12% to 16%, respectively, whereas the cost of extruding increases the price by 30% to 40%.

Recent advances in corn genetics have produced corn containing up to 7.0% oil and crude protein 1.0% higher than the current average (higher lysine). As the sum of oil and protein percentage increases, the starch content decreases by approximately the same percentage.[25] Genetic research also has improved the phosphorus availability by greater than 50%, thus reducing the need to increase the total phosphorus in the diet, and changed the starch structure to remove the amylose and replace it with amylopectins.[24] More will be heard about "environmental friendly" corn in the future, as well as the feed industries' addition of the phytase enzyme (to improve digestibility of phosphorus) into grain mixtures.

Molds in the soil lay dormant until the right environmental conditions are present, and then they propagate. All grains grown under drought or stressed conditions and harvested under moist conditions are prone to contain molds. Corn is the most susceptible because it is harvested in the autumn. Outbreaks of aflatoxicosis and leukoencephalomalacia in horses, caused by toxins produced by *Aspergillus flavus* and *Fusarium*

moniliforme, respectively, have raised concerns.[26] In response, most feed companies voluntarily began screening corn shipments for the presence of aflatoxins, reducing the threat of commercial feed contamination.[15]

23.2.1.3 Barley

In certain parts of the world barley is the only cereal grain fed to horses. In the United States, barley is grown 1553
primarily on the East and West Coasts and therefore commonly is used in horse grain mixtures in those 1554
regions. However, with the increasing cost of oat, more barley is being fed to horses throughout the United States. Barley, depending on its weight per bushel, contains between 9.0% and 12% crude protein, 0.33% and 0.44% lysine,[20] and 4.0% and 6.0% crude fiber. The starch content of barley averages 50%, and ease of digestibility falls between corn and oat; therefore barley is a good choice for horses. The grain is larger in diameter than oat but has a tenacious seed coat; therefore barley should be cold or heat processed crimped to improve digestibility.[15]

23.2.1.4 Milo

In the southern United States milo is a common, high-calorie feed for horses. Milo, depending on its weight per bushel, contains between 9.0% and 11.0% crude protein, 0.27% and 0.33% lysine, and 2.0% and 3.0% crude fiber. The starch content of milo averages 57%, which is lower than corn and wheat but higher than all other cereal grains. The same cautions apply as for corn regarding overfeeding, and milo should be heat processed steam flaked or rolled to change the starch structure and improve digestibility.

23.2.1.5 Wheat

In Europe, where more wheat is grown than other cereal grains, heat processed rolled wheat is a mainstay in the formulae of horse grain mixtures. Wheat contains between 9.0% and 11.0% crude protein, 0.26% and 0.32% lysine,[20] and 2.0% and 3.0% crude fiber. The starch content of wheat averages 60%, which makes the calories per pound almost equal to corn. If wheat is not heat processed crimped or rolled, it should make up no more than 30% of the grain mixture because of the doughy consistency of its raw starch, which can affect palatability negatively.

TABLE 6 Protein Grains: Partial Analysis

PROTEIN GRAINS	% DRY MATTER	% CRUDE PROTEIN	% LYSINE	% ETHER EXTRACT	% ADF*	% NDF	% LIGNIN
Brewer's dried grain	90.7	27.0	0.90	4.7	20.0	43.0	4.5
Canola	90.3	18.4	1.04	36.4	10.4	16.0	4.1
Canola meal	89.9	34.1	1.92	4.9	18.5	26.9	6.7
Cottonseed meal solubles	90.5	41.0	1.68	1.7	18.0	27.9	6.9
Cottonseed, whole	90.1	21.0	0.92	17.4	36.1	45.3	11.6
Flaxseed	90.0	18.0	0.64	38.0	11.8	19.3	4.5
Linseed meal (mechanically extracted)	91.0	32.0	1.10	3.5	18.5	30.2	5.5
Linseed meal (solvent extracted)	91.0	33.0	1.30	0.5	19.9	32.5	5.9
Maize distiller's dried grain with solubles	90.2	27.0	1.08	9.0	17.8	35.0	3.9
Peanut meal solubles	92.3	48.0	1.60	1.3	12.5	19.8	4.2
Soybean meal, 48%	89.5	48.0	3.30	1.0	5.5	8.8	0.4
Soybeans, cooked	91.0	38.0	2.50	18.0	13.4	20.1	2.8

* *ADF,* Acid detergent fiber; *NDF,* neutral detergent fiber.

23.2.2 PROTEIN GRAINS

All protein grains are produced as high-oil seeds, heat processed, and fed as a high-protein and high-calorie feed, or the oil is extracted, using an expeller or solvent process, and fed as a by-product feed containing higher protein levels; or they are a by-product of the distilling or brewing industries. The true value of each of these depends on the cost per unit of protein, amino acid composition and bioavailability, fat level, and cost per ton delivered to the manufacturing facility (Table 6).

23.2.2.1 Soybean

Soybean meal is the most common protein grain fed in the horse industry today because of the amino acid balance and the low cost per unit of protein. *Whole soybeans* must be heat processed by roasting or cooking to inactivate the trypsin inhibitor, but not overheated, which negatively affects the availability of the amino acids in the horse. In the soybean oil industry, the whole soybean is heat processed, the oil extracted, and the hulls removed. This dehulled soybean meal contains up to 48% crude protein, 3.3% lysine, and 3.0% crude fiber depending on the amount of oil extracted. Soy hulls are added back into the meal to create a 46%, 44%, or 42% protein meal, depending on what the purchaser wants, and the crude fiber varies from 6.0% to 7.0%. Of course, the lower the percentage of protein (amino acids), the lower the cost per bag or per ton, but the cost per unit of protein increases. Heat processed whole soybeans, a legume, are used more in the equine 1554

industry today because of the value of the added oil with the high-quality amino acids. Heat processed whole or flaked soybean contains 38% protein (2.5% lysine), 18% oil, and 5.0% crude fiber.

23.2.2.2 Linseed Meal

Linseed meal for years was fed to increase crude protein and improve hair coat. However, when the flaxseed oil extraction process went from the press, or expeller process, to solvent extraction, the remaining oil in linseed meal decreased from 8.0% to 0.5%. Thus horses fed processed linseed meal today lose the extra bloom from their hair coats. *Flaxseed* is called linseed meal after the extraction of the oil, but the name was not changed after the reduction in the percentage of oil on the analysis tag. Because linseed is in the grass family, the percent of lysine is low; however, the high level of the ω-3 fatty acid is what imparts the extra health and shine in the hair coat of the horse. Linseed meal contains 33% crude protein (1.3% lysine), 0.5% fat, and 9.5% crude fiber, whereas flaxseed contains 18% crude protein (0.64% lysine), 38% fat, and 6.7% crude fiber.

23.2.2.3 Canola

Canola seed is starting to be used more in the Midwestern states as horse feed. Canola is a high-oil seed that can be used for its protein and high calories or as a high-protein grain after the oil has been extracted. Canola seed or meal may contain antinutritional components, such as erucic acid at varying levels. Canola seed contains 18% crude protein (1% lysine), 36% fat, and 14% crude fiber, whereas canola meal contains 34% crude protein (1.9% lysine), 4.9% fat, and 8.3% crude fiber.

23.2.2.4 Peanut Meal

Peanut meal has found favor in the horse industry in the southern states because that is where it is grown and harvested and the oil is extracted, so peanut meal is economical to use in that region. Although peanuts are legumes, they are a poor lysine source compared with soybean meal. Peanut meal contains 48% crude protein (1.6% lysine), 1.3% fat, and 10% crude fiber.

23.2.2.5 Cottonseed

Cottonseed meal has been included in horse diets for several years in the southern states. Cottonseed has been used extensively in dairy cow rations because of its high fat and high digestible lint content. The hulls are poorly digested but act as a good scratch factor in ruminant animals. The gossypol content of cottonseed meal limits its use. Cottonseed is a member of the grass family and so has a low lysine content; therefore one should supplement lysine when using cottonseed in a grain mixture for horses. Whole cottonseed contains 21% crude protein (0.9% lysine), 17% fat, and 29% crude fiber, whereas cottonseed meal contains 41% crude protein (1.6% lysine), 1.7% fat, and 15% crude fiber.

23.2.2.6 Maize Distiller's Dried Grains

Maize distiller's dried grains are made available to the feed industry as a by-product from the distilling industry. Basically, the hull, germ, and parts of the yeast are what remain after the starches have been used by the microbes in the distilling process and the liquid is removed. Once the grains have been dried to 10% moisture, they can be added as a protein grain and for the yeast content. One must take care during the drying

process because too much heat burns the grains, turns them black, and makes them unpalatable, and the protein is not available. Maize distiller's dried grains should be golden. Although made up of several different grains, the grains have a low lysine content but can increase fat and digestible fiber and give a pleasant aroma to horse feed. Maize distiller's dried grains contain 27% crude protein (1.0% lysine), 9.0% fat, and 14.5% crude fiber.

23.2.2.7 Brewer's Dried Grains

Brewer's dried grains are made available from the brewing industry. The grains are palatable once dried to 10% moisture and can be added to horse feed as a protein source. Because the lysine content is low, the grains should be supplemented with amino acids if used in horse feeds. Brewer's dried grains contain 27% crude protein (0.9% lysine), 4.7% fat, and 16% crude fiber.

23.2.2.8 By-Product Feeds

By-product feeds come from the food industry. They are normally low in starch, high in crude fiber, and vary in the ability of the horse to ferment and digest them. The only way to tell their nutritional value is to look at the level of fat and ADF, NDF, and lignin levels. These fiber levels allow one to see whether they are fermentable or whether they are added as a low-calorie ingredient that cannot be broken down and used by the horse.

Comparing the analysis of the by-product feeds in Table 7 to the cereal and protein grains, one sees that the by-product feeds have a higher ADF, NDF, and lignin percentages. These higher percentages, especially the percentage of lignin, reflect a fiber source the horse is less able to break down by fermentation in the hindgut.

23.3 Major Minerals, Trace Minerals, and Vitamins

The purpose of adding major minerals, trace minerals, and vitamins to a grain mixture is to provide those nutrients 1555
that are low or deficient in forages in an amount to meet the nutrient needs of the horse. The vitamin 1556
recommendations from the National Research Council (NRC) for horses only include vitamins A, D, and E; thiamine; and riboflavin.[15]

TABLE 7 By-Product Feeds: Partial Analysis

BY-PRODUCT FEEDS	% DRY MATTER	% CRUDE PROTEIN	% LYSINE	ETHER EXTRACT	% ADF*	% NDF	% LIGNIN
Beet pulp, dried	88.3	8.8	0.38	1.0	20.4	40.4	1.4
Citrus pulp	85.8	5.9	0.15	4.2	19.0	20.8	0.8
Cottonseed hulls	89.0	5.5	0.26	2.2	57.8	75.7	20.0
Oat hulls	93.0	2.0	0.08	3.5	39.0	72.5	7.4
Rice bran, 13% ether extract	90.6	14.0	0.65	13.8	11.9	23.6	4.2
Rice bran, 20% ether extract	90.6	13.1	0.61	20.0	11.0	22.0	3.9
Soy hulls	90.9	12.6	0.79	2.5	40.5	54.8	2.3
Wheat bran	89.1	15.4	0.62	3.8	13.8	37.9	2.7
Wheat middlings	89.5	16.6	0.60	4.0	10.8	32.8	3.8

* *ADF,* Acid detergent fiber; *NDF,* neutral detergent fiber.

Tables 8 and 9 list the most common sources of major and trace minerals used to formulate horse diets and their nutrient concentrations. Table 10 lists the vitamins commonly used in horse feed formulations.

23.3.1 FEED LABELING GUIDE

The AAFCO sets the standard for each state to accept and follow on what should be listed on the tag or bag of each horse feed.[28] The standard determines the following:

1. How much of the analysis to guarantee
2. How to list the ingredients used in the formula
3. The detail of the feeding directions to help the end user identify which feeds are best suited for their horses

TABLE 8 Major Minerals Analysis

	ELEMENT (PERCENT)						
MAJOR MINERAL	Ca	TOTAL P	Na	K	Mg	S	Cl
Calcium carbonate	38.0	—	0.06	0.06	0.5	—	0.03
Defluorinated phospahte	33.0	18.0	4.50	0.09	—	—	0.92
Dicalcium phosphate	22.0	18.5	0.08	0.07	0.6	—	1.00
Magnesium sulfate	—	—	—	—	10.0	13.0	—
Magnesium sulfate	—	—	—	—	20.0	26.6	—
Monodicalcium phosphate	16.0	21.0	0.05	0.06	0.5	—	0.70
Potassium chloride	—	—	—	51.0	—	—	49.0
Potassium sulfate	—	—	—	44.8	—	18.8	—
Sodium chloride	—	—	39.00	—	—	—	61.0
Sodium phosphate (monobasic)	—	21.8	32.30	—	—	—	—

The current AAFCO recommendations for horse feed are as follows:

Guaranteed Analysis

Crude protein	Minimum	Percent
Crude fat	Minimum	Percent
Crude fiber	Maximum	Percent
Calcium (Ca)	Mininum and maximum	Percent
Phosphorus (P)	Minimum	Percent
Copper (Cu)	Minimum	ppm
Zinc (Zn)	Minimum	ppm
Selenium (Se)	Minimum	ppm
Vitamin A	Minimum	IU/lb

Other nutrients can be listed on the tag or bag at the discretion of the manufacturer.

The most expensive ingredients used in horse feeds today are protein, phosphorus, fat, and vitamin E. The horse 1556
owner will benefit from comparing tags and purchasing the feed that compliments the forage and meets the 1557
nutrient needs of the horse at the amount per day the horse is fed to maintain desired body condition.

TABLE 9 Trace Minerals Analysis

	ELEMENT (ppm)						
TRACE MINERALS	Co	I	Mn	Fe	Cu	Zn	Se
Calcium iodide		650,000					
Cobalt carbonate	490,000						
Cobalt proteinate	90,000–100,000						
Cobalt sulfate	380,000						
Copper carbonate					570,000		
Copper oxide					800,000		
Copper proteinate					100,000–130,000		
Copper sulfate					250,000		
Ethylene-diaminedihy-droiodide		770,000					
Ferric ammonium citrate				170,000			
Ferric carbonate				480,000			
Ferric chloride				210,000			
Ferric oxide				700,000			
Ferrous fumarate				330,000			
Ferrous sulfate				200,000–300,000			
Iron dextran				20,000–50,000			
Manganese proteinate			150,000				
Manganese sulfate			230,000				
Manganous oxide			770,000				
Potassium iodide		760,000					
Sodium selenite							200 and 400
Zinc methionine						40,000 and 100,000	
Zinc oxide						800,000	
Zinc proteinate						150,000–220,000	
Zinc sulfate						330,000	

23.3.1.1 Ingredient Terminology

Individual ingredients and collective terms are the two approved ways to list ingredients on a feed tag.

23.3.1.1.1 Individual Ingredients

Individual ingredients are written out and listed in decreasing amounts used in the formula. This list permits the practitioner to assess the suitability of ingredients, which is important when one must consider the quality and consistency of major components or when specific ingredients may be contraindicated. For instance, one should consider the amino acid composition from protein grains, type of starch structure from cereal grains, and how fermentable (digestible) the fiber source is from the ingredients that make up the feed in question.

23.3.1.1.2 Collective Terms

Collective terms recognize a general classification of ingredient origin, which perform a similar function but do not imply equivalent nutritional values. When a collective term is used, individual ingredients within that group cannot be listed on the label.[27] Manufacturers are permitted to use the ingredient listing of their choosing as long as they meet the specified analysis. Box 1 shows the practitioner which ingredients can be used under each broad classification, called collective terms.

23.3.1.2 Feeding Directions

All horse feeds shall have the following:

1. Purpose statement

2. Directions for use and any warning or caution statements

    a. The statement of purpose shall contain the specific species and animal class(es) for which the feed is intended.

    b. The manufacturer shall have the flexibility in describing in more specific and common language the defined animal class, species and purpose, while being consistent with the category of animal class. This may include, but is not limited to, the weight ranges(s), sex, or ages of the animal(s) for which the feed is manufactured.

    c. The purpose statement may be excluded from the label if the product name includes a description of the species and animal class(es) for which the feed is intended. 1557

TABLE 10 Vitamins Commonly Used in Horse Feed Formulations

VITAMIN	SOURCE
Biotin	*d*-Biotin
Choline	Choline chloride
Cyanocobalamin (B_{12})	Cyanocobalamin
Folacin (folic acid)	Folacin
Niacin	Nicotinic acid
	Nicotinamide
Pantothenic acid	Calcium pantothenate
Pyridoxine (B_2)	Pyridoxine hydrochloride
Riboflavin (B_2)	Riboflavin
Thiamine (B_2)	Thiamine hydrochloride
	Thiamine mononitrate
Vitamin A	Vitamin A acetate
	Vitamin A palmitate
	Vitamin A propionate
	β-Carotene (precursor)
Vitamin C	Ascorbic acid
Vitamin D	Activated animal sterol (D_2)
	Cholecalciferol
	Ergocalciferol (D_2)
Vitamin E	α-Tocopherol acetate
Vitamin K	Menadione sodium bisulfite

23.3.2 FEED INDUSTRY TERMINOLOGY

To assist the equine practitioner in terminology generally used by the feed industry, Box 2 lists the terms and their explanations.[27]

23.4 Equine Digestive System

The equine digestive system is designed to function most efficiently and effectively on a forage-based diet. One should feed grain mixtures and ration balancers to provide the difference between what the horse needs and what nutrients are in their forage. Knowledge of the digestive system and how it functions enables equine practitioners to better understand the role of available feed stuffs, forages, and grain mixtures in the health and performance of

the horse. The horse is a nonruminant herbivore or hindgut fermenter with the ability to digest and use diets predominantly of forage. In contrast to the ruminant, the primary absorption sight for starches, amino acids, major and trace minerals, and vitamins precedes the fermentation vat. Therefore the quality of ingredients is far more important to the equine than to the ruminant, especially those that are stressed, that is, growing, reproducing, or performing.[18] The horse is classified as a “selective, continuous grazer” and is capable of consuming large quantities of forage while grazing up to 18 hours each day.[17]

The small size of the equine stomach in relation to the rest of the digestive tract limits meal size. The best recommendation is not to feed more than 2.25 kg of grain per feeding per 500 kg of body mass. The stomach empties rapidly after initial exposure to acidic gastric secretions; then the digesta becomes more alkaline when mixed with bile as it enters into the small intestine. Horses are best suited to handling small, frequent meals and should have continual access to forage. If allowed to graze pasture, hay, or haylage continuously, a 500-kg horse can produce 95 to 114 L of saliva per day.[17] The saliva assists the rate of passage through the gut, provides an important source of enzymes, and together with the bile provides a buffering solution to help maintain normal pH for optimal hindgut fermentation of forage.[28]

The chance of impaction colic is enhanced greatly if forage or water is limited. Lack of adequate forage limits saliva production and predisposes all horses to impaction, as does inadequate water supply. The small intestine is the primary absorption site of the amino acids, starches, fats/oils, minerals, and vitamins found in grain mixtures and ration balancers. The maturity (RFV) of the forage determines how much, if any, of these nutrients will be broken down and absorbed in the small intestine. Because the transit time through the small intestine is only 2 to 3 hours, the quality and bioavailability of these nutrients are critical for optimal absorption. Nutrients that are not digested or absorbed pass into the hindgut for fermentation and absorption or excretion.

The digestive tract of the horse has the ability to break down and use plant fiber because of the microbes contained in their fermentation vat, that is, the cecum, large intestine, and colon. However, these microbes are pH sensitive, and the vat must maintain a pH of 6.7 to 6.8. If too many rapidly fermentable carbohydrates, such as starch, reach the cecum or large intestine, the D-lactic acid–producing bacteria proliferate. The microbes necessary for optimal fermentation start to die when the pH is 6.5 or less. The visible results are loose stools and, if not corrected, diarrhea from D-lactic acidosis. If the acid-loving bacteria grow out of control, the pH continues to drop, and when the cecal pH reaches 6.0, 80% of horses founder.[28] The large volume and blind sac design of the cecum, along with slower transit time, give the protozoa and bacteria ample opportunity to facilitate fiber digestion. The end products of microbial action or fermentation are volatile fatty acids, ammonia, and B-complex vitamins, with the by-products being gas and heat. The large intestine is a continuation of fermentation and absorption of those nutrients made available by the microbial population. The colon is the site of water recycling and controls the water resorption and stool formation. The total transit time is approximately 50 hours, depending on the percentage of ADF and NDF in the forage.[18]

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The digestive system of the foal evolves from being monogastric and completely dependent on milk, to the ability to utilize starches, and then later to a fermentation vat that can use some nonstructural carbohydrates and cellulose or plant fiber. Enzymatic activities have been measured in the small intestine of fetuses and foals.[29] The lactase begins to rise within a few hours of birth. Lactase, maltase, and sucrase activities are present; however, the lactase is the predominant disaccharidase in the foal until 3 to 4 months of age (Figure 2). At this time, maltase activity continues to increase, surpassing lactase, and becomes the primary disaccharidase in the small intestine.[15] The growth of the gastrointestinal tract parallels the changes in enzyme activity.[30] Significant increases in the length and diameter of the small intestine occur within the first month, increasing the available villous surface area and making it possible for the foal to process increasing volumes of milk to meet its needs. During the next 5 months,

continued growth of the small intestine occurs, but the greatest increases occur in the lengths of the cecum and large colon at a time when foals traditionally begin mimicking adult grazing behavior and increasing their intake of forage and grain.[31] From >6 months on, most of the growth occurs in the cecum and large intestine, allowing for the increased consumption and use of fibrous feeds.[15]

23.4.1

BOX 1 INGREDIENT ORIGIN OF COLLECTIVE TERMS

Animal protein products may include one or more of the following:

Animal blood dried

Animal by-product meal

Buttermilk, condensed

Buttermilk, dried

Casein

Casein, dried hydrolyzed

Cheese rind

Crab meal

Fish by-products

Fish meal

Fish solubles, dried

Hydrolyzed hair

Hydrolyzed leather meal

Hydrolyzed poultry feathers

Lactalbumin, dried

Meat and bone meal

Meat meal

Meat meal tankage

Meat solubles, dried

Milk protein, dried

Milk, dried whole

Poultry by-product meal

Poultry by-products

Shrimp meal

Skimmed milk, condensed

Skimmed milk, dried

Whey product, dried

Whey solubles, dried

Whey, condensed

Whey, condensed hydrolyzed

Whey, dried

Forage products may include one or more of the following:

Alfalfa meal, dehydrated

Alfalfa hay, ground

Alfalfa meal, sun-cured

Coastal Bermuda grass hay

Corn plant, dehydrated

Flax plant product

Ground grass

Soybean hay, ground

Grain products in any of the normal forms such as whole, ground, cracked, screen cracked, flaked, kibled, toasted, or heat processed may include these:

Barley

Corn

Grain sorghum

Oats

Rice, ground brown

Triticale

Wheat

Plant protein products may include one or more of the following:

Algae meal

Beans, dried

Canola meal

Coconut meal

Cottonseed

Cottonseed flakes

Cottonseed meal

Linseed meal

Peanut meal

Peas

Soy flour

Soy protein concentrate

Soybean feed

Soybean meal

Soybeans, heat processed

Soybeans, ground

Whole yeast, active dry

Yeast brewers

Yeast, culture

Yeast, dried

Processed grain by-products may include one or more of the following:

Aspirated grain fractions

Brewer's dried grains

Buckwheat middlings

Condensed distiller's solubles

Corn flour

Corn gluten feed

Corn gluten meal

Corn grits

Distiller's dried grains

Distiller's dried grains/solubles

Grain sorghum grits

Hominy feed

Malt sprouts

Oat groats

Peanut skins

Rice bran

Wheat bran

Wheat flour

Wheat germ meal

Wheat middlings

Wheat mill run

Roughage products may include one or more of the following:

Almond hulls, ground

Apple pomace, dried

Bagasse

Barley hullsBarley mill by-product

Beet pulp, dried

Citrus meal, dried

Citrus pulp, dried

Corn cob fractions

Cottonseed hulls

Flax straw by-products

Husks

Oat hulls

Oat mill by-product

Peanut hulls

Rice hulls

Rice mill by-product

Soybean hulls

Straw, ground

Sunflower hulls

Tomato pomace, dried

Molasses products may include one or more of the following:

Beet molasses

Beet molasses, dried product

Beet pulp, dried molasses

Cane molasses

Citrus molasses

The Food and Drug Administration does not recognize *molasses* as a collective term.

23.4.2

BOX 2 OFFICIAL FEED TERMS

Additive An ingredient or combination of ingredients added to the basic feed mix or parts thereof to fulfill a specific need. Usually used in microquantities and requires careful handling and mixing.

(Note: A *food additive* is defined by federal law as any substance that becomes a component or affects the characteristics of a feed or food if such substance generally is not recognized among experts qualified by scientific training and experience to evaluate its safety as having been shown adequately through scientific procedures to be safe under the conditions of its intended use. Excepted are substances having prior sanction and pesticide chemicals under certain conditions. See Public Law 65-929 for details.)

Aerial parts (part) The above-ground parts of plants.

Antibiotics A class of drug. They usually are synthesized by living microorganisms and in proper concentration inhibit the growth of other microorganisms.

Artificially dried (process) Moisture having been removed by other than natural means.

Aspirated, aspirating Having removed chaff, dust, or other light materials by use of air.

Bagasse (part) Pulp from sugar cane. (See *pulp*.)

Balanced A term that may be applied to a diet, ration, or feed having all known required nutrients in proper amount and proportion based on recommendations of recognized authorities in the field of animal nutrition, such as the National Research Council, for a given set of physiologic animal requirements. The species for which the feed is intended and the functions such as maintenance or maintenance plus production (growth, fetus, milk, eggs, wool, feathers, or work) shall be specified.

Barn-cured (process) Forage material dried with forced ventilation in an enclosure.

Beans Seed of leguminous plants especially of the genera *Phaseolus, Dali Chos*, and *Vigna*.

Biscuits (physical form) Shaped and baked dough.

Blending (process) To mingle or combine two or more ingredients of feed. Blending does not imply a uniformity of dispersion.

Blocked, blocking (process) Having agglomerated individual ingredients or mixtures in to a large mass.

Blocks (physical form) Agglomerated feed compressed into a solid mass cohesive enough to hold its form and weighing more than 2 lb and generally weighing 30 to 50 lb.

Blood albumin (part) One of the blood proteins.

Bone (part) Skeletal parts of vertebrates.

Bran (part) Pericarp of grain.

Brand name Any word, name, symbol, or device or any combination thereof identifying the commercial feed of a distributor and distinguishing it from that of others.

Bricks (physical form) Agglomerated feed, other than pellets, compressed into a solid mass cohesive enough to hold its form and weighing less than 2 lb. (See *blocks*.)

Browse (part) Small stems, leaves, and flowers and fruits of shrubs, trees, or woody vines.

Buttermilk (part) All residue from churning cream.

By-product (part) Secondary products produced in addition to the principal product.

Cake (physical form) The mass resulting from the pressing of seeds, meat, or fish to remove oils, fats, or other liquids.

Chaff (part) Glumes, husks, or other seed coverings together with other plant parts separated from seed in threshing or processing.

Charcoal Dark-colored porous forms of carbon made from the organic parts of vegetable or animal substances by their incomplete combustion.

Chipped, chipping (process) Cut or broken into fragments; also meaning prepared into small thin slices.

Chopped, chopping (process) Reduced in particle size by cutting with knives or other edged instruments.

Cleaned, cleaning (process) Removal of material by methods such as scalping, aspirating, and magnetic separation or by any other method.

Cleanings (part) Chaff, weed seeds, dust, and other foreign matter removed from cereal gains.

Cobs with grain (part) The ears of maize without the husks, but consisting of the entire cobs and adhering grain.

Cobs with husks (part) Kernel-free fibrous inner portion of the ear of maize with enveloping leaves.

Complete feed A nutritionally adequate feed for animals other than human beings; by specific formula, complete feed is compounded to be fed as the sole ration and is capable of maintaining life and promoting production without any additional substance being consumed except water.

Concentrate A feed used with another to improve the nutritive balance of the total and intended to be diluted further and mixed to produce a supplement or a complete feed.

Condensed, condensing (process) Reduced to denser form by removal of moisture.

Conditioned, conditioning (process) Having achieved predetermined moisture characteristics and/or temperature of ingredients or a mixture of ingredients before further processing.

Cooked, cooking (process) Heated in the presence of moisture to alter chemical and physical characteristics or to sterilize.

Cracked, cracking (process) Particle size reduced by a combined breaking and crushing action.

Crimped, crimping (process) Rolled by use of corrugated rollers. Crimping may curtail tempering or conditioning and cooling.

Crumbled, crumbling (process) Pellets reduced to granular form.

Crumbles (physical form) Pelleted feed reduced to granular form.

Cubes (physical form) See *pellets*.

Cull Material rejected as inferior to the process of grading or separating.

Culture Nutrient medium inoculated with specific microorganisms that may be in a live or dormant condition.

Cultured, culturing (process) Biologic material multiplied or produced in a nutrient media.

Customer-formula feed Consists of a mixture of commercial feeds and/or feed ingredients each batch of which is manufactured according to the specific instructions of the final purchaser.

D-activated, D-activating Plant or animal sterol fractions that have been vitamin D activated by ultraviolet light or by other means.

Defluorinated, defluorinating (process) Having had fluorine removed.

Degermed (process) Having had the embryo of seeds wholly or partially separated from the starch endosperm.

Dehulled, dehulling (process) Having removed the outer covering from grains or other seeds.

Dehydrated, dehydrating (process) Having been freed of moisture by thermal means.

Diet Feed ingredients or mixture of ingredients including water that is consumed by animals.

Diluent (physical form) An edible substance used to mix with and reduce the concentrate of nutrients and/or additives to make them more acceptable to animals, safer to use, and more capable of being mixed uniformly in a feed. (Diluent also may be a carrier.)

Dried, drying (process) Materials from which water or other liquids have been removed.

Drug (as defined by FDA as applied to feed) A substance (a) intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in human beings or other animals or (b) a substance other than food intended to affect the structure or any function of the body of a human being or other animals.

Dust (part) Fine, dry pulverized particles of matter usually resulting from the cleaning or grinding of grain.

Ears (part) Fruiting heads of Zea maize, including only the cob and grain.

Egg albumin (part) Whites of eggs of poultry.

Enzymatic activity The catalytic activity required to convert a given amount of assay substrate to a given amount of product per unit time under the standard conditions set forth in the assay procedure.

Enzyme A protein made up of amino acids or their derivatives that catalyzes a defined chemical reaction. Required cofactors should be considered an integral part of the enzyme.

Enzyme product A processed, standardized enzyme-containing material that has been produced with the intention of being sold for use in animal feed and feed ingredients.

Emulsifier A material capable of causing fat or oils to remain in liquid suspension.

Endosperm (part) Starchy portion of seed.

Ensiled (process) Aerial parts of plants that have been preserved by ensiling. Normally the original material is finely cut and blown into an airtight chamber such as a silo, where it is pressed to exclude air and where it undergoes an acid fermentation that retards spoilage.

Expanded, expanding (process) Subjected to moisture, pressure, and temperature to gelatinize the starch portion. When extruded, its volume increases because of abrupt reduction in pressure.

Extracted, mechanical (process) Having removed fat or oil from materials by heat and mechanical pressure. Similar terms are *expeller extracted, hydraulic extracted*, and *old process*.

Extracted, solvent (process) Having removed fat or oil from materials by organic solvents. Similar term is *new process*.

Extruded (process) A process by which feed has been pressed, pushed, or protruded through orifices under pressure.

Fat (part) A substance composed chiefly of triglycerides of fatty acids and that is solid or plastic at room temperature.

Fatty acids (part) Aliphatic monobasic acids containing only the elements carbon, hydrogen, and oxygen.

Feed(s) Edible material(s) that are consumed by animals and contribute energy and nutrients to the diet of the animal. (Usually refers to animals rather than human beings.)

Feed grade Suitable for animal consumption.

Feed mixture See *formula feed*.

Fermentation aid A substance added to assist in providing proper conditions that result in action by yeasts, molds, or bacteria in a controlled aerobic or anaerobic process used for the manufacture of certain products.

Fermented, fermenting (process) Acted on by yeasts, molds, or bacteria in a controlled aerobic or anaerobic process in the manufacture of products such as alcohols, acids, vitamins of the B-complex group, or antibiotics.

Fiber (part) Any of a large class of plant carbohydrates that resist digestion hydrolysis.

Fines (physical form) Any materials that will pass through a screen the openings of which are immediately smaller than the specified minimum crumble size or pellet diameter.

Flaked, flaking (process) See *rolled, rolling*.

Flakes (physical form) An ingredient rolled or cut into flat pieces with or without prior steam conditioning.

Flour (part) Soft, finely ground and bolted meal obtained from the milling of cereal gains, other seeds, or products. Flour consists essentially of the starch and gluten of the endosperm.

Formula feed Two or more ingredients proportioned, mixed, and processed according to specifications.

Free choice A feeding system by which animals are given unlimited access to the separate components or groups of components constituting the diet.

Fresh (process) Ingredients(s) having not been subject to freezing, to treatment by cooking, drying, rendering, hydrolysis, or similar process, to the addition of salt, curing agents, natural or synthetic chemical preservatives or other processing aids, or to preservation by means other than refrigeration.

Gelatinized, gelatinizing (process) Having had the starch granules completely ruptured by a combination of moisture, heat, and pressure and in some instances by mechanical shear.

Germ (part) The embryo found in seeds and frequently separated from the bran and starch endosperm during the milling.

Gluten (part) The tough, viscid nitrogenous substance remaining when the flour or wheat or other grain is washed to remove the starch.

Gossypol (part) A phenolic pigment in cottonseed that is toxic to some animals.

Grain (part) Seed from cereal plants.

GRAS Abbreviation for the phase *g*enerally *r*ecognized *a*s *s*afe. A substance that generally is recognized as safe by experts qualified to evaluate the safety of the substance for its intended use.

Grease Animal fats with a titer below 40° C.

Grits (part) Coarsely ground grain from which the bran and germ have been removed, usually screened to uniform particle size.

Groats (part) Grain from which the hulls have been removed.

Ground, grinding (process) Reduced in particle size by impact, shearing, or attrition.

Hay (part) The aerial portion of grass or herbage especially cut and cured for animal feeding.

Heads (part) The seed or grain-containing portions of a plant.

Heat-processed, heat-processing (process) Subjected to a method of preparation involving the use of elevated temperatures with or without pressure.

Hulls (part) Outer covering of grain or other seed.

Hydrolyzed, hydrolyzing (process) Complex molecules having been split to simpler units by chemical reaction with water, usually by catalysis.

Ingredient, feed ingredient A component part or constituent of any combination or mixture making up a commercial feed.

Iodize, iodized (process) To treat with iodine or an iodide.

Irradiated, irradiating (process) Treated, prepared, or altered by exposure to a specific radiation.

Kernel (part) A whole grain. For other species, dehulled seed.

Kibbled, kibbling (process) Cracked or crushed baked dough, or extruded feed that has been cooked before or during the extrusion process.

Lard (part) Rendered fat of swine.

Leached (process) The condition of a product following subjection of the material to the action of percolating water or other liquid.

Leaves (part) Lateral outgrowths of stems that constitute part of the foliage of a plant, typically a flattened green blade, and primarily function in photosynthesis.

Lecithin (part) A specific phospholipid. The principal constituent of crude phosphatides derived from oil-bearing seeds.

Liver (part) The hepatic gland.

Malt (part) Sprouted and steamed whole grain from which the radicle has been removed.

Mash (physical form) A mixture of ingredients in meal form. Similar term is *mash feed*.

Meal (physical form) An ingredient that has been ground or otherwise reduced in particle size.

Medicated feed Any feed that contains drug ingredients intended or presented for the cure, mitigation, treatment, or prevention of diseases of animals other than human beings or that contains drug ingredients intended to affect the structure or any function of the body of animals other than human beings. Antibiotics included in a feed growth promotion and/or efficiency levels are drug additives, and feeds containing such antibiotics are included in the foregoing definition of medicated feed.

Microingredients Vitamins, minerals, antibiotics, drugs, and other materials normally required in small amounts and measured in milligrams, micrograms, or parts per million.

Middlings (part) A by-product of flour milling comprising several grades of granular particles containing different proportions of endosperm bran and germ, each of which contains different levels of crude fiber.

Mill by-product (part) A secondary product obtained in addition to the principal product in milling practice.

Mill dust (part) Fine feed particles of undetermined origin resulting from handling and processing feed and feed ingredients.

Mill run (part) The state in which a material comes from the mill, ungraded and usually uninspected.

Mineralize, mineralized (process) To supply, impregnate, or add inorganic mineral compounds to a feed ingredient or mixture.

Mixing (process) To combine by agitation two or more materials to a specific degree of dispersion.

Molasses (part) The thick, viscous by-product resulting from refined sugar production or the concentrated, partially dehydrated juices from fruits.

Natural A feed or ingredient derived solely from plant, animal, or mined sources in its unprocessed state or having been subject to physical processing, heat processing, rendering, purification, extraction, hydrolysis, enzymolysis, or fermentation, but not having been produced by or subject to a chemically synthetic process and not containing any additives or processing aids that are chemically synthetic except in amounts as might occur unavoidably in good manufacturing practices.

Nutrient A feed constituent in a form and at a level that helps support the life of an animal. The chief classes of feed nutrients are proteins, fats, carbohydrates, minerals, and vitamins.

Offal (part) Material left as a by-product from the preparation of some specific product, less valuable portions, and the by-products of milling.

Oil (part) A substance composed chiefly of triglycerides of fatty acids and that is liquid at room temperature.

Parboiling A hydrothermal process in which the crystalline form of starch is changed into the amorphous form because of the irreversible swelling and fusion of starch. This is accomplished by soaking, steaming, drying, and milling to produce physical and chemical modifications.

Pearled, pearling (process) Dehulled grains reduced by machine brushing into smaller, smooth particles.

Pelleted, pelleting (process) Having agglomerated feed by compaction and forcing through die openings.

Pellets (physical form) Agglomerated feed formed by compacting and forcing through die openings by a mechanical process. Similar terms are *pelleted feed* and *hard pellet*.

Polished, polishing (process) Having a smooth surface produced by mechanical process usually by friction.

Pomace (part) Pulp from fruit. See *pulp*.

Premix A uniform mixture of one or more microingredients with diluent or carrier. Premixes are used to facilitate uniform dispersion of the microingredients in a large mix.

Premixing (process) The preliminary mixing of ingredients with diluents or carriers.

Preservative A substance added to protect, prevent, or retard decay, discoloration, or spoilage under conditions of use or storage.

Pressed, pressing (process) Compacted or molded by pressure; also meaning having fat, oil, or juices extracted under pressure.

Product (part) A substance produced from one or more other substances as a result of chemical or physical change.

Protein (part) Any of a large class of naturally occurring complex combinations of amino acids.

Pulp (part) The solid residue remaining after extraction of juices from fruits, roots, or stems. Similar terms are *bagasse* and *pomace*.

Pulverized, pulverizing (process) See *ground, grinding*.

Range cubes (physical form) Large pellets designed to be fed on the ground. Similar term is *range wafer*.

Ration The amount of the total feed provided to one animal over 24 hours.

Raw Food in its natural or crude state not having been subjected to heat in the course of preparation as food.

Rolled, rolling (process) Having changed the shape and size of particles by compressing between rollers. Rolling may entail tempering or conditioning.

Roots (part) Subterranean parts of plants.

Rumen inert Refers to a nutrient that does not result in a change in rumen fermentation parameters yet is available to the animal in the intestine.

Rumen protected Refers to nutrient fed in such a form that it provides an increase in the flow of that nutrient, unchanged, to the abomasum yet is available to the animal in the intestine.

Scalped, scalping (process) Having removed larger material by screening.

Scratch (physical form) Whole, cracked, or coarsely cut grain. Similar terms are *scratch gain* and *scratch feed*.

Screened, screening (process) Having separated various sized particles by passing over and/or through screens.

Seed (part) The fertilized and ripened ovule of a plant.

Self-fed A feeding system by which animals have continuous free access to some or all components of a ration, individually or as mixtures.

Separating (process) Classification of particles by size, shape, and/or density.

Shoots (part) The immature aerial parts of plants, stems with leaves, and other appendages in contrast to the roots.

Shorts (part) Fine particles of bran, germ, flour, or offal from the tail of the mill from commercial flour milling.

Sifted (process) Materials that have been passed through wire sieves to separate particles in different sizes. The separation of finer materials than would be done by screening.

Sizing (process) See *screened, screening*.

Skimmed (process) Material from which floating solid material has been removed. Skimmed also is applied to milk from which fat has been removed by centrifugation.

Skin (part) Outer coverings of fruits or seeds, as the rinds, husks, or peels. May also apply to dermal tissue of animals.

Solubles Liquid containing dissolved substances obtained from processing animal or plant materials. Solubles may contain some fine suspended solids.

Solvent extracted (process) A product from which oil has been removed by solvents.

Spray dehydrated (process) Material that has been dried by spraying on the surface of a heated drum and is recovered by scraping from the drum.

Spray dried Material that has been dried by spraying or atomizing into a draft of heated dry air.

Stabilized (process) To retard degradation of ingredients. (The process used to be specified.)

Stalk(s) (part) The main stem of a herbaceous plant often with its dependent parts as leaves, twigs, and fruit.

Starch (part) A white, granular polymer of plant origin; the principal part of seed endosperm.

Steamed, steaming (process) Having treated ingredients with steam to alter physical and/or chemical properties. Similar terms are *steam cooked, steam rendered*, and *tanked*.

Stem (part) The coarse, aerial parts of plants that serve as supporting structures for leaves, buds, fruit, etc.

Sterols (part) Solid cyclic alcohols that are the major constituents of the unsaponifiable portion of animal and vegetable fats and oils.

Stillage (part) The mash from fermentation of grains after removal of alcohol by distillation.

Stover (part) The stalks and leaves of corn after the ears, or sorghum after the heads, have been harvested.

Straw (part) The plant residue remaining after separation of the seeds in threshing, including the chaff.

Sun-cured (process) Material dried by exposure in open air to the direct rays of the sun.

Supplement A feed used with another to improve the nutritive balance or performance of the total and intended to be

1. Fed undiluted as a supplement to other feeds,
2. Offered free choice with other parts of the ration separately available, or
3. Further diluted and mixed to produce a complete feed.

Tallow (part) Animal fats with titer above 40° C.

Titer A property of fat determined by the solidification point of the fatty acids liberated by hydrolysis.

Toasted (process) Browned, dried, or parched by exposure to a fire or to gas or electric heat.

Trace minerals Mineral nutrients required by animals in minute amounts only (measured in milligrams per pound or smaller units).

Tubers (part) Short, thickened fleshy stems or terminal portions of stems or rhizomes that usually are formed underground; bear minute scaled leaves, each with a bud capable under suitable conditions of developing into a new plant; and constitute the resting stage of various plants.

Vitamins Organic compounds that function as parts of enzyme systems essential for the transmission of energy and the regulation of metabolisms of the body.

Wafers (physical form) A form of agglomerated feed based on fibrous ingredients in which the finished form usually has a diameter or cross-section measurement greater than its length.

Whey (part) The watery part of milk separated from the curd.

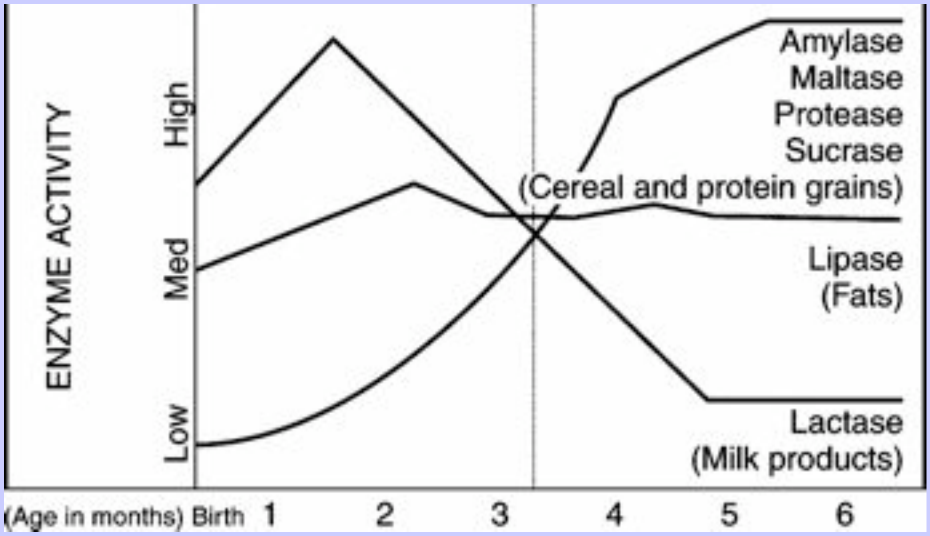
Whey solids (part) The solids of whey (proteins, fats, lactose, ash, and lactic acid).

Whole (physical form) Complete, entire.

Whole pressed, whole pressing (process) Having the entire seed to remove oil.

Wilted (physical form) A product without turgor as a result of water loss.

Figure 2 Digestive enzyme activity of the young, growing horse.



After the hindgut has been inoculated with microbes, the young foal can start using plant fiber, which occurs at 10 to 12 weeks of age. Their fermentation vat is not efficient or effective at this age, so one should feed the softest, most immature forage available to the suckling and early weanling. A RFV of 115 or higher in the forage is recommended. A distended abdomen, or hay belly, becomes visible if the forage is too mature. As the body increases in size, so does the fermentation vat. The larger the capacity to ferment, the more efficient the vat becomes. Therefore as young horses grow, their need for forage increases in their diet, their need for milk is eliminated, and grain mixtures are decreased. The NRC recommends that from 12 months of age, greater than 50% of their total diet, by weight, should be forage or forage equivalent (on a dry matter basis)[1] (Table 11). The amount of grain mixture required per day to maintain desired body condition is related directly to the quantity and quality of forage being consumed.

TABLE 11 National Research Council Recommendation of Diet Proportions for Young, Growing Horses

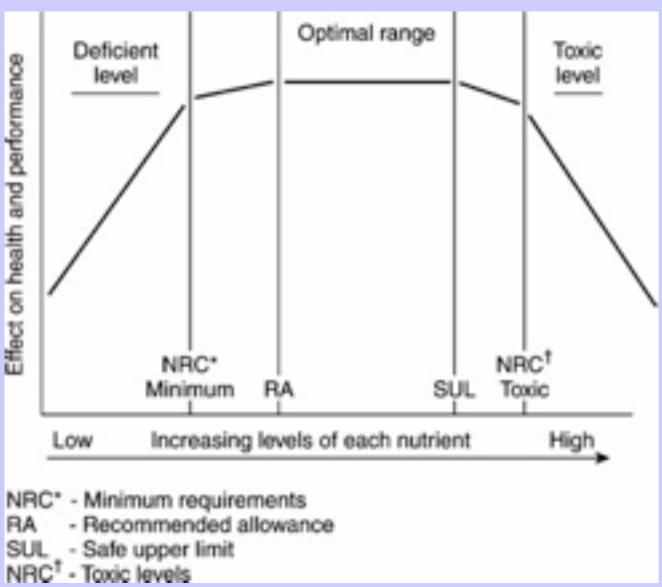
AGE	FORAGE	GRAIN MIXTURE
Birth to 2 months	0	100% (milk only)
3 months	20%	80%
6 months	30%	70%
12 months	50%	50%
18 months	60%	40%
24 months	70%	30%

From Nutritional Research Council: *Nutrient requirements of horses,* Washington, DC, 1989, National Academy Press.

The research has not been completed on the absorption of the essential amino acids from different ingredients, grains and forages, or the optimal level of ADF and NDF to maintain hindgut function and intestinal health. Therefore horsemen feed forage and grain mixtures to achieve the desired body condition in their horses. What is needed today is an easy way to measure how much and of which feed is necessary to meet nutrient needs of horses, whether they are growing, reproducing, or performing. Feed manufactures in the United States now are required to have a purpose statement and feeding directions explaining the minimum amount to feed to each horse per day, depending on their age, size, growth rate, reproductive status, and performance level.[27] Feeding less than the manufacturer recommended amount per day provides the horse with a deficient diet. (See the section Feed Labeling Guide.)

Figure 3 shows how the amount of each nutrient fed has an effect on the health and performance of the horse. The NRC has listed individual nutrients and their minimum amounts in the total ration to be fed per day to prevent deficiencies. The NRC also has listed the known toxic levels of those nutrients. The owner/manager, veterinarian, and nutritionist are responsible for balancing the diet and staying between the deficient and the toxic levels to maintain optimal health for growth, reproduction, and performance. Because of the varying quality of different forages, nutritionists balance diets on recommended allowances. If one uses the NRC minimum to balance a forage and grain combination, and one's forage quality decreases, one will be feeding a deficient diet and not
know it until the health, performance, or appearance of the horse declines. So grain mixtures and ration balancers 1565
formulated for horses need a recommended allowance built into their formula and feeding directions. An optimal 1566
range for each nutrient in the equine diet exists. Before reaching toxic levels, nutrients will antagonize or interfere with one another, reducing their availability. This antagonism also will affect the health and performance of the horse negatively. So the manager needs to know what the minimum pounds are per day of the recommended allowance and the safe upper limit (SUL) of their selected grain mixture or ration balancer to stay within the optimal range.

Figure 3 Nutrient effects on health and performance. *NRC*, National Research Council requirements; *RA*, recommended allowance; *SUL*, safe upper limit.



23.5 Feeds and Feeding Amounts: Digestive Upset and Abnormal Behavior

James B. Rowe

23.5.1 ACIDIC GUT SYNDROME

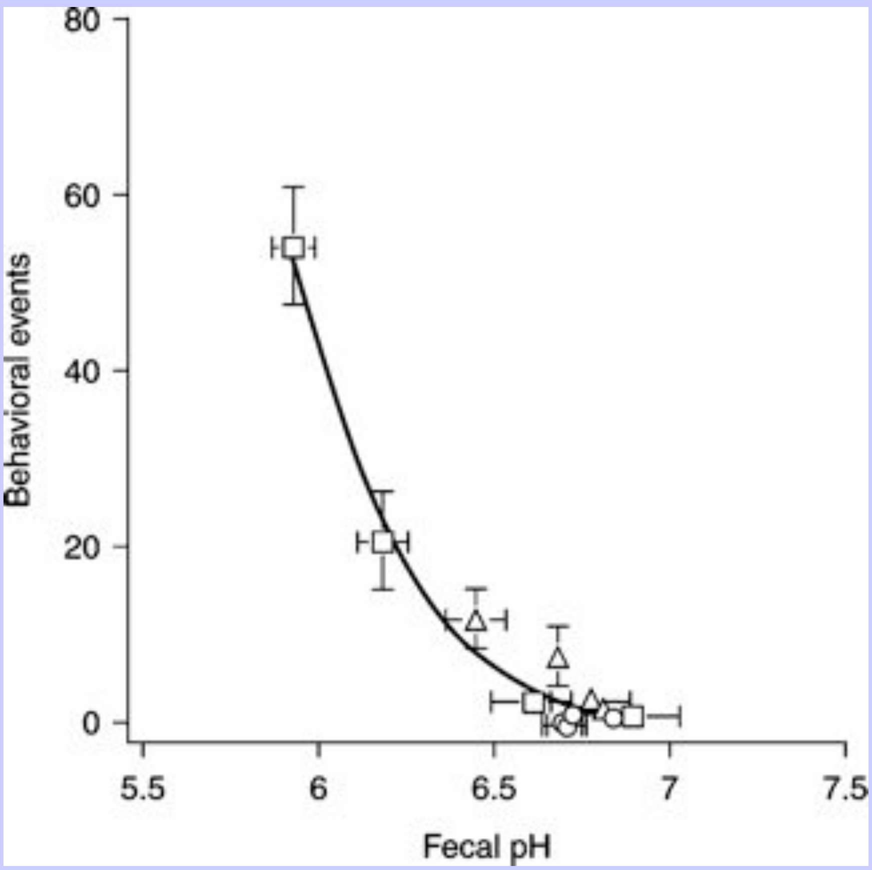
Acid accumulation in the gut occurs when a number of dietary factors and animal digestive responses interact to deliver readily fermentable carbohydrates to parts of the digestive tract colonized by bacteria capable of rapid fermentation and multiplication. Lactic acidosis is a well-documented condition characterized by the absorption of lactic acid from the hindgut, the slow metabolism of D-lactic acid, and the effect of these processes on the acid-base balance in the tissues of the horse. Acidic gut syndrome is characterized by the accumulation of acid in the gut at concentrations that previously have not been considered harmful to horses. The detrimental effects initiated by lactic acid and low pH may be mediated through direct action on the gut wall, through the production and absorption of bacterial endotoxin, through the combination of acid and endotoxins, or through other factors. The adverse effects associated with acidic gut syndrome are not defined conclusively but may include behavioral changes, increased risk of gut infections, skin and respiratory conditions, and a range of other problems that traditionally have been attributed to food allergies or reactions to stress. Acidic gut syndrome may impact or influence the immune system and in this way forms a basis for understanding a range of secondary diseases of previously unknown origin. Acute lactic acidosis still is acknowledged as a serious problem in extreme circumstances, but possibly acidic gut syndrome may be more common and may affect production, health, and welfare in significant ways.

A number of dietary and animal digestive factors interact to influence the possibility of acid accumulation in those parts of the digestive tract adapted for microbial fermentation where the normal pH is between 6.6 and 6.8. The problem of lactic acidosis is recognized widely in ruminants, and although lactic acidosis is attributed largely to the fermentation of starch and sugars in the rumen, it is also a significant problem in the hindgut of equines.[32,33] Hindgut acidosis is also well recognized in horses, for this condition is related closely to founder or laminitis.[34,35] In lactic acidosis, or D-lactic acidosis, considerable amounts of lactic acid are absorbed through the wall of the fermentation vat and some undoubtedly moves into and is absorbed from the intestinal tract.[36] D-lactic acid is metabolized more slowly than L-lactic acid and therefore accumulates in the tissues, where it causes severe D-lactic acidosis. This author suggests that endotoxin released with the death of gram-negative bacteria in the gut may play a role in the pathogeneses of lactic acidosis. Acidic gut syndrome, however, may not depend on metabolic acidosis as a primary or secondary factor in its pathogenesis, but rather on acidity within the gut. The adverse toxic effects may be mediated through the direct effect of acid on the gut wall or through microbial endotoxins, or through a combination of these factors, or through some other factor initiated within the gut or gut wall and acting systemically.

A recent study on adverse behavioral changes in the horse in response to increasing levels of grain in the diet showed that the frequency of unusual behavioral activities was related closely to fecal pH. Traditionally, normal fecal pH has been considered to be within the range of pH 6 to 7; however, the more acidic the feces, the higher the frequency of adverse behavior, that is, eating bedding or feces, wood chewing, and cribbing (Figure 4).[37] Conditions of low pH in the cecum and colon do not normally occur without accumulation of lactic acid. Therefore although lactic acid itself appears unlikely to be an important trigger in the chain of events leading to laminitis, it may be an important indicator of an acidic pattern of fermentation in the gut. 1566
Bacteria in the gut produce two isomers of lactic acid in almost equal proportions. These are D- and L-lactate. 1567
However, only one of these isomers, L-lactate, is produced by body tissue and can be detected at elevated levels

in blood during strenuous exercise or in response to pain or stress. No D-lactate is present in the blood unless a significant accumulation of lactic acid occurs in the gut. For this reason, measurable amounts, in millimoles per liter, of blood D-lactate always provide an accurate indication of abnormal fermentation/digestion and acidic conditions in the hindgut.

Figure 4 Adverse behavioral events versus fecal pH. Relationship between fecal pH and behavioral events (eating bedding, grasping or cribbing, wood chewing, and stall licking). Each point represents the weekly sum of observations for 2 hours every day of the week. Horses were fed increasing levels of grain over 4 weeks, and differences in fecal acid were related directly to the amount of grain consumed per day.



Good circumstantial evidence also exists for other disease conditions being linked to acidic gut syndrome. These conditions are associated mainly with high levels of grain (starch) feeding and circumstances in which a well-recognized risk of lactic acidosis exists but a low incidence of the frank disease condition occurs. A more sensitive assessment of acid accumulation, at least in the gut, could be the pH of feces that would allow investigation of the possible link between gut acidity and secondary disease problems.[37,102]

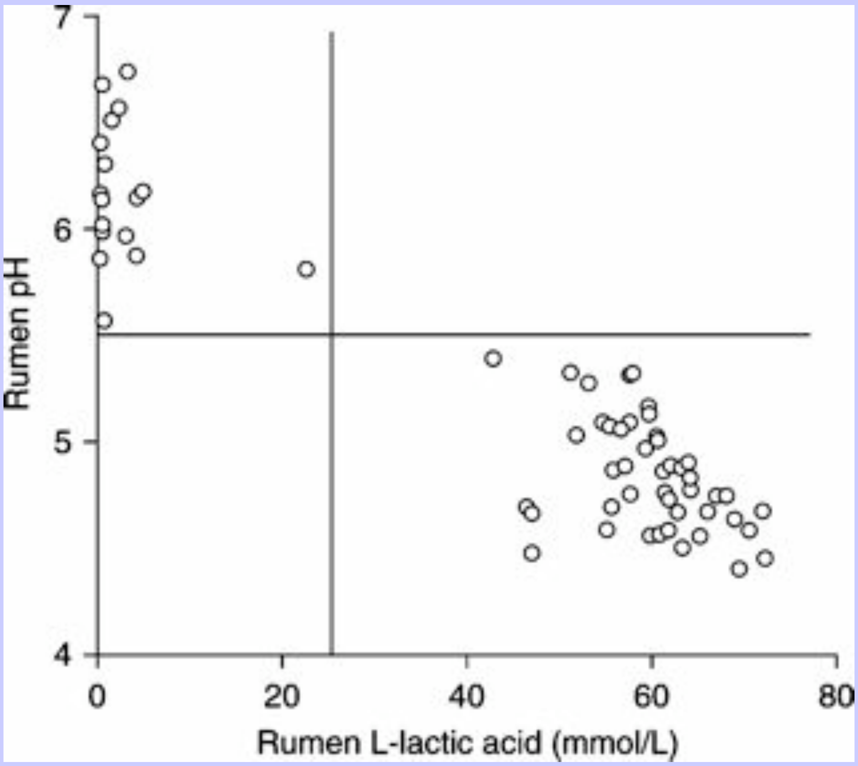
The results of a recent study suggest that TNF-α was released from epithelial cells of the gut into the gut lumen in substantial quantities after lactic acid accumulation and low pH in the rumen and cecum. This increase in TNF-α indicates that a significant immune response occurred with the development of lactic acidosis. This represents a substantial increase in understanding the etiology of lactic acidosis. It seems likely that the increase in acid load in the hind gut and decrease in pH acting on the gut wall may initiate a break down of integrity of the gut wall.[102] This agrees with similar evidence of the breakdown of the structural integrity of the cecum in horses during acidosis (Kruegar et al., 1986).

23.5.2 FERMENTATION ACTIVITY

Under normal conditions of fermentation in the hindgut, lactic acid is not present in measurable amounts even though it can be an important intermediate in the production of propionic acid. The conversion of lactic acid to propionate is thermodynamically favorable for microbes, and this process is normally rapid and complete. The accumulation of lactic acid only occurs when the bacteria responsible for the conversion of lactic acid to volatile fatty acids are in a reduced pH environment. The lactic acid–using bacteria are far more sensitive to low pH than the lactic acid producers, and once the balance is upset, the situation is exacerbated by further accumulation of lactic acid that results in even lower pH.[38] Thus any net production of lactic acid in the cecum leads to an accumulation of acid that lowers pH.

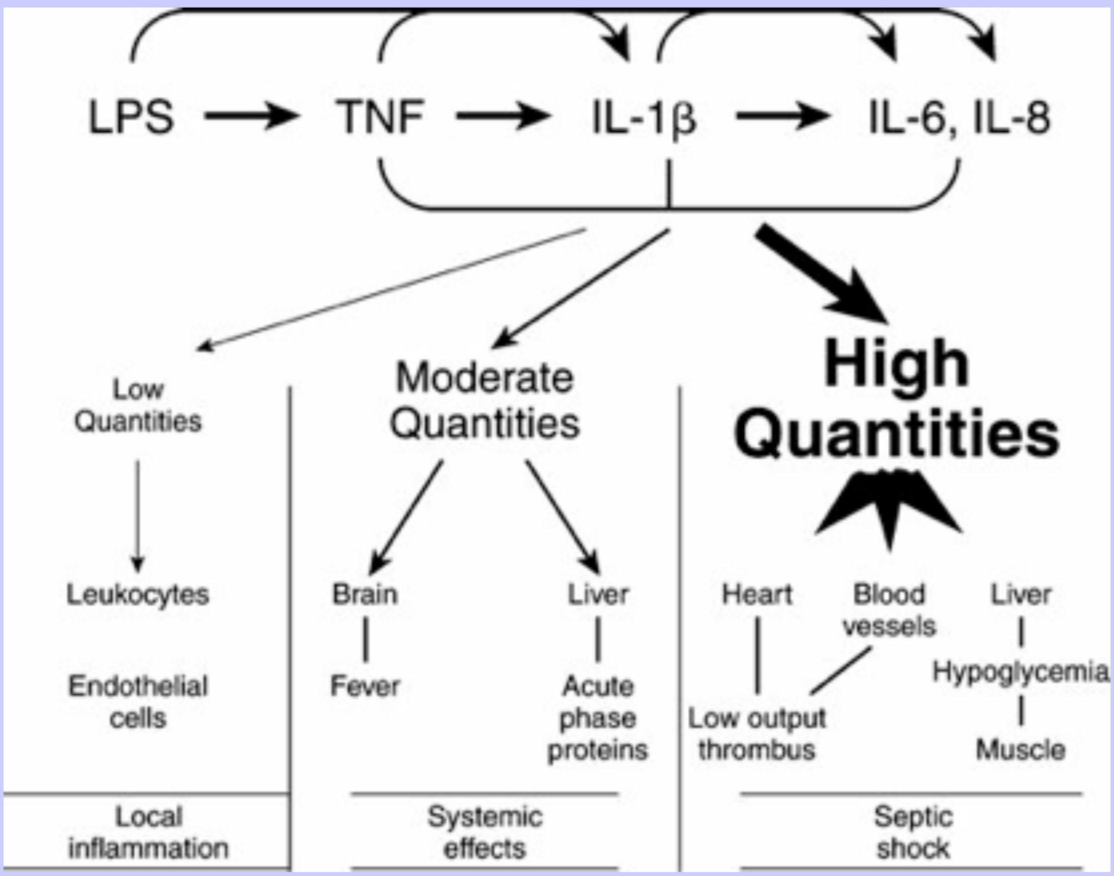
The accumulation of lactic acid therefore appears to be associated always with low pH and also signals the failure of the gram-negative lactate users. Figure 5 shows an example of the role of lactate in the accumulation of acid in the gut that demonstrates the relationship between lactic acid and pH. This figure suggests that when 1567
lactic acid is present in the hindgut at concentrations greater than 20 mmol/L, the pH normally is below 5.5. 1568
The primary lactic acid users are gram-negative bacteria, whereas the bacteria primarily involved in lactic acid production, *Streptococcus bovis* and *Lactobacillus* spp, are gram-positive.[38] Accumulation of lactic acid under these conditions is therefore likely to have a dual significance in contributing to a nonabsorbable acid to the gut contents and as an indicator of a decline in gram-negative bacteria, which is linked with bacterial endotoxin lipopolysaccharide release. Lipopolysaccharides are released during lyses of gram-negative bacteria. Lipopolysaccharide release can occur during the sudden decrease in pH associated with rapid fermentation of carbohydrates and has been demonstrated in the cecum of horses.[39] These results showed a tendency toward higher levels of endotoxin accumulation at lower pH. Considerable quantities of endotoxins can be released without the pH falling below 6.0. Studies by Hood and Stephens[40] and by Mullenax, Keeler, and Allison[41] suggest under normal conditions in the cecum, without acute lactic acidosis, little absorption of endotoxin from the gut is likely to occur.

Figure 5 Relationship between lactic acid concentration and pH. Apparent absorption of test solutions of acids from surgically sealed pouches in the rumen and cecum in the sheep. The apparent increase in lactic acid was caused by conversion from volatile fatty acids and tissue synthesis. (From Ding et al: 1997.)



Under conditions of acute lactic acidosis, gross structural changes to the cecal wall in horses[42] may allow bacterial endotoxins and lactic acid to gain access to the vascular system. Clearly lactic acid and endotoxins can be absorbed from the gut during acute lactic acidosis.[43–45] However, this situation is clearly different from acidic gut syndrome. The results from the two studies[33,43] indicate that significant absorption of lactic acid, and presumably endotoxins as well, only occurs under extreme conditions of fermentative acidosis in the gut. With lower levels of lactic acid and higher pH in the gut, little absorption of lactic acid from the gut occurred. The difference appears to be between the ratio of D- and L-lactate in the gut and in the blood. Measurable amounts of L-lactate always exist in the blood from tissue metabolism, and if lactic acid from the gut makes a major contribution to blood lactic acid, the ratio of D-lactate to L-lactate would approach that measured in the fermentation compartments in the gut.

Figure 6 Summary of the range of effects that varying levels of cytokine responses can have locally and systemically when stimulated by bacterial lipopolysaccharide *(LPS)* endotoxin. Although this model is based on systemic infection, the endotoxin *(LPS)* released with the death of gram-negative bacteria in the gut under acidic conditions likely triggers local and systemic cytokine effects. *TNF*, Tumor necrosis factor; *IL*, interleukin. (Adapted from Abbas AK, Lichtman AH, Pober JS: *Cellular and molecular immunology*, Philadelphia, 1996, WB Saunders.)



Bacterial endotoxins also stimulate tumor necrosis factor, which can initiate the cytokine cascade, releasing interleukins 1, 6, and 8.[46] The effects of these cytokines are widespread, acting locally and systemically depending on the level present.[47] Figure 6 summarizes the effects of cytokines. Pollitt[48] reported a range of histologic changes in the hooves of horses following carbohydrate overload and also the development of 1568
laminitis, both of which were consistent with cytokine activity. In the case of hoof damage at the lamellar level, 1569

Pollitt suggests metalloproteases specifically may be involved, but a sufficiently wide range of cytokine-initiated activities exists to explain damage and activation of other tissue types.

23.5.3 MANAGEMENT TO REDUCE THE RISK OF FERMENTATIVE ACIDOSIS

The factors that have the potential to contribute to lactic acid build up in the gut are numerous. They include any input or change that contributes to an increased supply of fermentable carbohydrates to any part of the digestive tract containing a dense bacterial population. Because a range of animal and feed factors affect the risk of acid accumulation in the gut, not surprisingly a significant variation exists between individual animals in the way in which they respond to similar quantities of dietary grains or other forms of fermentable carbohydrates. From this perspective, considering different management strategies for more susceptible individuals and for dietary regimens that are likely to be associated with higher risks of gut acid accumulation is appropriate. The challenge is to make this approach more practical and useful, to understand first the condition so that those individuals and diets that carry the greatest risk can be identified easily and managed appropriately. One should exercise caution concerning the potentially dangerous combination of adding probiotics to enhance lactic acid production while simultaneously increasing the amount of carbohydrates (cereal grains), causing more microbial fermentation.

The following summarizes dietary characteristics associated with an increase risk of fermentative acidosis:

Source of carbohydrates: The carbohydrate source is important because major differences exist in structure and characteristics of starch granules between cereal grains and the content of nonstarch polysaccharides. Resistance of starch to intestinal digestion increases the risk of greater hindgut fermentation and acid accumulation in the cecum and colon. The nonstarch polysaccharide fraction is highly fermentable and generates acid production in the hindgut. Nonstarch polysaccharides can also reduce starch digestion by increasing the viscosity of the digesta and in this way may act to increase hindgut fermentation.

Age of grain (storage): Aging reduces the endogenous enzyme and nonstarch polysaccharide contents of the grain and can have a significant effect in improving intestinal digestion of carbohydrates.[49] Changes from aging help explain why a sudden change in new season grain can cause production problems associated with reduced intestinal digestion and increased hindgut fermentation and fecal output.

Processing: Processing of the dietary ingredients affects particle size, solubility, gelatinization, and the rate at which food can be ingested. Any treatment that decreases particle size, reduces the resistance of starch to digestion, or increases its solubility increases the rate of carbohydrate absorption in the small intestine.

Frequency of feeding and meal size: Frequency of feeding and meal size determine the amount of fermentable substrate delivered to the stomach for acid digestion and to the hindgut for fermentation. Large amounts of carbohydrates ingested once or twice per day can overload the stomach and small intestine, leading to acid accumulation in the hindgut. Overload may result in incomplete gastric digestion and subsequent increased fermentation in the hindgut. Factors that facilitate rapid ingestion of carbohydrates are likely to result in less intestinal digestion and more hindgut fermentation.

Rate of passage: Rate of passage has an important role in determining the site of digestion and can be influenced by hormonal and other systemic factors, such as stress, cold weather, and disease. Rate of passage is also influenced by the autonomic nervous system, disease status, drug exposure, and nature of the diet. Rapid passage of ingesta through the intestine may lead to incomplete digestion of carbohydrates, which influences hindgut fermentation.

Adaptation of the bacterial population: Bacterial adaptation for starch fermentation and lactic acid use forms the basis of all practical grain mixture feeding programs. A gradual increase in the amount of grain allows a build up of bacteria able to convert lactic acid to volatile fatty acids. Bacterial buildup is not only important in terms of preventing lactic acid accumulation and low pH but also from the point of view of the stability of the gram-negative population of bacteria that use lactic acid. If this population declines sharply in response to increased acidity and if a rapid expansion of the gram-positive population of bacteria that produce lactic acid occurs, a release of endotoxins may lead to acidic gut syndrome.

Enzyme activity: Enzyme activity in the gastrointestinal tract affects the extent of carbohydrate digestion and the range of substrates degraded and absorbed. Significant differences exist between individuals in enzyme activity that can have an important effect on site of digestion and the accumulation of acid in the intestine.

Intestinal adaptation: Intestinal adaptation to the digestion and absorption of various carbohydrate fractions has an important effect on the site of carbohydrate digestion and on the potential for increased hindgut fermentation. The requirement for digestive adaptation to different carbohydrates is important in horses in which health problems are encountered often when they are changed from one type of grain to another.

Salivary secretion: Salivary secretion is an important source of enzymes and buffers. The amount of 1569
saliva secreted is related directly to how many hours per day the horse actually is chewing, and its 1570
composition varies between individuals.

23.5.4 SUMMARY

The problems of acute D-lactic acidosis are well understood in ruminants and horses, but the characteristics and adverse side effects of acid gut syndrome are not yet recognized in animal nutrition. Possibly a closer study of acidic gut syndrome and its effects on microbes and on the gut wall will increase understanding of a range of production and health problems that are well described but for which no cause is known. The variability in the types of carbohydrates and the wide range of factors that affect their pattern of intake and passage to parts of the digestive tract where fermentation occurs have made identifying and understanding the problem of fermentative acidosis as a potential disease condition difficult.

23.6 Management Tools to Help the Horse Owner

The growth and performance chart in Figure 7 provides feeding recommendations for all horses that will mature between 100 kg and more than 1000 kg in body weight. The growth curves, which indicate from birth to 3 years

of age and mature body weights with each physiologic status, are taken from the tables from the NRC.[1] Proper use of these charts assures that nutrition is not the limiting factor in growing, reproducing, and performing horses today. Feeding the correct amount of a selected balanced grain mixture or ration balancer allows all foals to reach their inherent genetic potential and reduces the nutrition-related growth and performance problems seen in horses.

23.6.1 YOUNG, GROWING HORSES

Weighing each horse and recording its body weight on a chart once a month allows the horse owner to track the growth rate of the young horse and compare it to established normal growth rates of horses with a known mature body weight. Because of differences in breeds and genetics, not all horses follow these respective growth curves. Some grow at a faster rate and some slower. Therefore one must monitor growth and then manage and feed the horse to meet its optimum growth rate. If growing horses are fed as a group, one must feed for the highest requirement in the group. If an owner is feeding for the average horse, his average horses should achieve their genetic potential. Rapidly growing horses are more susceptible to development of growth abnormalities because their mineral needs are not adequately met. Using information gained from using these charts and following an established feeding program assures adequate mineral and vitamin levels to meet their growth requirements. The number inside each rectangle reflects the amount of minerals and vitamins necessary to maintain the recommended allowance for that particular growth rate. This number is a balanced nutritional unit (BNU) and does not reflect the calories to maintain desired body condition, because the forages being consumed vary in calories and the owner/trainer/manager will feed varying amounts of a grain mixture to meet the desired body condition in the horses. What these persons need to know is what the minimum pounds are to achieve the recommended allowance (RA) and what the maximum pounds are not to exceed the safe upper limit (SUL) of the selected grain mixture they are feeding. The growth and performance chart provides this information.

The chart does not recommend trying to speed up or to slow down growth rate but states simply: “Let the young horses grow up to their genetic potential, but provide the nutrients to match their current growth rate.” On the larger, faster growing foals, the author recommends weighing and recording their weight, height, and body condition score on this chart every 2 weeks for the first 6 months of life. Their physiologic development is so great at this age, the author recommends monitoring them more closely to avoid any potential growth problems. As they become older, the growth rate slows but also may vary; therefore the author recommends weighing once a month for those more than 6 months of age to assure that their dietary needs (calculated in BNUs) are met accordingly.

23.6.2 REPRODUCING AND PERFORMANCE HORSES: MATURE/IDLE/OPEN

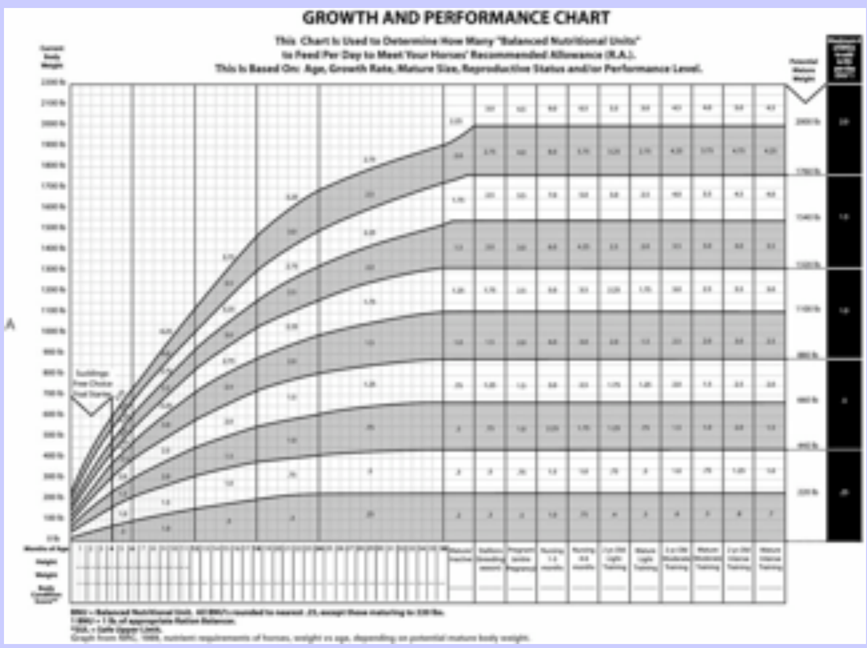
Knowing the ideal body weight of the mature, idle horse (see the section Body Condition Scoring) is essential and helpful to determine how many calories to feed per day, and use of the growth and performance chart assures that their recommended allowance is met. To this baseline maintenance, one must add the additional nutrient requirements for pregnancy, lactation, activity level, etc. This chart helps determine the recommended allowance and the SUL for each physiologic status.

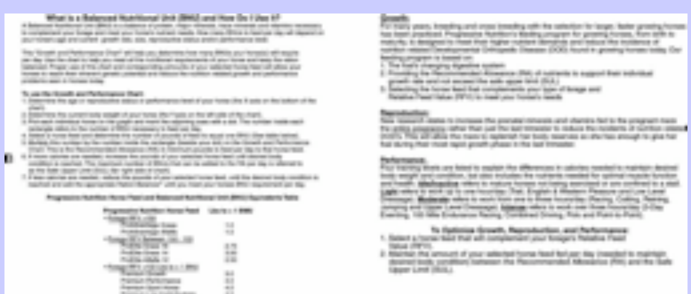
23.6.3 PREGNANCY

Implementation of research from the veterinary departments of Cornell University and University of Illinois stating that the nutrition received in the middle trimester of pregnancy is just as important as that received in the last trimester has helped many breeding farms improve the health of the mares and reduce early developmental

problems in foals. Changes in feeding practice have allowed the mare to replenish and build her body reserves to ensure she can supply the foal during their most rapid growth phase in the last trimester.[50] If the mare is not provided adequate nutrients during pregnancy, she has the ability to pull them from her body reserves to supply 1570
the fetus. Foals are not born with nutrient deficiencies until the mares' body reserves are depleted. This scenario 1573
excludes, of course, the genetic inability to manufacture quality cartilage,[51] placentitis, or older mares having reduced ability to absorb or use certain nutrients. One should note that the trace minerals copper and zinc in forages today do not meet the NRC minimum requirements for maintenance, let alone for pregnancy (see Table 3.) Management may look at the body condition score of pregnant mares on pasture and elect not to feed them any grain mixture because they are fat and shiny. Unfortunately, the major and trace minerals needed for fetal cartilage development have nothing to do with fat stores and little to do with shiny hair coats. For ease of management, improved health and reducing the incidence of nutrition-related fetal developmental problems, the author recommends providing all pregnant mares their required BNUs the entire pregnancy, rather than the age-old practice of just during the last trimester. If too much body weight is a concern, feeding the appropriate ration balancer with the forage and no cereal grain or grain mixtures is recommended.

Figure 7 **A**, Growth and performance chart. **B**, Growth and performance chart.





23.6.4
LACTATING MARE

During the first 3 months of lactation, most mares produce between 3.0% and 3.5% of their body weight in fluid milk each day.[52,53] Peak lactation usually occurs between 4 and 10 weeks post partum, with the average being 5 to 6 weeks.[52] As lactation continues, milk yield declines to 2% of body weight between the fourth and fifth months. The amount of milk a mare produces depends on her (1) genetic potential, keeping in mind that maiden mares produce less than mares with previous lactations, (2) nutrient supply (especially protein, calories, and water), and (3) individual foal intake. The nutrient composition of milk also changes during lactation. Mare's milk becomes more nutrient dilute as lactation progresses. Protein, fat, major and trace minerals, and vitamin content decrease and lactose increases.[15] Water is the major constituent of mare's milk, being approximately 90%,[52–54] and the total feed consumption of the mare of forage and grain mixture increases according to individual milk production. Table 12 shows how much total feed is consumed per day by pregnant and lactating mares. Managers must watch the nutrient input-output relationship of lactating mares. If the mare is providing more nutrients in her milk than she is taking in, she will draw from her body reserves until her nutritional reserves are depleted. If calories are in short supply, her fat stores will be used up and she will loose body condition. If protein is in short supply, her muscle mass will be sacrificed because proteins are stored in the muscle. This negative balance, major and trace minerals included, will deplete her body reserves, lower those nutrients in her milk, and may have a negative effect on her conception rate or current fetal development, in addition to her health.

TABLE 12 Total Feed Consumed Per Day by Pregnant and Nursing Mares

REPRODUCTIVE STATUS	AMOUNT OF HAY AND GRAIN MIXTURE TO FEED PER DAY AS PERCENT OF BODY WEIGHT
Pregnant	1.5–2.0
Lactating, first 3 months	2.5–3.5
Lactating, 4 to 6 months	2.0–3.0

23.6.5
PERFORMANCE

As the activities of the performance horse increase, so do the nutritional requirements: calories needed per day increase to maintain desired body condition, the amount of minerals (electrolytes) and vitamins needed for optimal muscle function and health increase (see Figure 7), and the total amount of feed consumed per day increases. Table 13 shows trainers how much total feed to expect to feed per day based on activity level, whether feeding forage and grain mixture or forage, oats, and a ration balancer.

23.6.6
SAFE UPPER LIMITS

In the last column on the right of the growth and performance chart (see Figure 7) is the maximum number of BNUs that can be added to the minimum BNUs listed in the individual rectangles, to stay within the optimal range of nutrients in the total diet. This number of BNUs ensures that the SULs are not exceeded, with no antagonisms or decreased absorption of nutrients because of major or trace mineral interrelationships or acidic gut syndrome from too much soluble carbohydrates or inadequate fermentable fiber. The chart provides the

horse owner/manager the maximum number of BNUs per day, which then can be converted to the maximum number of pounds of a grain mixture to be fed per day. The best rule of thumb to offer clients is not to feed more than 50% of the total diet, by weight, of a grain mixture per day. Therefore the maximum amount of a 1573
grain mixture to feed, to maintain normal gut function, is half of what Tables 12 and 13 show. If one feeds these 1574
amounts and the horse does not maintain body weight, one should consider using vegetable oil as a supplemental calorie source instead of more grain mixture or cereal grain.

TABLE 13 Total Feed Consumed Per Day by Horses in Training

ACTIVITY LEVEL	AMOUNT OF HAY AND GRAIN MIXTURE TO FEED PER DAY AS PERCENT OF BODY WEIGHT
Laid up	1.5–2.0
Light training	2.0–2.5
Moderate training	2.5–3.0
Intense training	3.0–3.5

The BNU is made up of protein (amino acids), fats (fatty acids), major minerals, trace minerals, and vitamins necessary to complement the analysis of different forages. A ration balancer is this concentrated source of nutrients, which means one BNU is equal to one pound of the appropriate ration balancer. How many BNUs to feed per day depend on the size, current growth rate, reproductive status, and performance level of the horse. Which ration balancer to feed depends on the type of forage fed. Ration balancer “grass” is a ration balancer formulated to be fed when the forage is over 50% grass. Ration balancer “alfalfa” is formulated to complement forage over 50% alfalfa. (In Tables 2 and 3 one may see the protein and mineral levels found in these forage types and may compare them to how each ration balancer is formulated to complement them.)

23.6.7 FEEDING EXAMPLES USING BALANCED NUTRITIONAL UNITS WITH APPROPRIATE RATION BALANCERS AND AD-LIB FORAGE

Table 14 provides analyses of two ration balancers.

TABLE 14 Two Different Ration Balancers and Their Individual Analysis to Complement Different Forage Types

NUTRIENT	FEED WITH GRASS FORAGE RATION BALANCER “GRASS”	FEED WITH LEGUME FORAGE RATION BALANCER “ALFALFA”
Dry matter (%)	90.0	90.0
Protein (%)	30.0	14.0
Fat (%)	5.5	5.5
Crude fiber (%)	5.0	15.0
Calcium (%)	3.0	1.5
Phosphorus (%)	1.5	200
Potassium (%)	1.5	3.0
Magnesium(mg/kg)	0.4	1.5
Manganese (mg/kg)	260	0.9
Copper (mg/kg)	200	240.0
Iodine (mg/kg)	3.0	0.4
Iron (mg/kg)	240.0	260
Selenium (mg/kg)	1.5	1.5
Zinc (mg/kg)	500	500
Vitamin A (IU/kg)*	48,400	48,400
Vitamin D (IU/kg)*	4840	4840
Vitamin E (IU/kg)*	770	770

* IU/kg is the same as mg/kg and ppm.

23.6.7.1 Example 1

If a grain mixture is made of 33% oats, 33% corn, and 33% ration balancer “grass” and fed with grass forage to a pregnant mare weighing 1100 lb, the manager would multiply the number inside the rectangle on the performance chart (which is 2) by 3, to see how many pounds of this grain mixture to feed per day. This grain mixture is 33% ration balancer, and so 3 pounds of this mixture make one BNU or 1 lb of ration balancer. The daily recommended allowance would be 6 lb/day. The SUL of BNUs to feed per day (see the last column on the right of the performance chart) would be one additional BNU per day, which equals 9 lb/day of the grain mixture. The minimum amount of this grain mixture to feed to this pregnant mare would be 6 lb/day, and the maximum would be 9 lb/day.

23.6.7.2 Example 2

If a grain mixture is made of 30% oats, 30% barley, and 40% ration balancer “grass” and fed to the same pregnant mare, the owner would multiply the number inside the rectangle on the performance chart by 2.5 to see how many pounds of this grain mixture to feed per day. This grain mixture is 40% ration balancer and so 2.5 pounds of grain mixture makes one BNU or 1 lb of ration balancer. For example, 2 × 2.5 = 5 lb/day. The maximum number of BNUs to feed per day would be 3, so that 3 × 2.5 = 7.5 lb/day. The minimum amount to feed per day is 5 lb, and the maximum is 7.5 lb of this grain mixture to feed this pregnant mare.

These two different grain mixtures, when correctly fed, provide similar amounts of prenatal nutrition. The body condition of the mare should determine which one to feed. The individual body condition score of the mare tells the manager how many pounds of a grain mixture to feed per day and that tells which grain mixture to select, the grain mixture that takes 3 lb to equal one BNU or the grain mixture that takes 2.5 lb. The higher the percentage of ration balancer in the grain mixture, the fewer pounds need to be fed per day, and visa versa, to meet the prenatal requirements of the mare.

23.6.7.3 Example 3

If the owner wants to feed straight oats as the calorie source with forage, the first step is to find the number of BNUs needed to feed per day and provide that many pounds of the appropriate ration balancer. If more calories are needed, one should add oats to reach the desired body condition of their horses. One BNU equals 1 lb of ration balancer.

One should use the correct ration balancer to complement their forage and, if mixed with grain, know what
percent of the grain mixture is ration balancer. One then can figure how many pounds of this grain mixture 1574
equals one BNU, or 1 lb of ration balancer, and can multiply that number by the number inside the rectangle 1575
on the growth and performance chart. In this way the manager can ensure a diet that stays between the recommended allowance and the SUL. However, one must select the appropriate ration balancer to complement the forage to meet the nutritional needs of the horse.

23.7 Body Condition Scoring

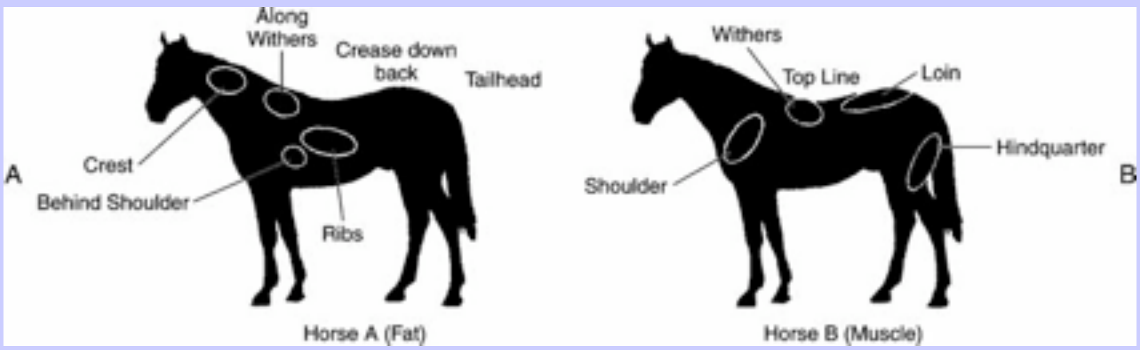
Body condition scoring is a visual and hands-on method to evaluate the amount of body fat a horse is carrying.[55] Developed at Texas A&M University by D.R. Henneke and others, this system is a good management tool to determine the optimal amount of body fat for every type of horse (Figure 8).

According to the diagram for horse A, one evaluates the amount of fat covering areas of the crest, withers, ribs, behind the shoulder, and down the back and around the tailhead. One must evaluate all of these areas because they are where the horse first deposits and stores body fat. Also, because different breeds and body types store fat in different locations, one must evaluate all of these areas.

Body condition scores range from 1 to 9, with a score of 1 being emaciated with no fat stores and 9 being extremely fat. A score of 5 indicates a moderate condition and the ideal body weight for most horses. One should be sure not to confuse body fat stores with gut fill or “hay belly.” The following provides a complete description of the body condition scores.

By regularly evaluating the condition of each horse, one can adjust the feeding and exercise program to maintain the desired condition. Each class of horse—based on age, growth rate, reproductive status, and performance level—has an optimal body condition score. When body condition scoring young, growing horses each month, one should record their weight, height, and body condition score on the growth chart. When scoring a performance horse, one should remember that they need enough body fat stores to use as an energy source to maintain performance, although too much fat can decrease performance. This system sets a standard for evaluating and communicating the condition of a horse. The BNUs on the growth and performance chart are set up for a body condition score of 5. The two most common management problems are overfeeding and underfeeding horses. To help prevent either of these problems from developing, one should evaluate the body condition score of the horse (Table 15), adjust the feeding program while assuring adequate BNUs are fed per day, and monitor the score frequently.

Figure 8 Body condition scoring. Horse **A**, Evaluating the locations of fat stored. Horse **B**, Evaluating locations of muscle stored.



In the diagram for horse B, one should not confuse body fat stores with muscle deterioration caused by a protein deficiency. Horses fed a protein-deficient diet have the ability to tear down muscle, where protein is stored, to keep the body functioning normally. The first visible loss in muscle mass is the withers, followed by the loin, the hindquarter, and then the shoulder area. In other words, the top line is the first place to look for protein deficiency and not fat stores. To round out the back area with fat, the horse will have to have a body condition score of 8 or better.

The optimal feeding program considers *all* the nutrients from the forage and the grain mixture or ration balancer. Nutrients are water, protein, fat, carbohydrates, minerals, and vitamins. A successful diet balances the recommended allowances of *all* these nutrients, including fiber, which is necessary for a healthy horse, rather than increasing any single nutrient alone. As horses age, their ability to absorb protein and phosphorus diminishes and their top line is the first area to show a deficiency, thus causing a weakened back to sag. To reduce this occurrence and meet the requirements of the older horse, Sara Ralston of Rutgers University recommends increasing the protein, phosphorus, and vitamin C quantities above the NRC minimum in the diet of the geriatric horse to meet nutritional needs.[56] 1575

TABLE 15 Description of Body Condition Scores

SCORE	NAME	DESCRIPTION
1	Poor	Animal extremely emaciated. Spinous processes, ribs, tailhead, and hooks and pins project prominently. Bone structure of withers, shoulders, and neck easily noticeable. No fatty tissues can be felt.
2	Very thin	Animal emaciated. Slight fat covering over base of spinous processes; transverse processes of lumbar vertebrae feel rounded. Spinous processes, ribs, tailhead, and hooks and pins prominent. Withers, shoulders, and neck structures faintly discernible.
3	Thin	Fat buildup about halfway on spinous processes; transverse processes cannot be felt. Slight fat cover over ribs. Spinous processes and ribs easily discernible. Tailhead prominent, but individual vertebrae cannot be identified easily. Hook bones appear rounded, but easily discernible. Withers, shoulders, and neck accentuated.
4	Moderately thin	Negative crease along back. Faint outline of ribs discernible. Tailhead prominence depends on conformation; fat can be felt around it. Pin bones not discernible. Withers, shoulders, and neck not obviously thin.
5	Moderate	Back level. Ribs cannot be distinguished visually but can be felt easily. Fat around tailhead beginning to feel spongy. Withers appear rounded over spinous processes. Shoulders and neck blend smoothly into body.
6	Moderately fleshy	May have slight crease down back. Fat over ribs feels fleshy-spongy. Fat around tailhead feels soft. Fat beginning to be deposited along the sides of the withers, behind the shoulder, and along the side of the neck.
7	Fleshy	May have crease down back. Individual ribs can be felt, but filling between ribs with fat is noticeable. Fat around tailhead is soft. Fat deposited along withers, behind shoulders, and along the neck.
8	Fat	Crease down back. Difficult to feel ribs. Fat around tailhead very soft. Area along withers filled with fat. Area behind shoulder filled with fat. Noticeable thickening of neck. Fat deposited along inner thighs.
9	Extremely fat	Obvious crease down back. Patchy fat appearing over ribs. Bulging fat around tailhead, along withers, behind shoulders, and along neck. Fat along inner thighs may rub together. Flank filled with fat.

Modified from Nutritional Research Council: *Nutrient requirements of horses,* Washington, DC, 1989, National Academy Press.

23.8 Nutritional Needs for Life Stages and Activity

Age, growth rate, reproductive status, and activity level may affect nutritional requirements significantly. Consideration of the affect of these variables on energy, protein, and mineral requirements is essential for establishing appropriate dietary recommendations for any horse.

23.8.1 NUTRITIONAL NEEDS FOR REPRODUCTION

23.8.1.1 Stallion

Nutritional management of the stallion differs little from that described for the mature horse (Table 16). During breeding season, stallions generally need more energy to compensate for the increase in activity and stress. Adjustments in feed intake should be gradual. One should increase feed to provide enough calories to maintain the stallion at the body condition score desired by the owner/manager. A calorie increase of 25% above maintenance may be needed during the breeding season. The usual digestible energy requirement for stallions in the nonbreeding season is about 15 kcal/lb body weight and during the breeding season is about 20 kcal/lb body weight.

Obesity can be a problem for all horses, especially stallions, in the off season. If the quantity of the grain mixture fed is not reduced when activity level decreases, the resulting is overfeeding of calories. The consensus of stallion managers is that stallions that are kept in good body condition through dietary control, exercise, or both, are more sound, responsive, and fertile and remain healthier than stallions that are permitted to become obese. To help maintain optimal body weight, stallions should be turned out or exercised in hand each day throughout the year.[15]

23.8.1.2 Open Mare

One should maintain mares with a body condition score of 5 to 7 and should feed them the BNUs according to their individual size (see Figure 7). One should place mares that have retired recently from athletic competition or that may be in poor condition in a weight-gaining plan before breeding to increase the likelihood of conception and improve reproductive efficiency.[15] Such management increases the chance of conception and reduces the number of covers compared with mares with a body condition score of less than 4.[50]

23.8.1.3 Pregnant Mare

For years, calcium and phosphorus (and their ratios) have been considered two of the most important
minerals in the equine diet. The reason these two major minerals received so much attention is because they 1576
are associated closely with bone development and overall health. When young horses developed distended 1577
joints, physitis, lameness, or fractures, deficiencies in calcium and phosphorus were the first suspected.

TABLE 16 Comparison of Recommended Allowances for Stallions During the Off Season and Breeding Season on a Total Ration Dry Matter Basis (Including Forage)

NUTRIENT	OFF SEASON	BREEDING SEASON
Dry matter intake (% body weight)*	1.5–2.0	2.25–2.75
Protein (%)	8.5	10.5
Calcium (%)	0.30	0.36
Phosphorus (%)	0.21	0.26
Potassium (%)	0.40	0.50
Magnesium (%)	0.10	0.12
Sodium (%)	0.14	0.30
Manganese (mg/kg)	40	50
Iron (mg/kg)	40	50
Copper (mg/kg)	15	20
Zinc (mg/kg)	40	50
Cobalt (mg/kg)	0.20	0.20
Iodine (mg/kg)	0.25	0.25
Selenium (mg/kg)	0.15	0.20
Vitamin A (IU/kg)	2000	2000
Vitamin E (IU/kg)	50	80
Thiamine (mg/kg)	3	5
Riboflavin (mg/kg)	2	2

* Feed intake is determined by body capacity, physiologic status, activity level, relative feed value of forage, and environment. Needed digestible energy (Mcal/day) is determined by body size, metabolic rate, reproductive status, dry matter consumption, and body condition score.

In the 1980s, research completed at The Ohio State University,[57] followed closely by that of the University of Guelph,[51] included the roll of trace minerals in prenatal nutrition and in suckling and weanling diets. The first trace minerals reviewed were copper and zinc in utero and after parturition, because of their involvement in cartilage formation, the transformation of cartilage into bone, and tendon elasticity.

In the early 1990s, research completed at the veterinary departments of Cornell University and University of Illinois stated that the last two trimesters of pregnancy were equally important times to provide the mare with the protein and calories to maintain normal development of the fetus and health of the mare.[50] Insufficient nutrient levels force the mare to draw from her body reserves to maintain normal fetal development and maintain her pregnancy. Once her reserves are depleted, the fetus could suffer from early embryonic death.[60]

In the late 1990s, further research published by E.C. Firth,[58a] from Massey University, New Zealand, took the concept one step further. Stating the importance of the total ration in the diet of the pregnant and lactating mare, Firth was able to show the higher incidence of developmental orthopedic disease (DOD) in their foals when the prenatal and postnatal diets of mares and their respective foals contained adequate major minerals but with trace mineral levels below recommended allowances suggested by The Ohio State University and the University of Guelph (Table 17).

The nonlactating pregnant mare should be fed the BNUs according to her individual size (see Figure 7) to meet her prenatal requirements (see Table 17). The early growth of the fetus is minimal and does little to increase nutrient demand above maintenance; however, recent research suggests that weight gain of the mare occurs during midgestation for use as an energy source later in gestation and lactation.[50] One should include in the dietary plan adequate calories to maintain the body condition score desired by the owner/manager and the protein, minerals, and vitamins to meet the recommended allowance to support the mare in pregnancy, lactation, and/or athletic activity, whichever requires the most nutrition.

The greatest increase in fetal growth and nutrient demand occurs during the last 3 to 4 months of gestation[1,18] (Table 18), necessitating increases in most nutrients to support growth and development. Supplying the extra nutrients is often confounded, however, by the reduction in digestive capacity because of the increasing size of the fetus.[15] The solution is to increase the nutrient density concentration by switching to the appropriate ration balancer with the forage and to add cereal grain *only* if necessary to maintain or increase body weight. One should monitor mares as previously described to prevent undesired increases in body condition score. Decreases in energy intake of more than 10% in obese mares are not recommended to facilitate weight loss because of the potential risk of hyperlipidemia.[1,58]

A minimum increase by 10% of the protein intake has been recommended for protein needs of the fetus.[2] The effects of higher intakes of protein on fetal health have not been evaluated; however, the fact that many mares fed alfalfa consume nearly twice the NRC requirement for crude protein and produce healthy, vigorous foals suggests that little concern is necessary[15] as long as the total diet is balanced and prenatal nutrients are supplied adequately (see Table 17).

Recent studies of fetal growth, body composition, and postnatal development have provided some estimates of mineral needs during the last 3 to 4 months of pregnancy.[59] Data suggest that to provide for fetal mineral deposition, the calcium and phosphorus intake must increase by nearly 80% above maintenance; magnesium
and potassium must increase by 25%.[2] However, mares fed diets of grass hay and unfortified cereal grain are 1577
likely to be deficient in protein, calcium, phosphorus, copper, zinc, iodine, and selenium. One should 1578
compare current intake with the recommended levels in Table 17 to determine how much extra of each nutrient is needed.

TABLE 17 Comparison of the Recommended Allowance for Mares While Open, Pregnant, and Lactating on a Total Ration Dry Matter Basis (Including Forage)

NUTRIENT	OPEN	PREGNANT	LACTATION (FIRST MONTHS)	LACTATION (4–6 MONTHS)
Dry matter intake (% body weight)*	1.5–2.0	1.5–2.0	2.5–3.5	2.0–3.0
Crude protein (%)	8.50	11.50	15.00	13.00
Calcium (%)	0.30	0.53	0.70	0.50
Phosphorus (%)	0.21	0.35	0.40	0.34
Potassium (%)	0.40	0.50	0.80	0.60
Magnesium (%)	0.10	0.12	0.15	0.12
Sodium (%)†	0.14	0.14	0.20	0.16
Manganese (mg/kg)	40	60	60	60
Iron (mg/kg)	40	80	80	80
Copper (mg/kg)	15	25	25	25
Zinc (mg/kg)	40	70	70	70
Cobalt (mg/kg)	0.20	0.20	0.30	0.25
Iodine (mg/kg)	0.25	0.25	0.50	0.35
Selenium (mg/kg) g	0.15	0.20	0.30	0.20
Vitamin A (IU/kg)	2000	3000	3000	3000
Vitamin E (IU/kg)	50	80	80	80
Thiamine (mg/kg)	3	3	3	3
Riboflavin (mg/kg)	2	2	2	2

* Feed intake is determined by body capacity, relative feed value of forage, physiologic status, activity level, and environment.

† Under average conditions 0.35% to 0.50% NaCl is recommended in the total diet. Needed digestible energy (Mcal/day) is determined by body size, metabolic rate, reproductive status, dry matter consumption, and body condition score.

A great deal of interest has arisen in work concerning trace mineral supplementation of the broodmare and its role in the DODs of foals.[51,57,58,61] Though additional studies are needed, recent research has demonstrated that supplementation of copper in the gestational diet of the mare and the diet of the foal reduced the frequency of cartilage abnormalities in the foals compared with mares and foals consuming NRC-recommended concentrations of copper.[57,58,61] An important note is that copper was not the only nutrient increased in this study. Researchers also increased manganese, zinc, and selenium concentrations between

diets to prevent antagonisms, but maintain nutrient ratios extrapolated from NRC recommendations. Therefore adding copper or any mineral alone without evaluating the other minerals is not recommended. Selenium and iodine are also of importance to the broodmare. Foals from mares fed from 35 mg to 48 mg of iodine per day were born with enlarged thyroid glands, characteristic of iodine toxicity. The levels of trace minerals listed in Table 17 currently are recommended for broodmare diets on farms where nutrition is believed to be a contributing factor to the occurrence of the DODs. In most situations, the recommended allowance fed throughout entire gestation is sufficient.

TABLE 18 Length and Weight of Equine Fetuses by Gestational Age of 450-kg (990-lb) Mares

AGE (DAYS)	CROWN-RUMP LENGTH (cm)[*]	MASS (WEIGHT)
60	6	17 g
90	16	160 g
120	25	700 g (1.5 lb)
150	35	1.6 kg (3.5 lb)
180	48	4.0 kg (9.0 lb)
210	60	10 kg (22 lb)
240	75	17 kg (37 lb)
270	85	20 kg (44 lb)
300	95	29 kg (64 lb)
330	100	42 kg (92 lb)
Average birth weight		42–55 kg (92–120 lb)

* Straight line from tip of forehead to base of tail.

23.8.1.4 Lactating Mare

Calorie requirements for the lactating mare are related directly to the amount of milk she is producing. Milk production volume is influenced by the number of previous lactations, the month of lactation, genetic potential, nutrient input versus output, and foal intake.[15] Mares come into milk production slowly and do not reach peak daily production until 5 to 6 weeks post partum. One should not be in a hurry to feed the 1578
maximum amount of a grain mixture to the mare that has just given birth. One can increase the amount of 1579
forage by offering hay ad lib for the first few days. On the third day, after assurance that no complications exist, one can begin increasing the grain mixture at a rate of 1 lb per day. Too much grain too soon can cause digestive upsets and colic. The key to feeding and keeping the foaling mares healthy is slowly increasing the amount of grain mixture fed per day.

During the first 3 months of lactation, most mares peak in milk production between 3.0% and 3.5% of their body weight in fluid milk per day. Pony mares give slightly more than 4.0%.[15,52–54,62] Field experiences suggest maiden mares have lower milk yields and lower immunoglobulin G (IgG) levels in their colostrum. Peak lactation occurs between 4 weeks and 10 weeks post partum,[53,54,62] with an average of 5 to 6 weeks.

[53,54] As lactation continues, milk production declines to approximately 2% of the body weight of the mare by the twelfth week of lactation.

Milk nutrient composition changes and becomes more dilute as lactation progress (Table 19). The protein, fat, caloric, mineral, and vitamin content decreases, and lactose increases with time of lactation.[53] The increase in lactose content is not high enough to offset the decreases in protein and calorie-dense fat, resulting in lower calorie content.[15] The nutrients of the mare in greatest demand are water and calories, followed by protein, major minerals, trace minerals, and vitamins. Mare's milk is approximately 90% water. A 500-kg mare producing 15 kg of milk daily would have to increase her water intake nearly twofold to replenish this loss. Early in lactation, mare's milk contains approximately 560 kcal/kg of fluid milk.[52–54,59] Assuming mares convert 60% of feed digestible energy into milk gross energy, the mare must consume an extra 792 kcal of digestible energy for every kilogram of milk produced.[2] The energy requirement increases 72% above maintenance, which is why lactating mares must consume 2.5% to 3.5% of their body weight in total feed per day[15] (see Table 12). Protein needs have been estimated to be almost double from those of maintenance (see Table 17).

Calcium and phosphorus losses from the body reserves of the mare during lactation can be significant without adequate supplementation of these essential minerals.[15] Mare's milk in early lactation contains 112 mg of calcium and 68 mg of phosphorus per kilogram of fluid milk, decreasing to 80 mg and 50 mg, respectively, during the third month (see Table 19). The recommended allowance in Figure 7 allows for these changes in mineral concentration by lowering the recommended allowance in the fourth through the sixth months of lactation.

The trace mineral and vitamin content of mare's milk is now available from several studies.[52–54,62] All trace minerals are low in mare's milk and are believed to be one of the genetic components of DOD. One now can monitor mare's milk on a monthly basis by analyzing it and comparing it with Table 19. If the foal's legs are normal at birth and acquire leg deviations with age, but all the foal is consuming is mare's milk, one logical step would be to analyze the mare's milk and compare it with the expected mineral density average. If a mare has had problems with her foals in the past with DOD, the author would consider her a suspect or at risk mare. If the mare is suspect, one should analyze her milk on day 7 after foaling and every 30 days after that and compare her milk mineral density with Table 19. Several products are on the market today formulated to complement mare's milk and to be given orally to the foal, as a drench, once a day. These products provide trace mineral and vitamin supplementation and enable the broodmare manager to keep the nutrient-deficient foal on the mare rather than wean early. However, if the foal is older than 3 months of age when these acquired tendon contractures begin to manifest, weaning the foal and putting it on a balanced weanling formula is easier and more economical to ensure adequate consumption of all nutrients. The manager must then make sure the correct number of BNUs are fed per day to complement the growth rate of the foal.

The analysis of Rejuvenaide® (per 5 ml drench or 2 ml paste) is as follows:

Ascorbic acid	100.00 mg
Copper	3.20 mg
Zinc	9.40 mg
Selenium	0.25 mg
Vitamin A	3500 IU
Vitamin D	350 IU
Vitamin E	100 IU

Reliance on adequate prenatal nutrition is important and must be emphasized greatly to prevent nutritional deficiencies from occurring in the suckling, until solid food intake begins. The point at which mare's milk and the liver stores of the foal can no longer provide the trace mineral needs of the foal can be seen in rapidly growing foals consuming only mare's milk and showing signs of DOD before the suckling begins consuming an adequate amount of dry feed. Low mineral density in mare's milk is one of the genetic components of DOD. Research completed by The Ohio State University, the University of Guelph, and most recently by Massey University clearly state that trace mineral supplementation is beneficial in the diet of the suckling when the mare has previously produced a foal with what appears to be nutrition-induced DOD. Comparing the suspect mare's milk analysis with Table 19 helps the practitioner to explain why and how much of a trace mineral supplement is necessary and recommended per day.

Body condition scoring is the best gauge for determining the caloric needs of the lactating mare.[15] Mares that 1579
lose weight during lactation should have their total ration evaluated to ensure they are being fed between the 1581
recommended allowance and the SUL of a feed formulated for the nursing mare (see Figure 7). If more calories are needed, one can add vegetable oil to the diet of the mare rather than feed over the SUL of the grain mixture. Mares that gain weight during lactation are not channeling the nutrients fed toward milk production, but are storing the extra calories as fat instead. When feeding easy-keeping mares, one should make sure the recommended allowance is provided with a lower-calorie diet and should be sure to offer a milk-based foal starter to her foal in a separate creep feeder until the foal is 3 months old.

TABLE 19 Mare's Milk Nutrient Composition by Week, Dry Matter, and As-Fed Basis

AGE	% TOTAL SOLIDS	ENERGY (kcal/100 g)	% PROTEIN	% FAT	% LACTOSE	% ASH*	% CALCIUM	% PHOSPHORUS	% MAGNESIUM	% POTASSIUM	% SODIUM	COPPER (mg/kg)	ZINC (mg/kg)	IRON† (mg/kg)
DRY MATTER BASIS														
Birth	—	536	75.79	2.78	18.25	2.86	0.34	0.16	0.19	0.45	0.21	3.93	25.40	5.20
12 hours	—	557	33.04	20.87	41.74	4.35	0.68	0.35	0.12	0.84	0.32	7.22	24.40	8.26
24 hours	—	544	28.95	21.93	45.61	4.65	0.85	0.39	0.10	0.74	0.30	6.40	31.60	9.21
1–4 weeks	—	542	25.23	16.82	57.94	4.91	1.12	0.68	0.08	0.65	0.21	4.21	23.36	7.99
5–8 weeks	—	505	20.95	16.19	60.95	3.81	0.95	0.57	0.06	0.48	0.18	2.48	19.05	6.29
9–12 weeks	—	500	18.00	14.00	65.00	3.00	0.80	0.50	0.05	0.40	0.15	2.00	18.00	4.90
AS-FED BASIS														
Birth*	25.2	135	19.1	0.7	4.6	0.72	0.085	0.039	0.0473	0.11	0.052	0.99	6.4	1.31
12 hours*	11.5	64	3.8	2.4	4.8	0.50	0.078	0.040	0.0138	0.10	0.036	0.83	2.8	0.095
24 hours*	11.4	62	3.3	2.5	5.2	0.53	0.097	0.044	0.0110	0.08	0.034	0.73	3.6	1.05
1–4 weeks‡	10.7	58	2.7	1.8	6.2	0.53	0.120	0.073	0.0090	0.07	0.023	0.45	2.5	0.86
5–8 weeks‡	10.5	53	2.2	1.7	6.4	0.40	0.100	0.060	0.0060	0.05	0.019	0.26	2.0	0.66
9–21 weeks‡	10.0	50	1.8	1.4	6.5	0.30	0.080	0.050	0.0045	0.04	0.015	0.20	1.8	0.49

* Ullrey DE, Struthers RD, Hendricks DG et al: Composition of mare's milk, *J Anim Sci* 25:217, 1966.

† Ullrey DE, Ely WT, Covert RL: Iron, zinc and copper in mare's milk, *J Anim Sci* 38:1276, 1974.

‡ Nutritional Research Council: *Nutrient requirements of horses,* Washington, DC, 1989, National Academy Press.

23.8.2 NUTRITIONAL NEEDS FOR GROWTH

23.8.2.1 Sucklings

At birth, normal-sized foals should weigh between 10% and 12% of the body weight of the mare. The exception is the maiden mare: these foals usually weigh between 8% and 10% of the mare's body weight. The average foal should stand square on all four feet and nurse within 2 hours after birth. Deviations from normal may indicate something interfered with the nutritional absorption, prenatal growth, or positioning process in utero and requires evaluation by the veterinarian. Abnormalities can occur in foals born to mares that were malnourished or had chronic placental insufficiency or uterine sepsis. These foals are often born small and weak and have lower nutrient reserves. Because of the increase in nutrient demand following birth, injury, or disease, supplying ample, balanced, and complete nutrition is critical for health, development, growth, and recovery of the foal.[15] One should ensure that nutrition is not the limiting factor in raising sound, healthy equine athletes today.

The average foal drinks 7 to 10 times per hour for the first 30 days of life. The frequency is necessary because they are born with little energy reserves.[18,63] Thus a readily available food source is necessary to support thermoregulation and growth.[15,63]

Data collected from several studies have provided reasonable estimates for evaluating growth and growth rates of foals.[1,63] The greatest increase occurs in the body weight. The first 30 days is when the most rapid growth rate occurs, and the birth weight doubles in less than 60 days.[15]

Data collected from lactation and growth studies have provided some estimate of the protein, calories, and minerals needed to support the early growth of healthy foals for the first 3 months of age.[15] Between 3 and 4 months of age the enzyme activity of maltase equals that of lactase,[29] so the foal less than 3 months of age should have available a milk-based foal feed to optimize absorption and reduce the chance of diarrhea as a result of too much starch consumption before the foal can digest it. A milk-based foal starter feed should be formulated to complement the mare's milk rather than forage. The large intestine of the foal is not yet fermenting and therefore cannot digest forage. If the young foal consumes forage, it often passes undigested. The vegetable matter takes up space but provides little nutrition and may cause a distended abdomen. The only feed the foal can handle in large quantities without digestive upset is milk. The protein, calories, and minerals in the feed of the foal should complement the mare's milk analysis (see Table 19). The amount of supplementation a foal needs to consume per day is related directly to the quantity of milk the mare produces. The young foal may show an interest in eating other feed when its mare's milk is inadequate to satisfy its appetite.

When crossbreeding a smaller mare to a larger stallion, the larger, faster-growing foal may have a larger appetite. Providing every foal a milk-based foal starter feed formulated to complement the mineral density of mare's milk is critical to the health and quality of growth for the first 3 months.

As stated previously, the growth of various portions of the gastrointestinal tract parallel changes in enzyme activity. Significant increases in the length and diameter of the small intestine occur within the first month, increasing the available villous surface area and making it possible for the foal to process increasing volumes of milk to meet its needs. During the next 5 months, growth of the small intestine continues, but the greatest increases occur in the lengths of the cecum and large colon at the time when foals traditionally begin mimicking adult grazing behavior and increasing their intake of forage and grain.[30] From 6 months on, most of the growth occurs in the cecum and large intestine, allowing for the increased consumption and use of fibrous feeds.

23.8.2.2 Feeding Orphaned or Rejected Foals

Orphaned or rejected foals can be raised on a nurse mare or provided with a mare's milk replacer in a bucket in their stall. Evaluating several factors helps one make the decision:

- Pro Nurse Mare: The nurse mare may be turned out to pasture with the grafted foal with other mares and foals. This requires less time and effort to manage the orphan or rejected foal and has the added advantage of often resulting in more normal behavior in the foal.[15]
- Con Nurse Mare: The current cost for nurse mare rental is $1,500 plus transportation to and from the farm, plus returning the mare back in foal. The mare must be in the same stage of lactation as the mare

she is replacing because the natural mineral density declines in her milk. The longer the mare has been lactating, the more difficult switching foals onto her will be. The possibility exists of infectious disease transmission to the privately owned broodmare stock.[15] 1581

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- Pro Milk Replacer: Currently, at approximately $100/50 lb bag of powered or pelleted milk replacer with a total of five bags needed until weaning, the average cost will be $500. The nurse mare's milk mineral density question is ruled out because of the guaranteed analysis in the milk replacer. Foals can be turned out with a quiet gelding or mare until weaning or at weaning turned out with other weaned foals.

- Con Milk Replacer: Management must oversee feeding and clean the bucket every 12 hours. One must teach the foal to drink from a bucket in the stall, must teach acceptable behavior early, and must get the orphan to interact with other foals or horses as soon as possible. One must follow the feeding directions prescribed on each bag; the feeding directions for one brand do not apply to a milk replacer of another company.

Several milk replacers are currently available; however, the calf, lamb, and kid milk replacers are not nutritionally adequate or balanced for foals. Whole cow's milk and goat's milk have been advocated, but poor weight gains and metabolic acidosis were reported recently in neonatal foals consuming their required amount per day. Milk replacers containing maltodextrins, corn syrups, and glucose polymers are not recommended for foals less that 3 weeks of age because of the low level of maltase activity in the small intestine of the foal.[15,29] Also, milk replacers with a crude fiber of more than 0.15% indicate that the formula is not all milk and therefore is not recommended up to 30 days of age.

If the foal is more than 3 weeks of age when orphaned, liquid milk replacer is unnecessary and a pellet may be fed milk-based foal starter free choice. At this age the molars are in and the foal can chew the milk pellets and swallow them successfully. One should provide only milk-based pellets until the foals are 3 months old. Between 3 and 4 months of age, one should mix equal amounts of the milk-based pellets with a weanling formula that complements their forage.

Each foaling season, a number of foals end up as orphans or rejected. The following is a highly successful program for raising orphan foals that has been implemented in several universities and veterinary neonatal hospitals. Foals raised with this method grow just as well as nonorphans and attain their normal size. In fact, when orphans are raised according to the following recommendations, telling the difference between them and those raised with the mare is difficult. The program is also easy to implement and manage.

Research completed in 1999 compared the different growth rates of foals remaining on the mare and provided a milk-based pellet in a creep with foals weaned at 3 days and raised on the following feeding program. Researchers recorded weekly measurements of body weight, heart girth, body length, wither height, hip height, and cannon bone circumference of the foals. Results showed that foals developed similarly in skeletal size. Although the control group foals (remaining on the mare) were heavier, the foals in all groups received similar body condition scores and were healthy. Foals were not negatively affected by early weaning and did not develop unacceptable habits. Raising foals on an equine milk replacer is helpful to those who are managing orphaned, rejected, or early weaned foals. The program is a successful alternative to keeping the mare and foal together for 4 to 6 months if the mare is competing and can be used for aged (>20 years) brood mares to reduce the stress of raising a foal and being bred.

Colostrum, or the mare's first milk, contains high levels of antibodies to protect the foal from disease. After foaling, the mare secretes colostrum for 24 to 48 hours. Foals absorb colostrum for 12 to 24 hours after birth

or until an adequate of amount of whole protein antibodies are absorbed through the small intestine. The quicker one can get the colostrum into the foal, the faster the large openings in the small intestine will close. All foals, whether on the mare or orphaned, need colostrum. After birth, preferably within the first hour, the foal should begin to receive colostrum. A 100-lb foal should receive 250 ml (approximately 1 cup) of colostrum each hour for the first 6 hours after birth for a total of 1500 ml, or about 3 pints of colostrum per 100 lb of body weight. All breeding farms should have a minimum of 3 pints of frozen colostrum in storage. When needed, one should remove the colostrum from the freezer and thaw it at room temperature or in warm water, pour it into a bottle that has a nipple opening of at least ½ inch, and let the foal suckle. One should *never* warm colostrum in a microwave because the radiation destroys the whole protein antibodies.

Septicemia continues to be the leading cause of death in neonatal foals. Permeability of the bowel during the first 12 to 24 hours of life is increased, which allows the foal to absorb immunoglobulins such as IgG, IgM, IgE, and IgA from the colostrum but may also allow the absorption of harmful pathogens from the environment. Bacterial exposure to the "open gut" is considered a likely route for exposure to bacteria in neonatal foals. Early (rapid) gut closure may be equivalent to or more important than simple absorption of serum IgG in preventing neonatal bacterial infection.[64] If colostrum is unavailable, a suitable substitute must be provided. These substitutes and recommendations for their use are discussed in Chapter 19.

Foals with only 200 mg/dl IgG at 24 hours of age do not get sick on some farms, suggesting that low IgG alone is not sufficient to result in septicemia in all foals.[64] Lack of illness may result from good management as well as conditions that favor the ingestion of colostrum by the foal rather than a specific IgG level in the foal. 1582

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After colostrum or a colostral substitute has been provided, one should introduce the orphaned or rejected foal to an ad lib mare's milk replacer. One may start the orphan drinking from a plastic bowl or with a lamb nipple, depending on how aggressive they are. (If one uses a nipple, one should make sure the *opening* is a least ½ inch wide.) However, a nipple is not necessary, and the foal will learn to drink from a shallow bowl or bucket at any age. The foal does not have an esophageal groove like the calf, so nipple feeding offers no bypass benefit. The foal learns to drink readily if one places a finger in its mouth and then, while it is sucking, raises the small bowl containing the liquid milk replacer up to its muzzle. One slowly removes the finger from the mouth of the foal while it is drinking. If the foal stops, one should repeat the previous steps until the foal is drinking by itself. One always should bring the milk up to the foal and *should never force the head of the foal into a bucket*. The first day one can warm the mare's milk replacer to encourage consumption. When the foal drinks without assistance, one can hang a bucket from the stable wall at shoulder height that will allow the foal to drink whenever it wants. The bucket should be a contrasting color to the wall to make it easy for the foal to find.

Select an ad lib mare's milk replacer formulated to be mixed in water and fed at room temperature free choice. Two frequently used milk replacers for foals are Foals First® from progressive nutrition and Mare's Milk Plus® from Buckeye Nutrition. The ingredients in the milk powder help maintain the natural pH in the digestive system of the foal. Table 20 gives proportions for mixing Foals First® powder and water to ensure the correct amount of milk solids (10%) and a pH of 5.3.

When mixed with cool water, the milk replacer has a tart taste that discourages a foal from drinking too much at one time, even when offered free choice. One should make sure the mixing directions are carefully followed. Foals less than 30 days old drink from their dams an average of 7 to 10 times per hour. Feeding Foals First® insolution free choice allows the foal to follow this natural inclination to drink.

The average mare produces 3.0% to 3.5% of her body weight in milk per day, so a 1000-lb (454-kg) mare can produce 30 pounds (14 kg), or 4 gallons (18 L) of milk per day.

TABLE 20 How to Mix Foals First® Milk Replacer Powder With Water

	FOALS FIRST(R) POWDER	
WATER (GALLONS)	**ENCLOSED RECEPTACLE**	**POUNDS**
1.0	2.00	1.0
2.5	5.0	2.5
5.0	10.00	5.0

One should start feeding the foal just like the mare would—slowly. One begins by providing half of the recommended amount on the first day according to the size of the dam and then gradually increases the amount over the next 2 to 3 weeks, but no faster than 1 qt or 1 L per day, until the suckling is consuming the recommended amount. If the stools of the foal become loose, one should slow down; the rate of increase may be too fast. One should remember, however, that even orphaned foals may go through "foal heat" scours. One should mix the amount a foal should consume in 12 hours (one half of the daily amount) and make it available free choice. Giving a foal access to milk at all times is feeding the natural way, or on demand. Allowing a foal to drink a little at a time, as often as it wants, results in fewer digestive upsets, improved milk digestibility, dramatic weight gains, and improved overall foal health. Each time one mixes new formula, one should discard any milk not yet consumed and thoroughly clean the bucket before adding fresh milk replacer.

From birth to weaning, at 4 months of age, a foal that weighs 100 lbs at birth will need 3/50 lb bags of Foals First® milk replacer powder, 4/50 lb bags of Foals First® milk replacer pellets, and 7/50 lb bags of Foals First® starter and creep feed.

After the foal is drinking the recommended amount of equine liquid milk replacer, one should provide clean water in another bucket free choice along side the milk replacer bucket.

When the foal consumes the recommended amount of equine liquid milk replacer before the next feeding, one should add a handful of the milk replacer pellets into the bucket. Foals are creatures of habit, so one must teach them that eating dry feed is okay. At the time of the next feeding of liquid milk replacer, one should empty any milk replacer pellets left in the bucket and give the liquid milk replacer as usual. Once foals begin to eat the pellets from the bucket, one should provide them in a separate feed tub free choice. Because of the milk formula, one can offer them free choice. A cereal grain–based weanling ration is not recommended at this time because of the low maltase activity and it could cause acidosis and loose stools. This digestive upset decreases absorption of nutrients, predisposing the foal to nutritional deficiencies that could lead to DOD.

At 4 weeks of age one can begin weaning the foal by reducing the liquid Foals First® milk replacer, one gallon (4.5 L) at a time. As the liquid milk is reduced, the foal will increase the amount of milk pellets consumed per day. For every gallon reduced, the foal should consume 1 pound more of milk pellets per day.
This weaning off of liquid milk replacer should take 7 to 10 days. The pelleted milk replacer should be 1583
formulated for the young foal with a monogastric digestive system. One should let the foal eat as much of 1584

them as it wants until it is 2 months old. At that age the digestive enzymes of the foal are changing from lactase to maltase and amylase and the cecum begins to function (ferment), allowing the foal to start digesting forage (hay or pasture).

At 2 months of age, change to the Foals First® starter and creep feed. This is a transition feed and the ingredients include extruded soybean and steam rolled oats. This complements the foals changing digestive system and should be provided free choice up to 4 months of age. Between 3 to 4 months of age, one should select a high-quality weanling ration formulated to complement forages and should mix it 50/50 with the foal starter and creep feed for the next 2 to 4 weeks. After 4 months of age, feeding milk is not necessary. This management follows the normal changes in the digestive system of every foal and promotes optimal growth and maximum nutrient absorption while reducing the chance of digestive upsets.

23.8.2.3 Weanlings

Because no one in the United States continues to breed mares for milk production, some of the genetic lines of good milk producers have been lost. Thus earlier weaning times are more common. The following two management tools help determine the time to wean:

1. The amount of milk-based foal starter and creep feed consumed per day before 4 months of age or the amount of a growing ration consumed per day after 4 months. When the foal is consuming more than 3 lb per day of either, the mare is not producing enough milk to satisfy its appetite. At this time, foals are easy to wean no matter how old they are.

2. When the foal is not eating anything but the mare's milk and starting to show signs of nutritionally related growth abnormalities such as DOD, physitis or acquired contracted tendons.

If weaning time is before 4 months of age, one must remember that offering milk-based foal starter and creep feed ad lib is critical. Just because the foal is weaned does not change the enzymatic activity in its digestive system. One should follow the same feeding program for the early weaned foal as recommended for the orphaned foal, depending on their age at weaning.

TABLE 21 Estimated Body Weights for Growing Horses Maturing at 400, 500, and 700 kg While Growing at a Moderate Rate

	MATURE BODY WEIGHT 400 kg		500 kg		700 kg	
AGE	WEIGHT (kg)	PERCENT OF MATURE WEIGHT	WEIGHT (kg)	PERCENT OF MATURE WEIGHT	WEIGHT (kg)	PERCENT OF MATURE WEIGHT
Birth	44	11	55	11	77	11
1 month	72	18	85	17	112	16
2 months	92	23	110	22	147	21
3 months	120	30	140	28	182	26
4 months	145	36	175	35	225	32
5 months	161	40	193	39	245	35
6 months	180	45	215	43	275	39
9 months	225	56	272	55	350	50
12 months	265	66	325	65	420	60
18 months	333	83	400	80	525	75
24 months	365	92	450	90	600	85
36 months	400	100	490	98	650	93

From Nutritional Research Council: *Nutrient requirements of horses,* Washington, DC, 1989, National Academy Press.

After 4 months of age, no reason exists to feed quantities of milk to the growing foal. One should provide a growing ration according to desired body condition and recommended allowance (see Figure 7). Because the body capacity of the foal is small, the percentages of all nutrients must be high to ensure the foal consumes adequate amounts per day. As the body capacity increases, one can lower the percentages fed because the foal is consuming more pounds or kilograms per day (Table 21).

After 6 months of age the digestive system of the foal is changing more into that of a continuous grazer, and the author recommends managing the weanling to complement its digestive system. The larger the body capacity, the larger the fermentation vat and therefore the more forage that can be consumed. However, the increased body capacity for forage does not usually keep up with the increased mineral requirements for
skeletal development. The larger, faster-growing foals are more prone to skeletal problems because of their 1584
higher mineral needs to support their larger structure. Therefore using the growth chart (see Figure 7) to 1585
determine their individual recommended allowances is the managers' best tool to reduce the incidence of nutrition-related growth problems.

Because foals do not grow at the same rate or mature to the same size, their nutritional needs differ. Table 21 is the accumulation of different breeds and the average rates at which they mature. The small horses mature earlier, and the larger horses mature later. Even within breeds maturing rates differ. The only way to meet the nutrient needs of the growing horse is to monitor its growth and feed according to its own growth rate. This feeding program does not recommend trying to speed up growth or to slow down growth but to provide all the nutrients foals need to grow optimally up to their genetic potential.

To meet the weanling caloric requirements to maintain desired body condition, the diet is approximately 70% growing ration and 30% forage.[1] As the body capacity changes, the proportion of the growing ration to forage shifts to more forage. The owner/manager must make sure to meet the recommended allowance with the pounds of growing ration they are feeding per day.

23.8.2.4 Yearlings

One should balance the total ration for yearlings according to their size, growth rate, and age. Their size and growth rate determines the necessary recommended allowance of protein, minerals, and vitamins needed per day (see Figure 7). As the body capacity increases and/or the forage RFV improves, less growing ration is needed per day to maintain desired body condition. One should increase the forage portion of their diet and decrease the growing ration, but make sure their recommended allowance (RA) of nutrients is met.

TABLE 22 Comparison of the Recommended Allowance for Growing Horses on a Total Ration Dry Matter Basis (Including Forage)

NUTRIENT	WEANLING (4–6 MONTHS)	WEANLING (6–12 MONTHS)	YEARLING (12–18 MONTHS)	LONG YEARLING (18–24 MONTHS)	2-YEAR-OLD TO (MATURE)
Dry matter intake (% body weight)*	2.25–2.75	2.75–3.125	2.25–2.75	2.0–2.5	1.75–2.25
Crude protein (%)	18.00	16.00	14.00	12.00	11.00
Calcium (%)	0.90	0.80	0.70	0.60	0.53
Phosphorus (%)	0.65	0.55	0.45	0.40	0.35
Potassium (%)	0.90	0.80	0.70	0.60	0.53
Magnesium (%)	0.20	0.19	0.18	0.15	0.12
Sodium (%)	0.10	0.12	0.14	0.14	0.14
Manganese (mg/kg)	65	60	55	50	45
Iron (mg/kg)	100	90	80	70	60
Copper (mg/kg)	35	30	25	22	20
Zinc (mg/kg)	100	90	80	70	60
Cobalt (mg/kg)	0.20	0.20	0.20	0.20	0.20
Iodine (mg/kg)	0.25	0.25	0.25	0.25	0.25
Selenium (mg/kg)	0.30	0.30	0.25	0.25	0.20
Vitamin A (IU/kg)	3000	3000	2500	2500	2000
Vitamin E (IU/kg)	95	95	90	85	80
Thiamine (mg/kg)	4	4	3.5	3.5	3
Riboflavin (mg/kg)	3	3	2.5	2.5	2

* Feed intake is determined by body capacity, physiologic status, activity level, relative feed value of forage, and environment. Needed digestible energy (Mcal/day) is determined by body size, metabolic rate, growth rate, dry matter consumption, and body condition score.

By 12 months of age their diet should be approximately 50% growing ration and 50% forage by weight[1,15] (see Table 11). The differences in amounts fed per day are related to the RFV of the forage (see Table 1), size

of the foal, metabolic rate, activity level, and environmental temperature. The sum of these determines how much of the growing ration the foal requires to maintain desired body condition, or calories needed per day.

One key management tool in developing easy-keeping yearlings is to provide the recommended allowance by feeding only the appropriate ration balancer and forage. On the opposite end of the spectrum, to manage the hard-keeping or the sales prep yearlings successfully, one should not feed over the SUL (see Figure 7) of the growing ration per day. This practice ensures optimal pH in the digestive system and maximum absorption of the nutrients fed per day.

Yearlings have attained nearly 90% of their adult height by 12 months and 95% by 18 months.[15] Table 22 lists, by percentage or nutrient per kilogram consumed per day, what the total ration nutrients should be from 4 months to 24 months of age. As the total ration dry matter consumed increases, one can lower the percentages or milligrams per kilogram of the nutrients and still meet the nutrient needs of the foal. 1585

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The 2-year-olds have slowed in their growth rate per day but are still growing, and one must meet the individual recommend allowance to ensure optimal growth, health, and performance.

23.8.3 NUTRITIONAL NEEDS OF THE PERFORMANCE HORSE

The largest increase in nutrient needs for horses in training is calories per day to maintain desired body condition. Body condition scoring is the best way to determine if one is meeting the individual caloric needs of the horse. The recommended allowances of protein, major and trace minerals, and vitamins do not increase at the same rate as the caloric requirements. Therefore to prevent possible digestive upset and acidosis from too much cereal grain (starch), one must know the SUL for every feed given to performance horses (see Figure 7). Table 23 allows for the increase in feed consumed per day, depending on the individual workload, and lists how the protein, major and trace minerals, and vitamins change with work intensity.

To help one understand the different levels of work, the NRC has put together four different, broad terms to evaluate them[1]:

- *Laid up/Idle/Inactive:* Refers to horses not being exercised or confined to a stall (limiting activity level while giving them time to heal or recuperate).
- *Light:* Refers to work up to 1 hour per day, 6 days per week (trail, English and Western pleasure, and low-level dressage).
- *Moderate:* Refers to work from 1 to 3 hours per day, 5 days per week (racing, cutting, reining, jumping, and upper-level dressage).
- *Intense:* Refers to work more than 3 hours per day, 3 days per week (100-mile endurance racing, Three-Day Eventing, combined driving, polo, and point-to-point).

TABLE 23 Comparison of the Recommended Allowances for Performance Horses While Layed Up or in Light, Moderate, or Intense Training on a Total Ration Dry Matter Basis (Including Forage)

	LAID UP OR IDLE		LIGHT		MODERATE		INTENSE	
NUTRIENT	2-YEAR-OLD	MATURE	2-YEAR-OLD	MATURE	2-YEAR-OLD	MATURE	2-YEAR-OLD	MATURE
Dry matter intake (% body weight)*	1.5–2.0	1.5–2.0	1.75–2.25	2.0–2.5	2.0–2.5	2.5–3.0	2.5–3.0	3.0–3.5
Crude protein (%)	11.00	8.50	12.00	10.50	12.50	11.00	13.00	12.00
Calcium (%)	0.53	0.30	0.55	0.40	0.56	0.42	0.57	0.48
Phosphorus (%)	0.35	0.21	0.36	0.27	0.37	0.28	0.38	0.30
Potassium (%)	0.53	0.40	0.70	0.70	0.80	0.80	0.90	0.90
Magnesium (%)	0.12	0.10	0.19	0.18	0.21	0.20	0.23	0.22
Sodium (%)	0.14	0.14	0.16	0.16	0.18	0.18	0.20	0.20
Manganese (mg/kg)	45	40	50	45	50	45	50	45
Iron (mg/kg)	60	40	80	80	80	80	80	80
Copper (mg/kg)	20	15	20	15	20	15	20	15
Zinc (mg/kg)	60	40	60	55	60	55	60	55
Cobalt (mg/kg)	0.20	0.20	0.30	0.30	0.30	0.30	0.30	0.30
Iodine (mg/kg)	0.25	0.25	0.30	0.30	0.30	0.30	0.30	0.30
Selenium (mg/kg)	0.20	0.15	0.20	0.20	0.25	0.25	0.30	0.30
Vitamin A (IU/kg)	2000	2000	2500	2500	2750	2750	3000	3000
Vitamin E (IU/kg)	80	50	80	80	80	80	80	80

Thiamine (mg/kg)	3	3	3	3	3	3	3	3
Riboflavin (mg/kg)	2	2	2	2	2	2	2	2

* Feed intake is determined by body capacity, physiologic status, activity level, relative feed value of forage, and environment. Needed digestible energy (Mcal/day) is determined by body size, metabolic rate, growth rate, dry matter consumption, and body condition score.

The author realizes that one needs to consider time and intensity of work, as well as ambient temperature and environmental conditions, when evaluating these workloads. The author also realizes that all trainers train intensely; however, the duration at that level of training determines the term. These names were given to help define caloric needs to maintain desired body condition and the recommended allowance needed to maximize muscle function, animal health, and performance.

Calories may be provided by carbohydrates, fats, digestible fiber, and protein. Carbohydrates and fats (vegetable oil) are the most concentrated sources of energy. Carbohydrates are abundant in cereal grains and in forages with a RFV greater than 115. Cereal grains average 3.0% to 4.0% fat, whereas forages contain between
1.5% and 5.0% fat, depending on their maturity when harvested or eaten. Horses easily digest and use vegetable 1586
oils, even though they have no gall bladder. The continuous flow of bile from the liver to the small intestine 1587
allows this, but for that reason also the author recommends not exceeding 8% fat in the total diet. If overfed fat, the horse either stops eating or develops diarrhea from not being able to emulsify the level of fat being consumed. Interference with calcium absorption and fiber fermentation may occur also with overfeeding. Forages provide fiber, but how fermentable the fiber source is relates directly to the RFV of the plant. The higher the RFV, the easier fermentation is for the bacteria and protozoa in the hindgut, to break down the plant cells and produce usable energy sources (volatile fatty acids). Protein can be used as an energy source if other calorie sources are deficient or protein is in excess in the diet, but protein is not an efficiently converted energy source.

Water and the necessary electrolytes are critical in moderate and intensely trained horses (see the section Practical Approach to Management of Fluid and Electrolyte Balance later in this chapter).

Body condition and the performance of the individual horse is still the best way to evaluate a feeding program. But one must remember to evaluate the total diet, forage and grain mixture.

23.9 Developmental Orthopedic Disease From a Nutritional Standpoint

23.9.1 MULTIFACTORIAL PROBLEM

Research continues to try to find the answers to perplexing problems dealing with the growth and development of foals today. Researchers realize a *genetic* component exists; for example, the inability of the mare to produce quality cartilage in utero. This inability can be passed on to the foal and could affect its ability to form healthy cartilage and strong bone as it grows.[51,57] Researchers also realize that *management* is involved. How do veterinarians handle the old, debilitated mares with chronic illness or injuries and mares with placentitis? When a foal is born compromised, for whatever reason, or it acquires the problems, attention to early foal nutrition is indicated. If the foal responds to nutrition therapy, one can use the flow chart on DOD (Figure 9)[18,65] to correlate the age of onset with its possible nutritional cause. This is the *nutrition* component of DOD.

This section discusses only the nutritional aspects of DOD and what recommendations have shown the most promise with nutritional intervention.

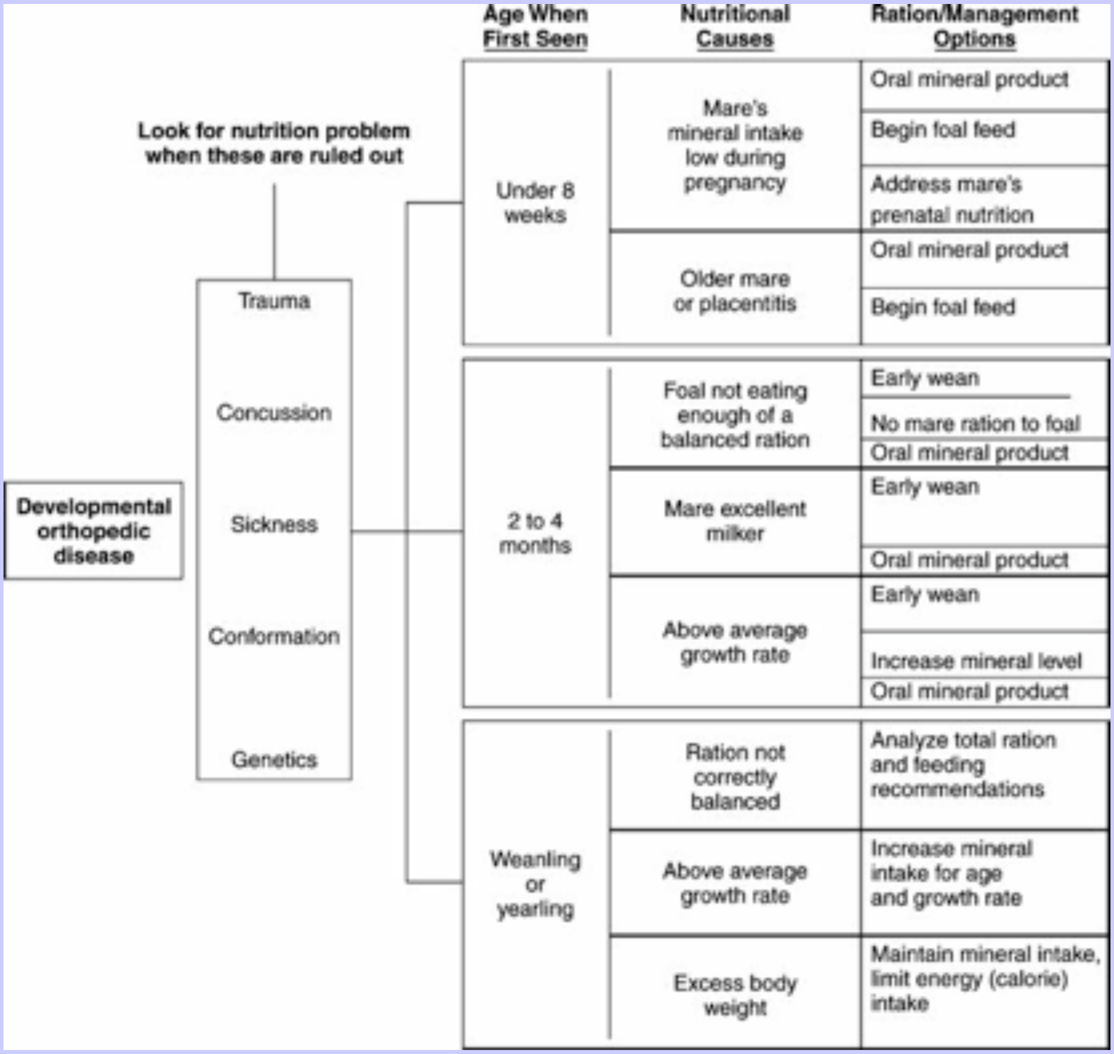
Several research projects have been completed to prove that adequate or higher amounts of protein do *not* cause DOD. However, Ott and Asguith[66] have proved that feeding protein levels below NRC recommendations decreases bone density and has a negative affect on tendon and ligament strength. When they increased the mineral density in the diet, the increase had no affect on the growing horse as long as the protein was inadequate. The only link between the more than adequate protein and DOD is when one or more of the minerals calcium, phosphorus, copper, or zinc was inadequate. Unfortunately, protein continues to be blamed, and horsemen remain afraid to feed an adequate amount.

In the field, two types of growing horses with increased incidence of DOD are common. They are the rapidly growing and the very small foals.

Very large, growing foals have an increased requirement for all nutrients and minerals to support their larger, faster-developing skeletal structures.[1] Often accompanying this type of foal is a lower metabolic rate, causing it to be an easy keeper. Fewer pounds of a grain mixture fed per day may be recommended to reduce calories; however, along with the decrease in calories comes a decrease in all the nutrients needed to support the developing cartilage and bone. If a deficient amount of protein accompanies this decrease, the horsemen effectively can create many of the forms of DOD known today. The other end of the spectrum is also true. When the horseman overloads the digestive system with too much of a grain mixture (starch) trying to fill out this large frame, the result is acidic gut syndrome, which deceases the absorption of nutrients fed and causes mineral deficiencies and digestive upset.[45,67,68]

The small foal weighing at birth less than 7% of the body weight of the mare but normal in all other aspects also is predisposed to skeletal problems if the small size is caused by the mare being fed a deficient diet. Such deficiencies usually occur because the owners did not want to overdo nutrition. Figure 10 is from a breeding farm where workers measured growth and development from birth and recorded the growth rates of all foals. These 3 years of observations showed the mares were fed little to no grain mixture because they were too fat while turned out for 24 hours per day in their pasture. Therefore the owners selected a 12% protein grain mix (which was formulated for mature, idle horses) because they thought protein was causing more than 60% of their foals to have osteochondritis dissecans, requiring surgery. After year 1, the owners made a concerted effort to ensure that the recommended allowances were met on prenatal nutrition during the entire pregnancy and lactation. The graph shows the first year before the plane of nutrition increased, which resulted in the smallest foals having the highest percentage of DOD. The second year reduced the incidence, and the third year had the largest foals in body size, with the fewest, 16%, requiring surgery for osteochondritis dissecans. The mares remained the same and the management remained the same, except for feeding a ration balancer for the entire pregnancy and ensuring the recommended allowance was met during lactation. 1587

Figure 9 Developmental orthopedic disease problem-solving flow chart.



23.9.2 INVESTIGATING GROWTH-RELATED PROBLEMS ON A FARM

The following procedures allow one to investigate growth-related problems (DOD) on a farm. Numbers 1 to 7 consider nutrition and management, whereas 8 and 9 address genetics.

1. Record the ages, heights, weights, and body condition scores of all horses on the growth and performance chart (see Figure 7).

2. Complete the Ration Balancing Client Information Worksheet (Figure 11). Record the feed and supplement tag analysis in detail. If the mares are on pasture, then record the number of hours they are outside eating fresh grass.

a. For the mares, record the pounds of hay, grain mixture, and pounds/ounces of any supplement fed to the mares while pregnant and nursing: first 3 months and the second 3 months.

b. For growing horses, record the pounds of hay, grain mixture, and pounds/ounces of any supplement fed to the sucklings, weanlings, yearlings, and long yearlings while on the farm.

3. Record or make copies of the analysis of all supplements provided free choice and note on the Ration Balancing Client Information Worksheet which horses had access to them. How much has been consumed of each supplement and over what time frame?

4. On the Ration Balancing Client Information Worksheet, record how long the pregnant mares and growing horses were on the current feeding program.

5. Get accurate samples for analysis of their appropriate (a) hay, (b) pasture, (c) grain mixture, (d) supplements, and (e) water (Table 24).

6. Because veterinarians look into every nutrient and certain mineral combinations when they evaluate the 1588
total ration, send all samples to a laboratory that can analyze the following: dry matter, total protein, 1589
available protein (heat-damaged protein), fat (ether extract or acid hydrolysis if cooked/extruded/milk based), equine digestible energy in Mcal/kg (not ruminant), acid detergent fiber, neutral detergent fiber, relative feed value, calcium, phosphorus, potassium, magnesium, sulfur, sodium, chloride, dietary cation:anion difference, manganese, iron, molybdenum, copper, and zinc. These are the first nutrients to evaluate, but if further investigation is necessary, then one also should evaluate selenium, iodine, vitamin A, vitamin D, and vitamin E. The selenium and iodine in the forage and grains is related directly to the soil type and location (state or country) where they were grown. The amounts of the oxidizable vitamins A and E found in forages are related to time in storage, whereas the levels in cereal grains, grain mixtures, and supplements are related to nutrients that antagonize them, such as iodine and choline chloride or any processing (cracking, crimping, rolling) that breaks their protective hulls.

7. Enter the amounts of everything fed, and the analysis of each feed, into an equine ration balancing program and check for deficiencies, interferences, or toxicities of each nutrient.

8. Review the pedigrees of all sires and dams, looking for similar names in affected foals.

9. Review and record, on the Ration Balancing Client Information Worksheet, leg conformation of all growing foals. Look for deviations from normal: base wide, base narrow, toe-in, toe-out, bench kneed, too upright in their pasterns and shoulders, post-legged, sickle hocked, etc. All of these conformation faults increase the trauma on the growth plates and joint surfaces because of the uneven weight distribution placed on them.

Figure 10 A breeding farm that measured growth and development from birth and recorded growth rates of all foals.

23.9.3 RATION BALANCING CLIENT INFORMATION WORKSHEET

23.9.3.1 Forage Samples

When one sends a forage sample (hay or pasture) to a laboratory to be analyzed (e.g., Holmes Laboratory), several tests are available. Selection of tests for analysis are dependent on the clinical problems being recognized on the farm or training center.

The minimum amount of information needed of forages, grain mixtures, and supplements: percents dry mutter, protein, acid detergent fiber, MCAL DE (horse), calcium, phosphorus, magnesium, potassium, sodium; and parts per million of copper, manganese, zinc, and iron. Add sulfur if you suspect a selenium deficiency. Add neutral detergent fiber (NDF) if you need relative feed value (RFV) and non-structured carbohydrates (NSC). Include (DCAD) dietary cation:anion difference if you suspect electrolyte problems or skeletal problems. Have heat damaged protein in the hay analyzed if you smell the carmalization which occurs during the browning reaction. This occurs during the first 30 days in storage only and is associated with too much moisture when baled.

23.9.4 MANAGEMENT AND FEEDING RECOMMENDATIONS

Once all of the information is available and potential nutritional problems have been identified, one then can make management and feeding recommendations. If deficiency or mineral interference is the culprit, the most

successful nutritional approach, based on field experience, has been to meet the requirements for protein, adjust the major and trace mineral and vitamin levels above their requirements for a short period of time until one sees a positive response, and then adjust down to meet the requirements. This process needs to be completed on the lowest-calorie diet possible, which can be accomplished by providing grass forage ad lib, *without* feeding any cereal grain (straight oats, barley, rice, etc.) but providing only the grass ration balancer for the horses to eat other than hay. Using the growth and performance chart (see Figure 7), one can find how many BNUs are needed per day to meet the needs of the horses according to their ages and sizes. If mineral deficiency or unbalanced diets are found, one should provide one half BNU more than the chart recommends per day for the first 30 to 60 days. When one can see and record improvements, one should reduce the BNUs to the required amount for each horse per day. One should remember that the growth and performance chart is for all horses with a body condition score between 5 and 6. One must adjust accordingly. If mineral or vitamin toxicities are identified as the cause, one should feed only the required BNUs per day. 1589

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The younger the horse, the faster the response, if nutrition is the limiting factor, because of the rate of tissue turnover in younger horses. If the inflamed physis or acquired contracted tendons results from a nutritionally deficient diet, foals under 30 days of age show a positive response within 10 to 14 days; weanlings up to 6 months of age, within 30 to 45 days; and yearlings, within 60 to 90 days. Horses more than 2 years of age with tendon contractures respond minimally to a change in nutritional status.

Figure 11 Ration Balancing Client Information Worksheet.


Form submitted by ____ Phone ____
Owner's name ____ Farm/Training center ____
Address ____ City ____ State ____ Zip ____
Phone ____ Fax or e-mail ____
Buckeye dealer ____ City ____ State ____ Zip ____
Veterinarian ____ Clinic name ____
Address ____ City ____ State ____ Zip ____
Phone ____ Fax or e-mail ____
What form of feed do you want to use? Pellet Sweet Ration Balancer w/oats
Level of Training definitions ____
Light: Up to 1 hour (English and Western Pleasure, Lower Level Dressage, Hack)
Moderate: 1 to 3 hours (Reining, Roping, Cutting, Jumping, Upper Level Dressage)
Intense: Over 3 hours (Endurance Racing, 3 Day-Eventing, Combined Driving, Polo)
What problems, if any, are they having and do they think they are nutrition related?

Add information that would help identify problems with these horses.

Sucklings	How many? ____	Weanlings	How many? ____
Breed ____		Breed ____	
Height range ____ to ____		Height range ____ to ____	
Weight range ____ to ____		Weight range ____ to ____	
Leg conformation ____		Leg conformation ____	
lbs Hay/day ____	Pasture ____	lbs Hay/day ____	Pasture ____
lbs Grain/day ____	Grain used ____	lbs Grain/day ____	Grain used ____
Supplements ____	Qty/day ____	Supplements ____	Qty/day ____
How long on this feeding program? ____		How long on this feeding program? ____	

Yearling	How many? ____	Pregnant mares	How many? ____
Breed ____		Breed ____	
Height range ____ to ____		Height range ____ to ____	
Weight range ____ to ____		Weight range ____ to ____	
Leg conformation ____		Leg conformation ____	
lbs Hay/day ____	Pasture ____	lbs Hay/day ____	Pasture ____
lbs Grain/day ____	Grain used ____	lbs Grain/day ____	Grain used ____
Supplements ____	Qty/day ____	Supplements ____	Qty/day ____
How long on this feeding program? ____		How long on this feeding program? ____	

TABLE 24 Analysis of Water Supplies

ITEM	EXPECTED RANGE[1]	POSSIBLE PROBLEMS[2]
Total dissolved solids	500 or less	Over 3,000
Total hardness	0–180	0–60 Relatively Soft
		61–120 Moderately Hard
		121–180 Hard Water
		Over 181 Very Hard
Calcium	0–43	Over 500
Magnesium	0–29	Over 125 ppm, is laxative and diuretic
Alkalinity (phenothalein	0-trace	High is unusual
Alkalinity (total)	0–400	Over 5,000
Carbon dioxide	0–50	Over 300 ppm
Chlorides	0–250	Over 250 ppm
Iron	0–0.3	Over 0.3 off-odor, taste, staining
Manganese	0–0.05	Over 0.05 (taste)
		Over .2 may stain
Sulfate	0–250	Over 250 may be laxative
		Over 2000 in some mine drainage wastes
Fluorides	0–1.2	Over 1.5 may stain
		Over 2.4 mottling of teeth
Silica	0–10	
Copper	0–0.6	Over 1.0 gives bitter taste
Phosphate	0–1.0	High when contaminated from sewage, agricultural wastes and some industrial waters
pH	6.8–7.5	Under 5.1 or Over 9.0. Wide values may indicate contamination with industrial or mine wastes
		Over 8.0 may give drying effect on skin and soda taste
Stability index	6.0–7.5	Below 6.0 is scale-forming
		Above 7.5 is corrosive
Sodium	0–3	Response to lower salt intake might result at about 40 ppm
Potassium	0–20	

Arsenic	0.05	Over 0.20
Cadmium	0–0.01	Over 0.05
Chromium	0–0.05	
Mercury	0–0.005	Over 0.01
Lead	0–0.05	Over 0.10
Hydrogen sulfide	0–2	Over 0.1 (taste)
Barium	0–1	Over 0.1 (health)
Zinc	0–5	Over 25
Molybdenum	0–0.068	
Nitrites as NO_2	0–0.33	Over 4.0 ppm may be toxic
Nitrates as NO_3	0–44	High values may indicate pollution with organic matter
		Over 45 may cause methemoglobinuria in infants

EXPECTED EFFECTS OF NITRATES IN WATER

CONTAMINANT	RECOMMENDATION	EFFECTS
NITRATE CONCENTRATION (ppm)		
0–45	Approved	None
46–135	Doubtful	May cause methemoglobinuria
136–225	Risky	Reproductive problems and subclinical rickets are possible
226–450	Do not use	Definite interference syndrome, vitamin deficiencies, arthritis
451–675	Do not use	Serious health problems
676–900	Do not use	Reduced resistance to disease
More than 900	Do not use	Possible heavy acute toxicity and death losses
BACTERIAL COUNTS/POLLUTANTS		
Total bacteria/100 ml	Less than 200	More than 1 million
Total coliform/100 ml	Less than 1	More than 1 for young; more than 15–50 for mature
Fecal coliform/100 ml*	Less than 1	More than 1 for young; more than 10 for mature
Fecal streptococcus/100 ml	Less than 1	More than 3 for young; more than 30 for mature

Content developed by Dr. Richard Adams, Penn State University Department of Dairy Science.

* If pollution is from human wastes, fecal coliform should exceed fecal streptococcus by several times. If pollution is from an animal source, streptococcus should exceed coliform in refrigerated samples tested soon after taking.

23.9.5 CONDITIONS THAT RESULT FROM A NUTRITIONALLY DEFICIENT DIET

Physitis and acquired contracted tendons are the only DODs in the early stages that are visible to the client. The following identification and management recommendations can assist the horse owner/manager.

23.9.5.1 Physitis

If inflammation is caused by a nutritional deficiency, mineral interference, or toxicity, it will be evident on all four legs, medial and lateral. Unequal weight distribution, caused from a deviation in leg conformation and trauma, causes only medial or lateral inflammation on the affected legs. If only one leg is inflamed, inflammation probably came from an injury and is trauma induced. The author recommends handling all of these physis problems by limiting calories while providing adequate protein, minerals, and vitamins to support healing and skeletal development. The previous feeding program provides such a diet. The owner/manager must understand the only physis one can see is in the ankle and knee/hock areas. If the inflammation is nutrition induced, other physes also will be inflamed but not visibly so.

To assist the horse owner/manager in understanding where they are starting and to see improvements, the veterinarian should have them measure the circumference of the inflamed area with a cloth measuring tape and record. Measurements should be completed once a week to reinforce that healing is taking place. If the inflamed area stays the same or becomes smaller, the problem is under control. If the inflamed area becomes larger, one then must limit trauma by limiting activity or changing the hardness of the surface area where the horse exercises.

23.9.5.2 Acquired Contracted Tendons

When presented with the deep digital or the superficial digital flexor contractures, one must try to eliminate the pain and provide adequate nutrition at the same time.

If the contractures worsen with exercise, one should limit activity to hand walking and completely bandage the area.

Support from the foot to the knee or hock is warranted, and if the heels are not touching the ground, one should tape on a wedge pad for 7 days, which will raise the heel and eliminate the pain caused from stretching. Once the pain is alleviated, relaxation occurs, and proper nutrition can assist the healing and growing process. One can apply the wedge pad in 7-day intervals but should not leave it on for more than 7 days (thus on 7 days and then off for 7 days). One can reapply the wedge pad if necessary. Once the pain is gone, the farrier can begin lowering the heels of the foot, but no more than 2 degrees per month. A toe extension may cause increased pressure and pain will return. Toe extensions are not recommended when pain is involved.

If the contractures improve with exercise, one can recommend a tip shoe or a toe extension. One achieves success by not being in a hurry to fix this problem. Too much stress on the flexors may cause a set back and extend the healing time by months.

The foregoing management and nutrition programs are recommended for tendon contractures also. The age of the affected horse has a great deal to do with the amount of success with this management and nutritional intervention.

23.9.5.3 Wobblers

If the inflamed physis in the vertebra is causing a narrowing of the spinal canal, the foregoing feeding program is recommended. Reducing caloric intake while increasing the plane of nutrition is critical for skeletal growth and healing. This nutrition program is recommended for all postoperative cases to ensure optimal results.

23.9.5.4 Osteochondritis Dissecans and Bone Cysts

To ensure adequate nutrition after surgery and provide every opportunity to heal, the foregoing nutrition program is recommended. Reducing caloric intake while providing the nutrients for skeletal growth and healing is critical.

23.10 Practical Approach to Management of Fluid and Electrolyte Balance

Gayle Ecker

The goal of electrolyte and fluid supplementation should be to “maintain optimal performance through optimal health.” One should view fluid and electrolyte supplementation as a means to maintain health and performance by *preventing* the problems associated with losses. One should use electrolytes *and* water to support rheostasis of water and ions in the fluid compartments. Physiologically, one *must* use water with electrolytes to replace the losses that occur with sweating. One must consider those electrolytes that are effective for maintaining or restoring extracellular fluid volume—sodium and chloride—and to those that help maintain or restore intracellular fluid volume—potassium and chloride. 1592

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The maintenance of fluid and electrolyte balance within the intracellular and extracellular fluid compartments plays a critical role in muscle function and in virtually all processes within the body. Under normal resting conditions, the body strives to maintain the fluid and electrolytes in balance inside and outside the cells. Disturbances in this balance can predispose the horse to a myriad of health and performance problems such as fatigue, dehydration, heat injury, muscle cramping, and reduced gut function. With vigilance, hydration, and health monitoring and a deeper understanding, experienced riders can avoid and have avoided these disturbances, thus maintaining the optimal health and performance of the horse before, during, and after the event.

The veterinarian's role at an equine event is to be the advocate for the horse. Helping clients, owners, and riders of horses understand good management practices of water and electrolyte supplementation can help prevent many problems and needless suffering of the animal.

Fifteen years ago, only a few sectors of the horse industry were using electrolytes and with highly variable effects: misinformation on their use was prevalent. Today, thanks to the efforts of many researchers worldwide, veterinarians have a great deal more information. However, this information has not permeated the equine industry to the point of providing consistent, beneficial effects for the horse. Fluid and electrolyte research has made significant inroads most notably to the endurance sector and to a lesser extent to other disciplines such as

eventing. The veterinarian's role is important for helping to educate the client on effective use of electrolytes and dispelling misinformation or old horsemen's tales that have been proved incorrect.

23.10.1 HOW DEHYDRATION AFFECTS THE BODY

With exercise comes an increase in heat production. Some heat storage is desirable for enhancing certain metabolic reactions; however, beyond that, the heat must be dissipated. The main route for heat dissipation is through sweating, resulting in losses of water and electrolytes in the sweat. Depending on the intensity and duration, the losses of water and electrolytes can range from minimal, with a minimal effect on health and performance, to the profound, resulting in serious performance decrements and life-threatening health problems for the horse.

Dehydration, a deficit of body water, negatively affects virtually every process in the body. Dehydration is a disturbance of fluid and electrolyte balance that can result in premature fatigue and increased risk of heat illness. Dehydration compromises the production of energy in the muscles, increases stress on the heart, impairs the blood flow and perfusion to the tissues, and compromises dissipation of body heat. Proper hydration is critical, for even small losses can negatively affect performance. Losses as little as 3% have been shown to affect performance in horses, and losses of as little as 1% negatively affect performance in human beings.[69] In horses, losses of 1% to 5% are common because of exercise, transport, and even spending a day at a horse show in hot weather. Losses easily exceed this for more active or prolonged equine events such as endurance, Three-Day Eventing, polo, and racing. One must realize that sweat losses can be moderate to high even in horses that undergo slower but extended exercise in the heat, such as the pleasure trail horse and possibly the equine that is competing in a horse show that includes reining, games, and equitation style classes over the day. The horse is usually somewhat dehydrated from the trailer ride, followed by less than normal feed and water intake and combined with prolonged sweating because of standing in the sun for most of the day. So, although the losses are not nearly as profound as the endurance-type events, the losses can be at levels that potentially affect health and performance, which is particularly true when these losses are not corrected before the start of endurance-type activities.

23.10.2 WHY THE HORSE NEEDS FLUID AND ELECTROLYTE REPLACEMENT

In human beings, production and excretion of dilute sweat results in an increase in sodium concentration, and the associated increase in plasma osmolality triggers the thirst drive as the body tries to regain euhydration, through drinking, reduced urine excretion, and renal retention of sodium. The dilemma for horses is that sweat is not dilute, rather it contains high concentrations of Na^+, K^+, and Cl^-. Because of this, sweating does not produce an increase in plasma Na^+ and osmolality and thus no osmotic thirst response occurs. The thirst response is delayed and may be a function of reduced circulating volume and blood pressure. Thus many horses may not voluntarily drink until a 5% dehydration or greater has occurred, that is, clinical dehydration. Chronic dehydration and electrolyte depletion will increase the risk of heat illness and can lead to death. Typically, dehydrated horses only replace about two thirds of the sweat loss[70,71] when left to their own devices, that is, no electrolyte supplementation.

When a horse sweats, a loss of water, sodium, and other electrolytes occurs, and there is little change in sodium concentrations despite significant losses.[71] Water alone delays proper rehydration because the water intake dilutes the plasma sodium concentration and decreases plasma osmolality and the thirst drive before fluids are

replaced and may actually increase urine output. Also the increased urine output causes additional loss of Na^+, K^+, and Cl^-, further exacerbating the electrolyte deficits.

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Without a rise in sodium concentration and plasma osmolality, the thirst response is not triggered until a large volume deficit has occurred, resulting in baroreceptor response to decreases in central blood pressure. Electrolyte supplementation with the water helps prevent dilution of the sodium concentration and results in greater fluid consumption. Research has shown that fluid losses of more than 18 L can occur in as little as 20 miles[71,72]; therefore fluid and electrolyte intake should start before the event. When used effectively, this method has prevented dehydration and performance decrements in endurance horses by providing a reservoir in the gastrointestinal tract during performance; effective supplementation strategies during endurance events enhance recovery after the event. Waiting until the horse is thirsty results in a horse that is substantially dehydrated. Trying to catch up while exercise continues becomes more difficult, especially in the face of continued sweat losses. Little can be gained by prolonging dehydration; therefore the author holds that the health and performance of the horse are served best by prevention of losses as much as possible and rapid restoration of fluid and electrolytes during recovery. The longer the state of dehydration and electrolyte deficits linger, the greater the chances of health problems. 1594

23.10.3 FACTORS THAT INCREASE SWEAT LOSS AND INCREASE THE RISK OF HEAT STRESS/DEHYDRATION

23.10.3.1 Environment

High temperatures impede heat dissipation in the equine athlete. Heat and humidity compromise heat dissipation even further. Heat and especially humidity increase the sweat rate during exercise and increase water and electrolytes losses. Sweat losses during hot/humid conditions (temperature, 32° to 34° C; relative humidity, 80% to 85%) increased by 5% over the sweat rate during hot/dry conditions (temperature, 32° to 34° C; relative humidity, 45% to 55%) and were 32% higher compared with exercise in cool/dry conditions. Additionally, the composition of sweat changed with changing environmental conditions. McCutcheon, Geor, Hare, et al. found that osmolality and Na^+ concentration was high in the hot/dry conditions but lowest in the cool/dry conditions.[73] Also of practical importance, McCutcheon and Geor found that horses training in hot/humid conditions lost approximately twice the total sweat water and electrolytes compared with training during cool/dry conditions.[74] The authors concluded that the losses, particularly during training in hot/humid conditions, exceeded the dietary intake and that therefore electrolyte supplementation is warranted.

23.10.3.2 Intensity

The harder the horse works, the more body heat the horse generates and therefore the more sweating is needed to dissipate the heat. Intensity can increase because of speed, hilly terrain, and “giving” footing such as mud or sand. Electrolyte losses increased when mean ride speed or increased muddy terrain were encountered. For example, Cl^- losses (from the extracellular fluid compartment) increased from about 2% to 3% at an average ride speed of 6 mph to a loss of about 30% at an average ride speed of 12 mph in endurance rides. The losses could increase by about 5% to 10% roughly when the terrain included muddy conditions.[71]

23.10.3.3 Acclimatization

A horse that has been acclimatized to training in the heat actually loses more water and electrolytes because the response to acclimatization is increased sweating. Horses that have been trained in the heat (and humidity) undergo thermoregulatory adaptations that allow improved performance and heat tolerance; however, adaptation did not decrease the electrolyte losses during exercise.[75,76] The practical significance of this then is that one should not decrease electrolyte supplementation during and after acclimatization as many horse owners have believed in the past.

23.10.3.4 Surface Area

Covering the limited surface area of the horse minimizes the area available for sweating. The optimal method is to free up as much surface area as possible by minimizing coverage by saddle pads, loosening girths slightly and clipping areas on the neck, shoulders and upper legs for better evaporation of sweat. One should remove the saddle and pads when possible and keep the horse walking in a breeze to help the cooling process. Applying water frequently with a sponge or soaked towel increases heat loss and should continue until vasodilation of the skin decreases.

23.10.3.5 Preexisting Dehydration

Dehydration leads to decreases in blood volume, thereby decreasing perfusion to the muscles. Dehydration can affect heat dissipation negatively and therefore necessitates higher sweating rates to cool the horse. Wet feedstuffs, such as well-soaked beet pulp, soaked hay, soaked extruded/pelleted feeds, and fresh, wet grass are high in moisture content. One can use these types of feeds to help prevent dehydration and restore hydration. Adding water (and electrolytes as necessary) to this feed and giving it to the horse the night before and the morning of the competition can be useful in providing a reservoir of water and electrolytes in the gut for use during exercise.

23.10.4 DETECTION OF FLUID AND ELECTROLYTE DEFICITS IN THE FIELD

A common scenario is that a veterinarian is called in to an equine event to treat a horse for a metabolic or 1594
lameness-related disorder. Whenever pharmaceutical treatment is required, such treatment is well known to be 1595
potentially life-threatening for the dehydrated horse because of the potential damage on the kidney. So rehydration and correction of the fluid and electrolyte balance are critical when pharmaceutical treatment has been deemed necessary. If possible, at least beginning the process of restoring hydration before administering the pharmaceutical is important. The diuretic lasix and bute are the two most life-threatening under these conditions.

So how does one detect dehydration and its extent, before the administration of pharmaceuticals? The standard methods of detecting dehydration have been the skin pinch test and capillary refill time. For the skin pinch test, one grasps the skin on the shoulder between the thumb and first finger, pulls the skin away with a slight twist, and then releases it. Skin that snaps back quickly is believed to represent a condition of little or no dehydration. Skin that “tents” or stays in the pinched state for longer than 1 second before returning to a flat position indicates dehydration. Textbooks often suggest that a slight tenting represents 3% to 5% dehydration, and definite tenting represents 5% or greater dehydration. However, the experience of the author and many others is that this method is not dependable for many horses. Some horses with a definite skin pinch response (greater

than 3 seconds to return flat) had only minimal dehydration as measured by body weight change (less than 2%) at the end of an endurance ride (G.L. Ecker and M.I. Lindinger, unpublished observations) and plasma protein measured by refractometer (less than 6.7 g/dl). Yet others horses showed little change in skin tenting but had a 5% to 6% body mass loss because of dehydration (with elevated plasma proteins). Noting the breed, body condition, age, normal skin elasticity, and other factors that can affect the skin pinch test is important. The skin pinch test is not a reliable test for dehydration without looking at other factors such as plasma proteins, along with body mass losses if possible and the other parameters (Figure 12). One must consider the parameters assessed in Figure 12 altogether to present the whole picture on the state of the horse.

Figure 12 Parameters for checking the status of the endurance horse.

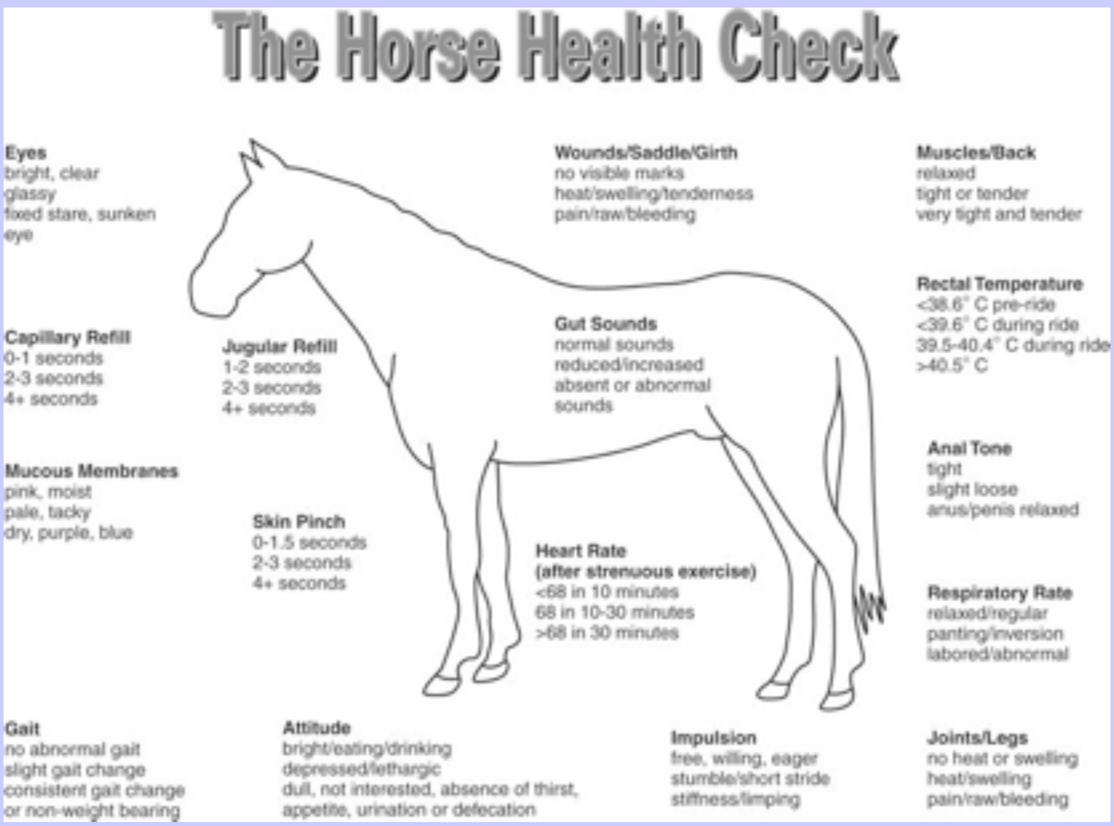

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Figure 12 represents the health check commonly performed on endurance horses. The parameters are outlined and color coded. Green indicates normal for a healthy horse. Yellow indicates that caution is necessary, particularly as the number of parameters that fall into the yellow category increase. Red indicates that a serious problem exists and all exercise should cease. Water and electrolyte deficits affect virtually all parameters because of loss of blood volume and therefore decreased perfusion and changes to the muscle membrane potential.

As water leaves the plasma compartment, plasma protein concentrations and hematocrit increase. Can one then use hematocrit alone to determine dehydration? Although this certainly is done, acknowledging the changes in hematocrit and how they come about is important. The spleen of the horse is capable of storing and then releasing up to one half of the red blood cells. Nervousness or stress can induce splenic contraction, but the response is not all or none. If the horse is stressed (and this is difficult to determine in many horses simply by

observing that many horses can be stressed for a variety of reasons without showing much outward sign), then the hematocrit may be raised. Therefore to assess dehydration of the extracellular fluid compartment more accurately, including measurement of plasma proteins is beneficial. Lindinger and Ecker found that during moderate-intensity exercise, 50% of the increase in hematocrit was caused by plasma water loss and 50% was caused by splenic release of red blood cells, thus emphasizing the importance of using the changes in plasma proteins.[72]

A quick calculation can give one a good indication of the plasma fluid compartment change in water content as follows:

$$\text{Percent change in plasma volume} = \frac{\text{Plasma protein at rest} - \text{plasma protein at finish}}{\text{Plasma protein at finish}} \times 100\%$$

This equation assumes no net change in plasma proteins.[71,72]

Knowing the average range of normal, rested, and fully hydrated horses is important before one begins so that one can determine if the horse is dehydrated at the first sampling. If the plasma protein is above normal, then the horse has preexisting dehydration and subsequent calculations will underestimate fluid losses. Waiting at least 15 minutes following the cessation of exercise before taking blood samples to assess plasma volume changes is important, so that the exercise-induced fluid shifts do not influence the calculations.[75] No direct method of detecting intracellular fluid volume loss exists, but one can estimate this from the difference between changes in total body water and the changes in extracellular fluid volume (ECFV) calculated from the change in plasma proteins.

One then can use the change in fluid content to calculate the change in electrolyte content. The calculations are as follows and use the typical values from an endurance horse.[71]

The following values are used:

- ECFV is assumed to be 0.222 L/kg.[70]
- Preride mass of the horse is 430 kg, and postride mass is 420 kg.
- Preride sodium concentration is 144 mmol/L, and postride it is 142 mmol/L.
- Preride plasma protein concentration is 6.2 g/dl, and postride it is 7.1 g/dl.
- Step one: The horse weighs 430 kg before the start of the ride. Extracellular fluid volume at rest would be 0.222 L/kg[70] × 430 kg = 95.5 L.
- ECFV ion loss = {plasma $[\text{ion}]_{rest} \times \text{ECFV}_{rest}$} − {plasma $[\text{ion}]_{finish}$}, where $\text{ECFV}_{finish} = \text{ECFV}_{rest}$ − (ECFV_{rest} × % change in plasma volume)

  $\text{ECFV}_{finish} = 95.5 - (95.5\ \text{L} \times 8.8\%)$

  $\text{ECFV}_{finish} = 87.1\ \text{L}$

Then,

$$\text{ECFV Na}^{+}\text{ loss} = \{144\text{ mmol/L} \times 95.5\text{ L}\} - \{142\text{ mmol/L} \times 87.1\text{ L}\} = 1383.8\text{ mmol}$$

Therefore the loss of Na^+ for this horse would be 1384 mmol or approximately 60 g Na^+ from the ECFV.

23.10.5 PREVENTION OF FLUID AND ELECTROLYTE DEFICITS DURING PROLONGED EXERCISE

Prevention of health and performance problems related to fluid and electrolyte imbalances is the goal to maintaining optimal performance.

The ideal would be to replace water and electrolytes at the same rate as they are lost; however, replacement is not always possible, given the circumstances of the competition. Therefore other management techniques are needed to prevent the dehydration and to restore the losses as soon as possible. Such techniques include using well-soaked feed (hay, extruded beet pulp, etc.) to put a large volume of water into the gastrointestinal tract, preloading with electrolytes before the event to help provide a reservoir and encourage drinking, the provision of small amounts frequently of electrolytes *and* water throughout the event if possible and immediately after events with prolonged sweating (i.e., longer than 2 hours).

23.10.6 SUMMARY

The veterinarian can give guidelines to help clients determine the best course of action for their horses, but 1596
unfortunately, trial and error still must play a role for the majority of horseowners to adjust the supplementation 1597
for the conditions of the day. A more detailed, individualized method has been used for individual consulting, and this includes blood samples and body weight measurements before, during, and after the event (or mock event), along with sweat electrolyte analysis. This allows designer electrolytes and individualized electrolyte supplementation for the horse owner based on objective data collection. One then can adjust supplementation for the conditions and develop guidelines that will help for future ride conditions.

23.10.7 PRACTICAL HINTS AND APPLICATIONS

The following section gives, in short form, some of the important points that clients need to understand. This collection is based on the questions most asked of the author.

- During transport, the horse loses water and electrolytes because of the stress. Because recovery can take several days, prevention is important. Introducing soaked beet pulp to the diet of the horse during the week before transport helps. Two to 4 hours before loading, one can add soaked beet pulp to the feed and then provide wet hay and access to fresh water. One to 2 hours before transport, one can administer electrolytes orally. If possible, one should add the electrolytes to the water during transport; the addition of more sodium keeps up the thirst drive.

- Any horse that is off feed also will drink less and therefore is likely to have varying degrees of dehydration. If the horse is eating less, adding electrolytes, well-soaked beet pulp, and wet hay to the feed may be beneficial. The beet pulp delivers large amounts of water to the intestinal tract, and the electrolytes help increase the thirst response.

- Water intake alone dilutes the Na^+ concentration and diminishes the thirst drive, which usually happens long before the water deficit has been replaced. Adding electrolytes, or in particular sodium, to the water helps prevent the sodium concentration from being diluted, and the horse will continue to drink more during the trip.

- A horse that will not drink despite clinical signs of dehydration needs assistance to replace the water and electrolytes. Although the belief is prevalent that one should not give electrolytes to a dehydrated horse, one should give the horse small amounts of water frequently and electrolytes orally, along with soaked beet pulp and soaked hay. The small amounts of electrolytes with a glucose source frequently help increase the sodium concentration without causing massive fluid shifts into the gut and away from the other tissues. This method often is enough to stimulate a thirst response and get the horse to eat and drink. If the condition is serious, then nasogastric administration of oral fluids with a balanced isoosmotic solution of electrolytes containing Na^+, Cl^-, K^+, Ca^+, Mg^{2+}, and glucose is required for rapid restoration. The oral approach has the advantage of providing badly needed fluids into the gastrointestinal tract in an effort to maintain or restore intestinal motility (improve gut sounds).

- Glucose is needed for the intestinal cells for absorption of Na^+, K^+, and water to occur. Therefore the ideal electrolyte solution contains the appropriate amounts of glucose, along with the electrolytes lost in sweat (Na^+, K^+, Cl^-, Ca^{2+}, and Mg^{2+}). The forms of electrolytes should be highly soluble, the pH should be balanced (7.4), and the taste should be palatable. One gains little by using an unpalatable electrolyte because the horse will find ways to refuse to swallow it and some may refuse to eat and drink following forced administration of the unpalatable supplement. Another important note is that some electrolyte supplements have been associated with burns in the mouth, particularly with copper bits, and one should avoid these electrolyte mixes.

- A conditioned horse often is believed to need fewer electrolytes. In fact, the research shows the opposite. Horses that have undergone an acclimation protocol had higher sweat losses and therefore a greater need for electrolyte supplementation to maintain fluid and electrolyte balance.

- Regaining water and electrolyte balance quickly is desirable. Nothing is gained by allowing dehydration to linger except a delayed recovery, inadequate hydration *and* energy status, and higher risk of health and performance problems. Simply allowing a horse to recover from prolonged exercise results in delays in restoring normal fluid, electrolyte, and energy balance.

- About 15 years ago, many competitors and veterinarians told this author that dehydration during endurance events was inevitable. With improved fluid and electrolyte supplementation regimens, such statements have been shown incorrect. Proper administration of water and electrolytes before, during, and after an event has been shown to maintain body weight throughout the ride, despite the challenges of demanding, international-level, 100-mile endurance rides.

- Anecdotal evidence suggests that some electrolytes cause a horse to develop a sour stomach and hence go off feed and refuse to drink. Continuing to use such an electrolyte is ill-advised because the electrolyte exacerbates the very state one hopes to correct.

- Ideally, to achieve optimal hydration state and performance, one needs to adjust the electrolyte supplement for each horse and for the specific conditions under which the horse is being transported or is performing. Factors such as heat, humidity, lack of feed intake, higher-intensity work, and hilly terrain

increase the need for higher supplementation. Each horse is an individual, and one must adjust the supplementation regimen accordingly. 1598

- The veterinarian can help the client improve the fluid/electrolyte balance of the horse by passing along helpful hints and guidelines. The following section includes a variety of helpful hints that the veterinarian can use to help increase the effectiveness of clients' electrolyte supplementation regimens.

- The veterinarian should encourage the client to use a quality electrolyte supplement that has been developed and supported with proper scientific procedures and can help the client select a quality electrolyte supplement by studying the label. The product should consist of about 60% electrolytes and 40% glucose (or glucose/fructose mixture). If the product lists glucose or other sugars as the top ingredient, then the product is unlikely to be an effective a supplement for replacing the salts lost in sweat. Conversely, if the product lists no glucose, then absorption likely will be less and the product will not be palatable to the horse.

- An important note is that potassium plays a critical role in maintaining muscle function. The considerable amount of K^+ lost in sweat is derived from cells, not from the plasma or extracellular fluid, because these compartments maintain the K^+ concentration. Many studies clearly indicate that potassium plays an important role for maintaining skeletal muscle function, but what is less clear is the effect of potassium loss on smooth muscle. However, one can reasonably assume that potassium loss from the entire body is a risk for skeletal and smooth muscle. Therefore when gut function is diminished, one must consider potassium replacement a priority. For optimal absorption of the potassium, one should administer sodium, glucose, and water orally at the same time.

- Following oral administration of electrolytes and water, one should monitor for changes in 30 to 60 minutes. If the supplementation has been effective, the horse should improve noticeably during this time. For example, the absorption and uptake of an effective electrolyte supplement should reach maximal levels in about 45 minutes, and visual examination of the horse should show changes in any or all of the following: gut sounds, capillary refill, jugular refill, and sometimes skin pinch, anal tone, muscle.

- Many horses will not drink despite obvious dehydration. The loss of sodium in the sweat contributes to this lack of drinking despite water volume losses. Administering small amounts of electrolytes frequently may encourage the horse to drink or, if the horse is interested, one may feed wet hay, for often the horse will begin to drink once it starts to eat hay. Then one can follow water ingestion with appropriate amounts of electrolytes.

- One should not depend solely on the skin pinch to indicate the level of dehydration. The skin pinch test works well for some horses but can be erroneous for others. For example, in the research conducted by Lindinger and Ecker, horses with less than 3% body mass loss from sweating have had distinct skin tenting, and yet more than one horse with greater than 5% body mass loss from sweating has shown no skin tenting.[72]

- Horses do not store electrolytes. Electrolytes that are ingested in excess of what the horse needs for restoring and maintaining ECFV, intracellular fluid volume, and cellular function are cleared *rapidly* from the bloodstream by the kidneys. Therefore one should give electrolyte supplementation according the sweat losses once the basic diet has been balanced. Moreover, the kidneys cannot be trained by depriving them of electrolytes during the week, in the hopes of better retention of electrolytes during the competition. One should make sure the horse is on a balanced diet that provides all the nutrients,

including adequate minerals, and then add electrolytes according to the sweat losses during training and competition.

- Horses cannot be toughened to withstand dehydration and electrolyte deficits. Such practice is likely to cause chronic deficits that will have an affect on performance and eventually on health. Using electrolytes during training/heat acclimation is necessary to restore the losses quickly for enhanced recovery and to get the horse used to the procedures on a routine basis.

- Provision of a salt block may not be adequate for sodium intake. Loose sodium chloride should be available along with fresh water at all times. Horses that lick salt blocks for prolonged times or chew or scrape salt blocks are likely candidates for sodium (and therefore water) deficits.

- One should meet the basic (nonexercise) requirements for all the electrolytes through a properly balanced diet that has been analyzed and balanced for the hay ration and geared toward the age, activity, and breed of the horse. Then for performance horses that are engaged in sports where activity is prolonged, or in other words where the horse is sweating because of exercise for longer than 2 hours, replacing the electrolytes lost in the sweat for rapid recovery and optimal health and performance is beneficial. The use of electrolytes does not make up for inadequate diet and preparation of the equine athlete. This holds true for horses in prolonged transport.

- More than 10 years ago, when this author began studying fluid and electrolyte balance in endurance
horses, many competitors and veterinarians generally believed that weight loss and dehydration were 1598
unavoidable. Some participants in the sport even felt that such weight loss and dehydration problems 1599
were not that serious and could be tolerated during the event and then corrected over several days by leaving the horse alone. Further research in human beings and in horses has demonstrated that maintaining fluid and electrolyte balance is a highly desirable goal in athletes, with positive outcomes on health and performance. Maintenance applies to before, during, and after the event.

- Although many previously doubted this, a horse can compete in a tough, 100-mile ride with little to no weight loss. Weights collected at the PanAm Championships in Vermont in 2001 and at other rides demonstrated that when effectively managed with feed, fluid, and electrolytes, horses can be competitive and experience little weight loss (M. Foss and G.L. Ecker, M.I. Lindinger and G.L. Ecker, unpublished observations).

- One should realize that horses pulled for lameness may have underlying and potentially serious metabolic/dehydration problems. Monitoring the electrolyte/hydration balance on horses pulled for lameness is important to ensure that they recover metabolically afterward. Lameness could result from fatigued muscles, leading to compensation or stumbling and less coordinated movement.

- Sweat concentrations have received more study during the last 7 years, and veterinarians now have a better understanding of the individual differences in sweat concentrations (M.I. Lindinger and G.L. Ecker, unpublished observations). Some horses have higher than average concentrations of sodium or potassium, and these horses have benefited from specially tailored electrolyte mixes to ensure rapid restoration of electrolyte balance.

- In some cases, gut sounds are present, yet other parameters such as capillary refill, jugular refill, and mucous membranes indicate dehydration. Possibly, strong concentrations of electrolytes or insoluble salts in the intestinal tract without adequate ingestion of water creates an osmotic draw that pulls water from the extracellular fluid and ultimately from the intracellular environment. This may not be an issue

for a resting, hydrated horse; however, for a dehydrated horse, this possibly could tip the horse into a serious state of dehydration.

- Potassium plays a key role in the functioning of smooth muscle in a manner similar to that in skeletal muscle. Therefore in the presence of reduced gut sounds, one must consider potassium a priority for replacement with water, other electrolytes, and volume in the gastrointestinal tract. Well-soaked beet pulp or wet hay along with water consumption and electrolytes may help restore gut function quickly, thus significantly reducing the risk of colic and other health concerns.
- One must emphasize that a fluid and electrolyte supplementation program during athletic events will not compensate for a poor daily diet, inadequate conditioning, or poor selection of genetic material for the type of event. However, when the horse is well-suited for the event, the daily nutritional intake is balanced, and the horse has been properly conditioned, then an effective fluid and electrolyte management program goes a long way to develop optimal performance and health.
- Laboratory research around the world has provided new insights into electrolytes, but from the practitioner's point of view, the results must be applied in the real world, and often the transition is difficult. With the new portable blood analyzers, real-world application is easier; however, this information rarely is available.
- The future may hold a different picture for monitoring of hydration and electrolyte state through the use of bioelectric impedance analysis, but for now field-based systems are limited.[77] Portable weigh scales are useful for monitoring body water losses if one obtains weights before and after the event.
- If one has access to a portable blood analyzer, then the veterinarian possibly can help clients determine optimal supplementation regimens. One should be sure to take the blood sample at least 15 minutes after the horse has stopped exercising to allow time for exercise-induced fluid and electrolyte shifts to revert. Disturbances persisting after 15 to 30 minutes are attributable to dehydration. When one assesses blood analysis for electrolyte and hydration, interpreting the results of concentrations relative to the fluid shifts is critical. Sodium and other plasma electrolyte concentrations commonly remain within normal values despite large losses of water and electrolytes because the sodium and water are lost are roughly the same rate; therefore the concentration may change little. One should interpret concentrations relative to plasma protein changes (see the foregoing equations).

If one is able to collect blood in the field for later electrolyte analysis, then proper handling of the blood sample is critical. One should centrifuge the blood as soon as possible and then pipette the plasma and store it on ice until analysis of electrolytes and plasma proteins is possible.

23.11 Glucose Intolerance and Hyperinsulinemia

Sarah Ralston

Abnormalities in glucose/insulin metabolism are not uncommon in horses. Fasting glucose and insulin concentrations, however, rarely are affected. The abnormalities are detected most easily when the system is 1599
challenged with a load of dextrose or glucose, absorbed from the small intestine or administered intravenously. 1600

23.11.1 ABNORMALITIES OF GLUCOSE/INSULIN METABOLISM

Glucose intolerance is determined by observing abnormally high responses in glucose and/or insulin to a standardized glucose challenge. In human beings such response most commonly are associated with insulin-dependent diabetes (type 1 diabetes), with high blood glucose, and low to normal insulin release following deficient β-cell function in the pancreas. Insulin deficiency/type 1 diabetes rarely, if ever, has been diagnosed in horses.

However, *hyperinsulinemia* (typical of type 2 diabetes and obesity in human beings) is not uncommon in equines. The higher than normal plasma insulin concentrations associated with this syndrome may precede actual glucose intolerance with the concommitant elevations in blood glucose and reflect a relative resistance to the action of insulin on cellular uptake of glucose.[78] Other actions of insulin (such as inhibition of lipolysis and stimulation of lipogenesis and growth hormone) may or may not be affected.

Most studies of glucose/insulin metabolism in horses have reported at least one hyperinsulinemic individual.[79–87] In studies using adult horses or ponies, pituitary adenomata and obesity were associated most commonly with the reported hyperinsulinemia.[80,83,86] In other studies the hyperinsulinemia was a serendipitous finding and appeared to be caused by genetic predisposition[81,82,84,85] or was unexplained by the authors.[79,88]

The potential causes of glucose intolerance/hyperinsulinemia in horses are many, with obesity and pituitary dysfunction being the most common. Daily injections of equine recombinant growth hormone in aged horses resulted in significant hyperinsulinemia in all of the treated animals that persisted for at least 4 weeks after cessation of the treatment.[89] A strong correlation exists between postprandial hyperinsulinemia and the radiographic presence of osteochondrosis lesions in Standardbred horses less than 9 to 12 months of age.[84,85] Horses between 3 and 12 months of age also were documented as having higher insulin responses to meals than those greater than 18 months of age.[84,90] These studies led to the hypothesis that abnormally high insulin, whether caused by an inherited insensitivity, age-related alterations, or response to large amounts of carbohydrates being fed, would alter bone metabolism, which contributes to the appearance of osteochondrosis lesions or other growth abnormalities in young, rapidly growing horses.

Postprandial hypoglycemia has been reported in horses with polysaccharide storage disease.[91–93] In these animals insulin secretion was within normal limits but they suffered profound hypoglycemia following a meal of starch, supposedly because of an enhanced sensitivity to the action of insulin.[91] As in human beings, dietary control of the disease consists of a high-protein, high-fat diet with limited starch intake.[94]

23.11.2 FACTORS AFFECTING BLOOD GLUCOSE AND INSULIN IN HORSES

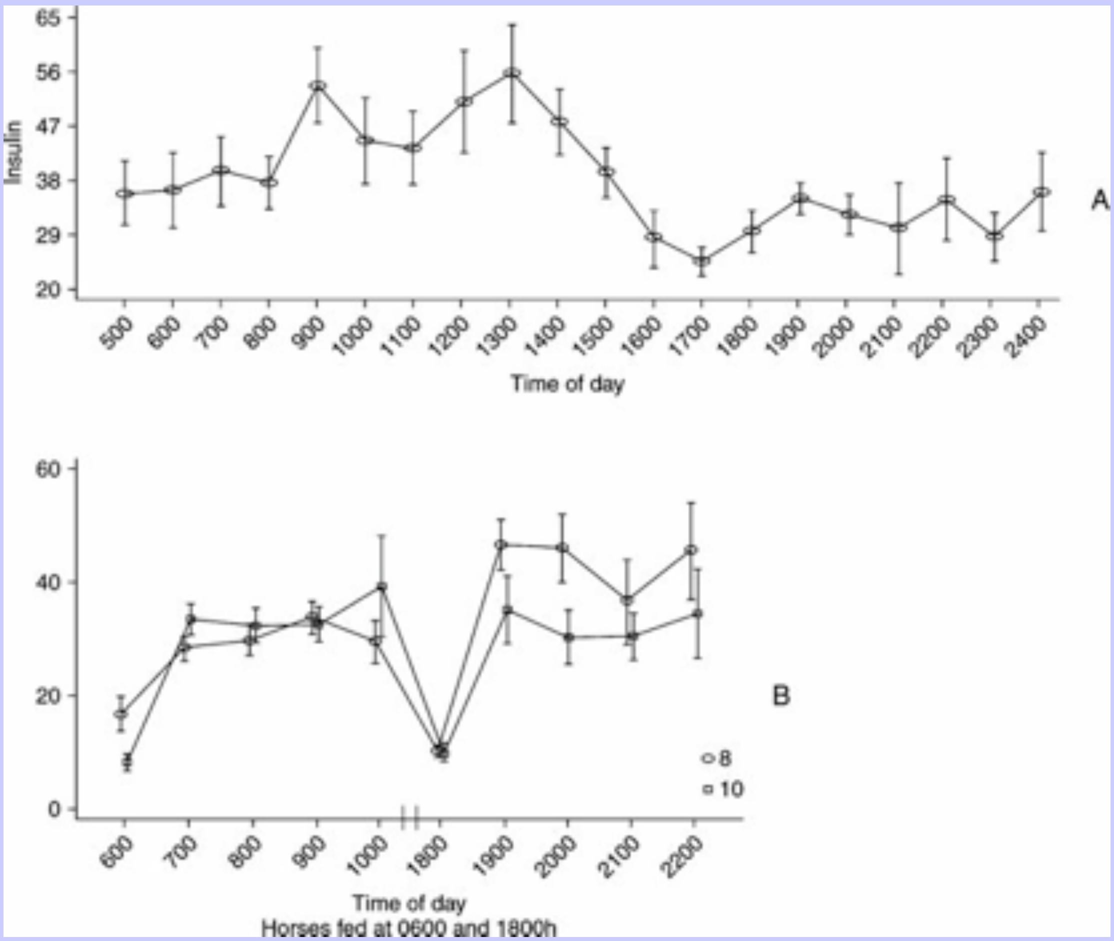
The regulation of insulin secretion in horses appears to be similar to that in human beings. Horses respond to insulin secretagogues such as arginine and lysine similarly to the way human beings do.[78] Glucose uptake differs with the type of ration the animals are fed, with lower insulin responses reported in animals adapted to only forage versus those fed higher-carbohydrate grains.[83,88,95,96]

In most studies of postprandial glucose/insulin responses in horses, however, the animals were fed their test meals in the morning.[83–85] In a 24-hour study of eight horses, researchers noted a striking difference in the

response to a meal of grain fed at 8 A.M. relative to the same amount fed at 4 P.M. The horses had significantly lower insulin responses to a meal of grain in the afternoon than in the morning (S.L. Ralston, H.F. Hintz, and T.J. Divers, 1998, unpublished data). The horses were fed 4 to 5 kg of hay and 1 kg of grain concentrate at 8 A.M. and 4 P.M. daily. They had been confined to their stalls for 4 days before the study and were well adapted to the routine. An important note is that while all the horses ate the grain concentrate meals within 30 minutes of feeding, they took 5 to 6 hours to consume the hay completely. This resulted in their having no feed available for more than 8 hours overnight but fasting only 1 to 2 hours before the afternoon feeding. Researchers hypothesized that the length of fast was more of a factor in their glucose/insulin responses than was the actual time of day.

In follow-up studies, horses were fasted for 0 (continuous access, fed at 4-hour intervals), 8, or 10 hours between feedings. This group of horses was not adapted to being confined to stalls for 24 hours and also were fasted for the allotted amount of time during the day before the initiation of sampling, starting at 10 A.M. (8-hour fast) or noon (6-hour fast) before the first feeding at 6 P.M. The continuously fed horses exhibited the same diurnal variation in insulin response observed previously (Figure 13, *A*), with higher insulin and cortisol release in the morning than in the late afternoon. When fasted for 8 hours or more before feeding in the afternoon, however, the horses did not exhibit diurnal variation in insulin response (Figure 13, *B*). Indeed, after the 8-hour 1600
fast, the horses had higher (P <0.05) insulin responses in the afternoon than they had the next morning, a 1601
reversal of the usual pattern. Researchers hypothesized that the animals were stressed by the daytime fast, which caused the higher than normal plasma cortisol at the first afternoon feeding. When the horses were kept in for a second afternoon feeding, the cortisol concentrations were lower, as were insulin responses to the concentrate feed. These results are comparable to the effects of stress and time of day on insulin sensitivity in human beings and emphasize the need to consider possible effects of diurnal variation in cortisol release higher in the morning than the afternoon in unstressed horses) and stress on glucose/insulin when testing for abnormalities.[97,98]

Figure 13 **A**, Diurnal variation in postprandial insulin (IU/ml) in horses offered feed every 4 hours for 24 hours. Values are means ± standard error. **B**, Morning versus late afternoon plasma insulin (IU/ml) response to feeding after 8 or 10 hours of fasting. Values are means ± standard error for eight horses.



23.11.3 TEST OF GLUCOSE/INSULIN METABOLISM

The use and interpretation of the traditional intravenous and oral dextrose challenges have been reviewed previously.[99] The focus of this section is novel or modified tests and interpretations.

23.11.3.1 Grain Test

In a study of the effects of chromium supplementation in aged mares, researchers found that a test meal of grain was a more sensitive test for abnormal glucose/insulin responses in aged horses than the traditional

intravenous glucose test.[87] One should feed 1 to 2 kg (depending on previous feeding history) of a grain concentrate to the subject horse and a control animal(s). The controls should be the same age and size and should have similar feeding histories and activity levels, because these factors influence the responses. The test should be done between 7 A.M. and 9 A.M. One then takes blood samples for glucose and insulin analysis before feeding and at 60, 90, and 120 minutes after feeding. These are the times at which one sees the peak glucose and insulin responses. Normal glucose responses are usually between 100 to 130 mg/dl, and insulin should not peak at more than 60 IU/ml in adults and 25 IU/ml in horses less than 9 months of age. 1601

Insulin responses 1 or 2 times higher than normal are associated with obesity and early pituitary dysfunction 1602
in adult horses and developmental orthopedic disease, specifically osteochondrosis, in horses less than 9 months of age. Insulin responses greater than 2 times normal usually indicate severe pituitary dysfunction. These results suggest only a potential problem, and one should perform definitive tests before making a diagnosis. In nonobese adults, one should consider a modified dexamethasone suppression test to confirm pituitary dysfunction. In horses less than 9 months of age, taking radiographs of the hocks and fetlocks to determine the presence or absence of osteochondrosis lesions is prudent. In all cases, one should limit and formulate concentrates to provide most of the calories in the form of fat, fiber, and protein rather than a highly soluble carbohydrate (i.e., sweet feeds with a high molasses content).

If neither glucose nor insulin concentrations increase following ingestion of the meal within 30 minutes of feeding, the horse may have delayed gastric emptying or a malabsorption syndrome. Again, one should perform more definitive tests before making a definitive diagnosis.

If blood glucose and insulin increase initially but then rapidly decline in a horse suspected of polysaccharide myopathy caused by chronic rhabdomyolysis, one should obtain muscle biopsies for a definitive diagnosis.[91] Placing such animals on a low-carbohydrate, high-fat, and high-fiber ration will not hurt them while one waits for results and may help the horses.[81,100]

Drawbacks of the grain challenge are variations in the rate at which horses consume the feed, differences in glycemic indices of feeds, and concerns in some horses regarding feeding large meals of grain as a risk factor for founder or colic.[100,101]

23.11.3.2 Low-Dose Oral Dextrose Challenge

The traditional oral dextrose challenge, which tests glucose absorption and metabolism, dictates that 1.0 g dextrose per kilogram body mass be delivered as a 50% solution by nasogastric tube after a 12-hour fast. One should take blood samples at 30-minute intervals for more than 3 hours. This test is not practical for standard use in practice and causes nonphysiologic elevations in blood glucose and insulin that may obscure more subtle alterations in glucose/insulin metabolism.[78]

Administering 0.25 gm dextrose per kilogram body mass as an oral paste even to nonfasted nursing foals and weanlings gives glucose and insulin responses that are indistinguishable from those observed after a standard meal of grain and allows a fairly nontraumatic, rapid assessment of glucose/insulin metabolism in a more controlled manner than the grain test. One should conduct the test between 7 A.M. and noon at the time that maximal responses are expected. One should be aware that horses adapted only to hay may have higher responses than those accustomed to more than 2 kg of grain per day. One should draw blood samples before dosing and at 60 and 90 minutes after dosing, the time at which peak responses are expected. In adult horses being screened for pituitary function, abnormally high responses also may be caused by obesity. In young

horses the responses even in abnormal animals are blunted after 10 to 12 months of age and may not reflect genetically induced abnormalities in glucose/insulin metabolism. One can intrepret and act on abnormal responses as for the grain test. Again, one should not consider this test definitive but rather a rapid screening for gross abnormalities.

23.12 Feeding and Management Today and in the Future: Environmental Concerns

Today phosphorus and nitrogen are recognized as potential environmental pollutants that can affect water quality negatively. The current Clean Water Act addresses the surface water runoff and groundwater contaminates as well as air pollutants. The federal government enforces the act under the Environmental Protection Agency and currently is evaluating dairy, swine, poultry, and horse farm operations. The Nutrient Management Plan affects horse farms with more than 8 horses or that have a gross farm income of more than $2,500 per year. Horses fed diets with excess amounts of nitrogen or phosphorus or fed any feed above the SUL produce manure with higher concentrations of phosphorus and possibly of nitrogen. Many states have enacted (or plan to enact) regulations concerning phosphorus and nitrogen to limit the amount of these nutrients that can be applied to cropland or pastures from the manure. These regulations provide a strong incentive in the horse industry to keep the phosphorus and nitrogen excretion under control. Each farm must write and submit an input and output balance plan for all ingredients brought onto the farm and how to handle the amounts excreted by the horse. These manure handling procedures will be written out for each farm and recorded in the county or regional office.

Feeding horses in the future will be more precise. The nutrient overages fed per day will be reduced because of the environmental concerns and regulations, and the nutrient deficiencies will be reduced because of new research and improved feed formulations and feeding directions. In the noble and appropriate attention to environmental concerns, not letting the proverbial pendulum swing too far in the direction that compromises animal health is important.

Proper diet formulation is now more important than ever. Diets should be formulated precisely to meet all the 1602
nutrient needs of the horse, and the feeding directions should be listed plainly on the bag. Each manufactured feed 1603
must have a tag or bag that lists a purpose statement and includes feeding directions to help the horse owner select the correct grain mixture to meet the needs of the horses. Such directions currently include the following:

- What horses should be fed this feed based on their physiologic status
- How much should be fed per day to meet the minimum nutritional requirements of the horse based on size and physiologic status; for example, idle, growing, reproducing, or performing

The NRC publication *Nutrient Requirements of Horses* remains an excellent starting point for developing diets and feeding programs involving specific levels of all nutrients for various physiologic stages. Ultimately, decisions about these nutrients are made on each farm. Those decisions will be based on the metabolic rate (genetics) of different breeds, the feed ingredients fed (forage and grain mixtures) with their daily consumption of each, the nutrient profiles of those ingredients, and other farm-specific factors including stress. No single publication presently addresses all of these issues. Management tools are now available within this textbook that include the following:

- Nutrient analysis of forages, hay, and pasture, including RFV
- Growth monitoring charts to determine individual growth rates and the amount of nutrients recommended to support that rate of growth

- Performance charts to determine individual nutrient recommendations based on size and reproductive status, such as open, pregnant, or nursing, and the nutrient recommendations based on performance level; for example, idle/laid up and light, moderate, and intense training

One management tool for everyone to use is the growth and performance chart. This management tool provides the minimum (recommended allowance) and maximum (SUL) amount to feed per day to meet the nutritional needs of the horse, without having a negative effect on the horse or the environment (see Figure 7).

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24 APPENDIX B DOSAGES OF HORMONAL PREPARATIONS*

HORMONE		DOSAGE	INDICATIONS
PGF2a analogues			Luteolysis
	Lutalyse	10 mg IM	Induced abortion (ineffective after 120 d)
			Sustained myometrial contraction
	Estrumate	250–500 mg IM	Uterine involution
			Induction of parturition
Progestins			
	Progesterone (oil)	150–300 mg IM	Estrous synchronization
			Delay ovulation
			Delay estrus
	CIDR		Maintenance of pregnancy
	Regumate	0.044 mg/kg PO	Behavioral changes
			Hasten cyclicity in transitional mares
		0.088 mg/kg PO	
GnRH agonist			
	Ovuplant	2.2 mg SQ	Induction of ovulation
hCG			
	Many	1500–5000 iu IM, IV	Induction of ovulation
Oxytocin		10–20 iu IM, IV, SQ	Uterine evacuation
			Induction of parturition
			Retained placenta
			Uterine involution
Estrogens			
	ECP		Estrous behavior
	Estradiol-17B		Synchronization of ovulation (with P)
PGE1	Misoprostol	100–200 μg	Cervical relaxation

* Many compounding companies provided hormonal preparations. Some preparations are not licensed for use in horses. Furthermore, they may not be available commercially.